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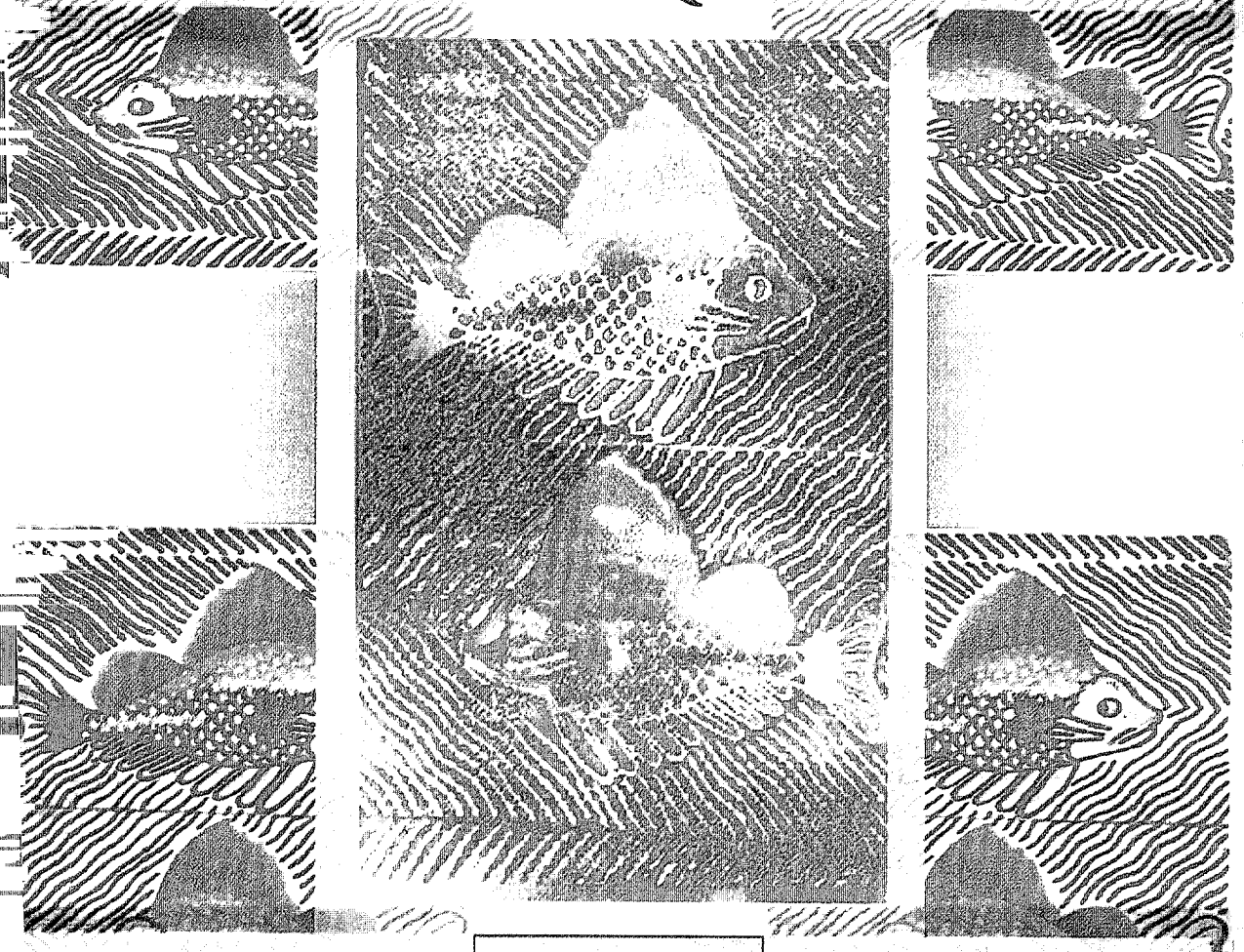
THE THIRD INTERNATIONAL SYMPOSIUM ON TILAPIA IN AQUACULTURE



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International Center for Living Aquatic Resources Management

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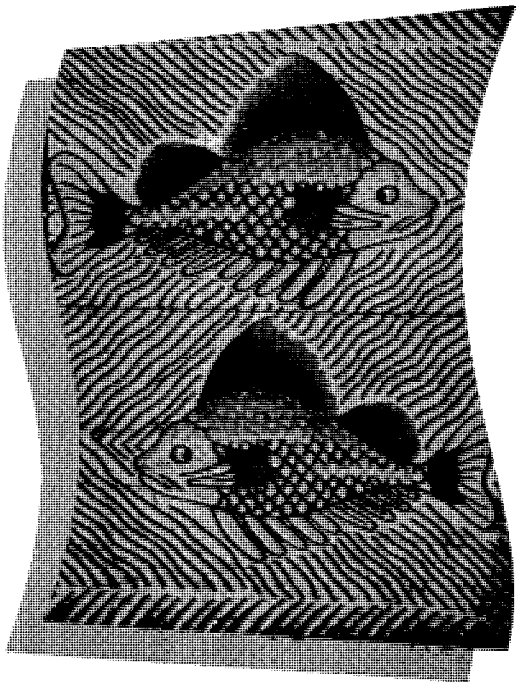

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Published by the International Center for Living Aquatic Resources Management (ICLARM), MCPO Box 2631, 0718 Makati City, Philippines; and
The Centre de recherches océanologiques (CRO) - Abidjan, 29, rue des Pêcheurs, BP V 18 Abidjan, Côte d'Ivoire;
The Institut français de recherche scientifique pour le développement en coopération (ORSTOM), 213, rue La Fayette, 75480 Paris cedex 10, France; and
The Centre de coopération internationale en recherche agronomique pour le développement/ Département d'élevage et de médecine vétérinaire (CIRAD-EMVT), Campus international de Baillarguet, B.P. 5035, 34032 Montpellier Cedex 1, France.

Printed in Manila, Philippines.

Pullin, R.S.V., J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly, Editors. 1996. Translated from the French by C. Lhomme-Binudin. The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Copyediting: Lilybeth Eleccion, Leticia B. Dizon, Marie Sol M. Sadorra and Marciana E. Araneta
Proofreading: Lilybeth Eleccion, Marie Sol M. Sadorra, Jessica A. Moya, Marciana E. Araneta and Rachel Africa
Layout: Ariel C. Aquisap
Cover: Tilapia design from an Ivorian batik. Cover design by Alan Siegfrid Esquillon.

ISSN 0115-4435
ISBN 971-8709-42-8

ICLARM Contribution No. 1325

Contents

Foreword viii
Preface ix

A. KEYNOTE PAPERS

World Tilapia Culture and Its Future Prospects • *R.S.V. Pullin* 1
Tilapia Culture in Francophone SubSaharan Africa: Current Status and Future Prospects • *J. Lazard* and *J.-Y. Weigel* 17

B. PAPERS PRESENTED

Session I: Culture Systems, Management and Production

Comparative Growth of *Oreochromis niloticus* and *Sarotherodon galilaeus* in Small Artificial Lakes in Burkina Faso and the Larger Lakes of Africa • *E. Baijot, I. Barry, S. Bouda, K.H. Kabore* and *A.C. Traore* 28
The Integration of Extensive Aquaculture (Acadja-enclos) into the Lagoon Village Environment in Côte d'Ivoire • *J.R. Durand* and *S. Hem* 33
Philippine Tilapia Farming Technologies and Their Relevance to Africa • *R.D. Guerrero III* 42
Acadja-enclos Used in Côte d'Ivoire as an Extensive Aquaculture System • *S. Hem* and *J.L.B. Avit* 46
Liming of Fishponds in Malaŵi: A Comparative Study of Limed and Unlimed Ponds • *D.M. Jamu* and *O.V. Msiska* 54
Stock Manipulation in Farmed Tilapias in Malaŵi • *E.K.W.H. Kaunda* 62
Strategies for Stocking Nile Tilapia (*Oreochromis niloticus*) in Fertilized Ponds • *C.F. Knud-Hansen* and *C. Kwei Lin* 70
The Spontaneous Reproduction of Tilapia: an Opportunity or a Handicap for the Development of African Aquaculture? • *J. Lazard* and *M. Legendre* 87
Selective Broodfish Exchange of *Oreochromis niloticus* in Large Breeding Hapas Suspended in Earthen Ponds • *D.C. Little, D.J. Macintosh* and *P. Edwards* 92
The Effect of Sex Ratio at Stocking on Growth and Recruitment in Nile Tilapia (*Oreochromis niloticus*) Ponds • *G.C. Mair* and *A.A. Van Dam* 100
Comparison of the Growth of *Oreochromis karongae* and *O. shiranus* in Fishponds in Malaŵi • *A.O. Maluwa* and *M.W. Dickson* 108
Growth, Survival and Sex Ratios of *Oreochromis urolepis hornorum, O. niloticus* and Their Hybrid Treated with 17α-Methyltestosterone • *D.E. Meyer* and *R.O. Smitherman* 112
Designing New Fish Farming Models Adapted to Rural Côte d'Ivoire • *P. Morissens, M. Oswald, F. Sanchez* and *S. Hem* 118
Growth Performance of *Oreochromis lidole, O. squamipinnis, O. shiranus* and *O. karongae,* New Candidate Species for Aquaculture in Open Waters and Fishponds in Malaŵi • *O.V. Msiska* and *B.A. Costa-Pierce* 129
Management of Tilapia (*Oreochromis shiranus* and *Tilapia rendalli*) in Ponds of Smallholder Farmers in Mwanza and Zomba West Districts of Malaŵi • *J.T.B. Mutambo* and *S.A. Langston* 135
Utilization of On-farm Resources for Aquaculture in Rural Africa • *R.P. Noble* 141

Effects of Pond Depth and Mechanical Mixing on Production of *Oreochromis niloticus* in Manured Earthen Ponds • *J.P. Szyper* and *K.D. Hopkins* 152
Production of Florida Red Tilapia (*Oreochromis* sp.) Fry in Brackishwater Tanks under Different Feeding Regimes and Stocking Densities • *W.O. Watanabe, S.J. Smith, W.D. Head* and *K.W. Mueller* 160
Production of Florida Red Tilapia (*Oreochromis* sp.) in Flowthrough Seawater Pools at Three Stocking Densities • *W.O. Watanabe, J.R. Chan, S.J. Smith, R.I. Wicklund* and *B.L. Olla* 168

Session II. Nutrition

Use of Terrestrial Plants in Aquaculture in Malaŵi • *F.J.K. Chikafumbwa* 175
Waste Vegetable Leaves as Feeds for Juvenile *Oreochromis shiranus* and *Tilapia rendalli* in Mono- and Polyculture • *S.K. Chimatiro* and *B.A. Costa-Pierce* 183
Effects of Varying Protein-energy Levels on Food Consumption, Growth and Body Composition of *Sarotherodon melanotheron* (Rüppel, 1852) • *A. Cissé* 193
Feeding Cottonseed Cake to Tilapia (*Oreochromis niloticus*) in Earthen Ponds with Catfish (*Clarias gariepinus*) as Police-Fish • *A.J. Middendorp* 198
Measurement of the Apparent Digestibility Coefficients for *Oreochromis niloticus* of Agro-industrial By-products Available in Côte d'Ivoire • *Y. Moreau* 204
Models for Estimating the Food Consumption of Tilapias • *M.L. Palomares* and *D. Pauly* 211

Session III. Reproduction and Genetics

Studies on the Effect of Manipulating Hapa Size on Broodstock Conditioning of *Oreochromis niloticus* in Fertilized Earthen Ponds • *A.J.D. Ambali* and *D.C. Little* 223
Significant Proportions of Unexpected Males in Progenies from Single Pair Matings with Sibling Sex Reversed Males of *Oreochromis niloticus* • *J.F. Baroiller* 229
Comparative Effects of a Natural Androgen, 11ß-Hydroxyandrostenedione, and a Synthetic Androgen, 17α-Methyltestosterone, on the Sex Ratios of *Oreochromis niloticus* • *J.F. Baroiller* and *A. Toguyeni* 238
Effects of High Rearing Temperatures on the Sex Ratio of Progeny from Sex Reversed Males of *Oreochromis niloticus* • *J.F. Baroiller, A. Fostier, C. Cauty, X. Rognon* and *B. Jalabert* 246
Cold Tolerance in Maternal Mouthbrooding Tilapias: Heritability Estimates and Correlated Growth Responses at Suboptimal Temperatures • *L.L. Behrends, J.B. Kingsly* and *M.J. Bulls* 257
Mitochondrial DNA Restriction Endonuclease and Isozyme Analyses of Three Strains of *Oreochromis niloticus* • *J.B. Capili* and *D.O.F. Skibinski* 266
Triploidy Induced by Heat Shock in *Oreochromis niloticus* • *S.L. Chang* and *I.C. Liao* 273
Effects of Substrate and Water Quality on Seasonal Fry Production by *Tilapia rendalli* in Tanks • *B.A. Costa-Pierce* 280
A Practical Quantitative Method to Estimate Relative Reproductive Activity in *Oreochromis niloticus* • *A.E. Eknath, J.B. Capili, J.C. Danting, M.S. Palada-De Vera, E.E. Dionisio, H.L. Bolivar, R.A. Reyes* and *M.M. Tayamen* 290

Searching for Behavioral Isolating Mechanisms in Tilapias • *U. Falter* 299
Plasticity in the Parental Cycle of *Oreochromis niloticus* • *J.Y. Gautier, M.A. Richard-Yris, B. Le Faucheux* and *M. Foraste* 308
Sex Reversal of Tilapia Fry by Immersion in Water Containing Estrogens • *C.J. Gilling, D.O.F. Skibinski* and *J.A. Beardmore* 314
Effects of Triploidy on Sexual Maturation and Reproduction in Nile Tilapia, *Oreochromis niloticus* L. • *M.G. Hussain, D.J. Penman* and *B.J. McAndrew* 320
Aspects of the Reproductive Strategy of *Sarotherodon melanotheron*: Comparison between a Natural Population (Ebrié Lagoon, Côte d'Ivoire) and Different Cultured Populations • *M. Legendre* and *J.M. Ecoutin* 326
Mouthbrooding Efficiency and Spawning Frequency of *Sarotherodon melanotheron* (Rüppel, 1852) in Culture Environments (Ebrié Lagoon, Côte d'Ivoire) • *M. Legendre* and *L. Trebaol* 339
A Search for Sex-specific DNA Regions in *Oreochromis niloticus* • *S.K.J. McConnell, D.O.F. Skibinski* and *J.A. Beardmore* 349
Comparative Growth of Hybrids (F_1, F_2 and F_3) of *Oreochromis niloticus* (L.) and *O. macrochir* (Blgr.) • *J.-C. Micha, R. Cuvelier, Ch. Tilquin, B. Muraille, M. Bourgois* and *U. Falter* 354
Comparison of Growth Performance and Electrophoretic Characteristics of Three Strains of *Oreochromis niloticus* Present in Côte d'Ivoire • *P. Morissens, X. Rognon* and *I. Dembele* 361
Genetic Differentiation in Several Stocks of *Sarotherodon melanotheron* and *Tilapia guineensis* from Côte d'Ivoire, Senegal and Gambia • *L. Pouyaud* and *J.F. Agnèse* 368
Growth and Gonadal Development of Triploid Tilapia (*Oreochromis niloticus*) • *B. Puckhaber* and *G. Hörstgen-Schwark* 377
Implications of Reproductive Behavior of Captive *Oreochromis* Broodstock on the Quality of Their Fry • *K.J. Rana* 383
Observations on Intergeneric Hybrids in Tilapias • *K.J. Rana, B.J. McAndrew, G. Wohlfarth* and *I. Macgowan* 391
Study of Genetic Variation in Farmed Populations of Some Species of the Genus *Oreochromis* • *X. Rognon* and *R. Guyomard* 398
Basis of the Sexual and Territorial Behavior in Males of *Oreochromis niloticus* and *Oreochromis mossambicus* • *Y. Rouger* 407
Truss Morphometric Characterization of Eight Strains of Nile Tilapia (*Oreochromis niloticus*) • *R.R. Velasco, M.J.R. Pante, J.M. Macaranas, C.C. Janagap* and *A.E. Eknath* 415
Estimation of Additive and Nonadditive Genetic Parameters in the Growth of Fry of Three Strains of *Oreochromis* spp. • *C.V. Yapi-Gnaore* 426

Session IV. Biology and Ecology

A New Method for Comparing the Growth Performance of Fishes, Applied to Wild and Farmed Tilapias • *D. Pauly, J. Moreau* and *F. Gayanilo, Jr.* 433
Survival of *Tilapia guineensis* under Conditions of Low Dissolved Oxygen and Low pH • *K. Wokoma* and *I.E. Marioghae* 442

Session V. Physiology

The Role of Prolactin in the Adaption of Tilapia to Hypo- and Hyperosmotic Environments • *B. Auperin* and *P. Prunet* 449

Physiological Adaptation of *Oreochromis niloticus* and *O. aureus* to Salinity • *M. Avella* and *T. Doudet* 461

Session VI. Economics and Socioeconomics

Economics of Tilapia Aquaculture in Small Waterbodies in Bangladesh • *M. Ahmed, M.P. Bimbao* and *M.V. Gupta* 471
Regional Trends in Tilapia Production and Prices in the Philippines • *M.A.P. Bimbao* and *M. Ahmed* 476
Tilapia Culture in the Senegal River Basin and the Causes of Its Failure • *P.S. Diouf* and *J.J. Albaret* 488
Nile Tilapia (*Oreochromis niloticus*) Culture in Small Waterbodies under Different Feeding and Fertilization Regimes • *M.V. Gupta, Md. Akhteruzzaman, A.H.M. Kohinoor* and *M.S. Shah* 500
Rural Development of Tilapia Culture in Africa: from Myth to Reality • *C. Koffi, M. Oswald* and *J. Lazard* 505
Which Research for Which Development of Tilapia Aquaculture in SubSaharan Africa? • *J. Lazard* 515
Peri-urban Aquaculture in Midwestern Côte d'Ivoire • *M. Oswald, Y. Copin* and *D. Montferrer* 525

C. POSTERS

Growth and Food Conversion of Five Strains of Nile Tilapia (*Oreochromis niloticus*) Fry • *B.O. Acosta, E.E. Dionisio* and *A.E. Eknath* 537
Historical and Technical Aspects of the Introduction of Tilapia Culture in Colombia • *L.F. Castillo Campo* 538
Studies on the Growth Performance and Gonadal Development of Triploid Tilapia *Oreochromis aureus* • *S.L. Chang, C.F. Chang* and *I.C. Liao* 539
The Malaŵi Central and Northern Regions Fish Farming Project: Research, Progress and Prospects • *M. Dickson* 540
Interactions Between Nile Tilapia (*Oreochromis niloticus*) and the Pond Community at Various Fish Densities • *F.A.R. Elhigzi* and *P. Larrson* 541
Studies on the Control of Tilapia Recruitment Using Tilapia-Predator Polyculture System in Southwest Nigeria • *O.A. Fagbenro* and *A.A. Salami* 542
Observations on the Possible Effects of Salinity, Pond Regime Practices and Behavior on the Culture of *Tilapia guineensis* and *Sarotherodon melanotheron* • *G.D. Igonifagha, S.N. Deekae* and *I.E. Marioghae* 543
Periphyton Composition and Physicochemistry in an Artificial Habitat (Acadja-enclos) for *Sarotherodon melanotheron*—Adiapoté Area, Ebrié Lagoon, Côte d'Ivoire • *A.A. Konan* 544
Production of Pseudo-females of *Oreochromis aureus* Using Ethynyloestradiol • *CH. Mélard* 545
Consumption of Phytoplankton by *Oreochromis niloticus* in Lake Muhazi (Rwanda) • *J.C. Micha, J.P. Descy* and *F. Laviolette* 546
Documentation and Evaluation of *Oreochromis niloticus* Populations in Ghana for Aquaculture • *J.K. Ofori* and *J.N. Padi* 547
Induced Spawning in *Oreochromis niloticus* L. • *E.E. Roderick, L.P. Santiago, M.-A. Garcia* and *G.C. Mair* 548
Acute Toxicity of Potassium Permanganate, Petroleum Product and Textile Effluent to *Oreochromis niloticus* • *A.A. Salami* and *O.O. Oguyemi* 549
Analysis of the Morphometrics of Three Tilapias (*Tilapia zillii, Sarotherodon galilaeus* and *Oreochromis niloticus*) and Their Intergeneric Hybrids • *E. Schwanck* and *K. Rana* 550

Development of an Autonomous Pilot System in Recycled Water for the Integrated Production of Tilapia and Garden Crops Behaving as Environment Purifier • *K. Souleymane, M. Didier* and *I. Quilleret* 551
The Development of a Tilapia Strain Registry as Part of FishBase • *W. Villwock, U. Sienknecht, R. Froese* and *L. Agustin* 553
Effects of Dietary Levels of Carbohydrate, Lipid, Phosphorus and Zinc on the Growth and Feed Conversion of Nile Tilapia (*Oreochromis niloticus*) • *L. Zhongzie, L. Wu* and *Y. Yunxia* 554

D. SPONSORS AND COLLABORATORS

Agence de coopération culturelle et technique (ACCT) 555
Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) 556
Centre de recherches océnologiques (CRO) 557
Technical Center for Agricultural and Rural Cooperation (CTA) 558
International Center for Living Aquatic Resources Management (ICLARM) 558
Institut national de la recherche agronomique (INRA) 559
The French Ministry of Cooperation 560
L'Institut français de Recherche Scientifique pour le Développement en Coopération (ORSTOM) 561

Author Index 563
List of Participants 565

Foreword

Interest in the culture of tilapia unites farmers and researchers on nearly all continents in a way more reminiscent of a major agricultural commodity than a fish. Despite having been cultured for over 2,000 years, the majority of research on tilapias has been in the field of ichthyology of natural populations with some emphasis on raising tilapia in aquaria. Since the 1980s, however, the world has seen a major upswing in the culture of tilapia, chiefly Nile tilapia (*Oreochromis niloticus*) and in related research, especially on tilapia biology, including genetics.

This volume of symposium proceedings shows a strong interest in production systems research and a dawning interest in socioeconomic research. Both of these fields of research are expected to receive much greater attention in the future as the economic and market importance of tilapia increases and as we seek to understand better the distribution of benefits of the different production technologies.

Tilapias, because of the low cost and relative ease of their production, are a potential food fish staple for many people in tropical countries and a globally traded commodity. Tilapias are used as live feed in the culture of some high-value predatory fish and are also marketed as value-added products (fillets, sashimi) in international trade. This diversity in potential end uses means that future research will have to address a wider spectrum of challenges.

With existing achievements and future challenges in tilapia research in mind, ICLARM is pleased to join with the Centre de recherches océanologiques (CRO), Abidjan, Côte d'Ivoire, the Institut français de recherche scientifique pour le développement en coopération (ORSTOM) and the Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) in publishing these proceedings of *The Third International Symposium on Tilapia in Aquaculture*. Thanks to the many individuals and organizations whose contributions and support enabled the holding of the symposium and the production of these proceedings; especially, the Ministère français de la Coopération, ORSTOM, CIRAD, the Centre technique de coopération agricole et rurale (CTA), the Agence de coopération culturelle et technique (ACCT), the Institut national de recherche agronomique (INRA) and the Institut des Savannes (IDESSA).

Dr. Meryl J. Williams
Director General
ICLARM

Preface

The Third International Symposium on Tilapia in Aquaculture (ISTA III) was one of the largest aquaculture conferences ever held in Africa. Building upon the work presented at ISTA I (Nazareth, 1983) and ISTA II (Bangkok, 1987) and upon the ever-increasing research efforts in support of tilapia farming, the proceedings of ISTA III will, we trust, be seen as another milestone in the development of tilapia as a globally accepted fish commodity and a contribution to the development of tilapia farming.

Africa, the "home of tilapias," has yet to benefit as much from tilapia farming as have other regions. However, African aquaculture research and development are producing promising results, despite the economic difficulties under which much of these are undertaken. Among the 64 papers and 17 abstracts of poster papers published here, 20 were contributed by African participants. We hope that support for the development of aquaculture in Africa—particularly using species like the tilapias-fish that feed low in the food chain and that can be farmed efficiently and without undue environmental impacts—will be increased and that Africa will become a more significant producer of farmed tilapias both for its own people and for export to the rest of the world.

ISTA III was generously supported by the Agence de coopération culturelle et technique (ACCT), the Centre de coopération internationale en recherche agronomique pour le développement-Département d'élevage et de médecine vétérinaire (CIRAD-EMVT), the Technical Center of Agricultural and Rural Cooperation (CTA), the French Ministry of Coopération, the Institut national de la recherche agronomique (INRA) and the Institut français de recherche scientifique pour le développement en coopération (ORSTOM). Information concerning these and others who assisted ISTA III is given at the end of this volume.

The long period that it has taken to publish these proceedings since ISTA III was held is regretted. Those concerned underestimated the difficulty of the task of compiling thoroughly edited, bilingual proceedings. This entailed very lengthy correspondence with some authors and among the editors, who wished to maintain high standards of scientific publishing and to include, as far as possible, all information presented. Despite the long hiatus, we hope that the value of these proceedings has not been diminished.

We applaud and thank all those who contributed to the success of ISTA III especially the organizing committee: Jean-François Baroiller, Adou Cissé, Jean-René Durand, Saurin Hem, Catherine Lhomme-Binudin, Pierre Luquet, Kassoum Traoré and Michael Vakily. We also thank Jean Baptiste Avit, Adou Cissé, Ziriga Josué Otémé, Sylvain Gilles, Rémy Dugué, Jesus Nunez-Rodriguez and Jean-François Agnèse for their help with the blueprint.

As we go to press, ISTA IV has been announced. It will be held in Orlando, Florida, on 9-12 November 1997. ISTA IV will doubtless be an opportunity for the world to see the giant steps that tilapia farming has taken since ISTA III and we wish its organizers and participants every success as they contribute further to one of the fastest growing sectors of world food production.

The Editors

A. KEYNOTE PAPERS

World Tilapia Culture and Its Future Prospects[a]

R.S.V. PULLIN
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PULLIN, R.S.V. 1996. World tilapia culture and its future prospects, p. 1-16. *In* R.S.V. Pullin, J. Lazard, M. Legendre and J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Tilapias are farmed worldwide in tropical and subtropical areas and occasionally elsewhere where warmwaters (thermal effluents or geothermal springs) are available. FAO statistics (1985-88) report tilapia culture in 68 countries. The annual global production of farmed tilapias, actually reported as such, has been static over this period: 263,000 t in 1985 and 264,000 t in 1988. If, however, estimates of production from Egypt and Vietnam (which are sometimes reported as "freshwater fishes") are included by assuming that this is nearly all tilapias for Egypt and 50% tilapias for Vietnam in 1988, then the global total for farmed tilapia production in 1988 was about 380,000 t. Over 95% of current production is *Oreochromis* spp. and their hybrids, with *O. niloticus* the predominant single species. Prospects for expansion of tilapia culture depend upon matching future Research & Development (R&D) efforts to the needs and circumstances of producers (most of whom will be new entrants, not only to tilapia culture but to any form of fish husbandry) and of consumers of domestic and export produce. The constraints to expansion of tilapia culture are, as in warmwater aquaculture: negative attitudes and policies, poor breeds, poor nonsustainable farming systems and possible adverse environmental impacts.

Recent Production Statistics for Tilapia Farming and Their Significance

Table 1 lists the best available statistics for farmed tilapia production from 1985 to 1988. In Africa, there are 29 tilapia farming countries but only seven produce > 100 t·year^{-1}. In Asia, 16 countries farm tilapia and nine produce thousands or tens of thousands of t·year^{-1}. Other regions have fewer tilapia farming countries (Caribbean, seven; Europe, one; Latin America, 10; Mediterranean/West Asia, five; Pacific, two; and the USA) and production in these regions rarely exceeds hundreds of t·year^{-1} for a single country. Such statistics are difficult to collect from remote areas and there is probably under-reporting. There are also some inclusions of catches from "enhanced" or "culture-based" fisheries; for example, nearly all the Cuban production derives from stocked reservoirs. What do such statistics mean? Is tilapia farming doing well? Does this production represent a good return on investment in R&D? The overall answer would be "probably no," or at least "not yet."

At the close of ISTA II (Pullin et al. 1988), tilapia farming seemed to have "come of age" because of the successes of ISTA I (Fishelson and Yaron 1983) and II: a growing enthusiasm for tilapia farming, a large supportive research effort and indications of progress towards solving long-standing technical problems such as population control. Moreover, tilapia farming is clearly not yet significantly hampered by diseases (there are only five papers on diseases out of a total of 259 in the three ISTAs) or by difficulties in persuading the fish to breed and to grow on a wide range of cheap feeds.

[a]ICLARM Contribution No. 841.

Table 1. Production of tilapias from aquaculture (1985-88). Entries in brackets are production data reported to FAO as "freshwater fishes," *not* tilapias *per se* and are included for those countries in which they include some significant tilapia production. All data are taken from the most recent information (FAO 1990), unless referenced otherwise. All remarks have been added by the present author. Blank entries here do not necessarily mean zero production; many reflect the difficulties of obtaining reliable information. This table is modified and updated from Pullin (1991).

Country	Species cultured	Production (t)				Remarks
		1985	1986	1987	1988	
AFRICA						
Angola	*Tilapia sparrmanii*	2	-	-	-	Culture of other species is anticipated.
Benin	Various tilapias	11	15	14	17	This probably includes *Oreochromis*, *Sarotherodon* and *Tilapia* spp. Exotic species including *O. niloticus* and *O. spilurus* have been recently introduced.
Burkina Faso	*O. niloticus*	43	40	36	7	Further development of aquaculture will probably concentrate on this species.
Burundi	Various tilapias	2	21	25	24	-
Cameroon	Various tilapias	91	87	96	116	The best prospect for future expansion is *O. niloticus* culture.
Central African Republic	*O. niloticus*	283	190	85	77	As for Cameroon.
Congo	*O. niloticus*	39	82	115	200	As for Cameroon.
Côte d'Ivoire	*O. niloticus*	559	503	648	740	There are good prospects for expansion of *O. niloticus* culture. There is also research to find suitable species or hybrids for brackishwater lagoon culture.
Ethiopia	*O. niloticus* *T. zillii*	-	-	-	1	Much of the country is too high and cold for tilapia culture.
Gabon	Various tilapias *O. niloticus*	(2)	(3)	(3)	(2)	There is scope for expansion of aquaculture using brackish- and freshwater tilapias.
Ghana	*O. niloticus*	321	225	270	297	Other cultured species include *S. galilaeus*, *S. melanotheron* and *T. zillii*.
Guinea	Various tilapias	2	1	1	-	-
Kenya	*O. niloticus*	113	114	121	169	Other cultured species include *O. spilurus* and *T. zillii*.
Liberia	*O. niloticus*	8	6	3	2	-
Madagascar	Various tilapias	74	36	38	42	Small quantitites of tilapia, principally *O. niloticus*, are grown with common carp.

continued

Table 1 (continued)

Country	Species cultured	Production (t) 1985	1986	1987	1988	Remarks
Malaŵi	Various tilapias	65	66	83	96	There is successful tilapia culture, principally *O. shiranus* and *T. rendalli*, on sugar estates and in small village ponds.
Mozambique	Various tilapias	-	5	17	20	Probably mainly *O. mossambicus*; culture of other native tilapias and possibly *O. niloticus* is likely to expand.
Niger	*O. niloticus*	7	8	14	16	*O. niloticus* is the only significant species cultured.
Nigeria	Various tilapias	4,573	3,274	3,602	3,962	Nigeria has good prospects for expansion of freshwater aquaculture (*O. niloticus*) and coastal aquaculture using native species, such as *S. melanotheron* and hybrids.
Rwanda	*O. niloticus*	35	24	55	28	*O. niloticus* was introduced.
Senegal	*O. niloticus*	1	2	2	10	Aquaculture is expanding using *O. niloticus*.
Sierra Leone	*O. niloticus*	9	14	18	20	As for Senegal.
Sudan	*O. niloticus*	30	41	43	45	There is probably some production of other native tilapias, such as *T. zillii*.
Tanzania	*O. niloticus*	21	32	35	37	Culture of native tilapias and exotics, particularly *O. niloticus*, is likely to expand.
Togo	*O. niloticus*	30	9	9	5	Culture of native species (such as *S. galilaeus* and *T. zillii*) and exotics, particularly *O. niloticus* and *O. macrochir*, is likely to expand.
Uganda	Various tilapias	13	21	27	24	Probably mainly *O. niloticus*.
Zaire	*O. niloticus*	180	689	723	759	Tilapia culture is expanding in Zaire. There is probably also significant culture of other tilapias, such as *O. macrochir*.
Zambia	*O. andersonii*	40	62	71	75	The statistics may now include other native species, introduced *O. niloticus* and hybrids.
Zimbabwe	Various tilapias	38	42	46	49	Now an expanding tilapia culture industry, changing from culture of native species, such as *O. mossambicus* and *T. rendalli*, to exotics, such as *O. niloticus* and *O. niloticus* x *O. aureus* hybrids.
Subtotals for 29 countries[a]		6,598	5,621	6,213	6,848	

[a]Excludes Gabon.

continued

Table 1 (continued)

Country	Species cultured	Production (t) 1985	1986	1987	1988	Remarks
ASIA						
Bangladesh	*O. niloticus*	-	-	-	-	*O. niloticus* culture is just beginning and will probably develop rapidly.
Cambodia	*O. niloticus*	-	-	-	-	Culture of *O. niloticus* in ricefields and ponds *O. mossambicus* is likely to expand.
China	*O. niloticus*	23,800	29,500	34,800	39,000	Tilapia culture is expanding especially in the warmer southern provinces.
Hong Kong	*O. niloticus*	1,500	1,435	1,700	1,690	Freshwater aquaculture in Hong Kong is disappearing as land values rise.
India	*O. mossambicus*	-	-	-	-	Traditional attitudes are changing. There is interest in acquiring good strains of *O. niloticus*.
Indonesia	*O. niloticus*	6,920	8,524	9,831	10,750	Tilapia culture is expanding, including now cage culture of red hybrids.
	O. mossambicus	35,410	15,487	25,228	25,228	
Japan	Various tilapias	4,180	4,113	4,624	4,760	Future expansion is unlikely because of the cold climate.
Korea (Republic of)	*O. niloticus*	118	120	56	92	As for Japan.
Laos	*O. niloticus* (and *O. mossambicus*)	-	-	-	-	These species are present for aquaculture.
Malaysia	Various tilapias	-	-	39	241	It has been difficult to separate species and hybrids in the statistics.
	O. mossambicus	313	575	539	2,350	*O. niloticus* culture is likely to expand.
Myanmar (Burma)	*O. mossambicus*	-	-	-	-	This species is present for aquaculture.
Pakistan	*O. niloticus*	2	-	-	-	There is new interest in tilapia culture in Pakistan, particularly in acquiring new stocks of *O. niloticus*.
	O. mossambicus	313	-	-	-	
Philippines	Various tilapias	27,206	30,602	44,682	48,327	There is interest in seawater culture of *O. mossambicus* and hybrids. Culture of *O. niloticus* is expanding.
	O. niloticus	15,434	25,217	31,087	26,719	

continued

Table 1 (continued)

Country	Species cultured	Production (t) 1985	1986	1987	1988	Remarks
Taiwan	Tilapias: mainly *O. niloticus* x *O. aureus* and other *Orechromis* hybrids	51,820	49,241	51,720	55,561	A large, dynamic tilapia culture industry using highly intensive systems. The statistics here are from the Taiwan Fisheries Bureau.
Thailand	*O. mossambicus*	1,432	879	476	476	*O. niloticus* is the prefered species.
	O. niloticus	15,128	18,886	16,920	16,920	Some red hybrids are also cultured.
Vietnam	Various tilapias	50,696	49,324	(115,000)	(115,000)	Tilapia culture (mainly *O. niloticus, O. mossambicus* and hybrids) is likely to expand.
Subtotals for 14 countries[b]		234,272	233,903	221,702	232,114	
CARIBBEAN						
Bahamas	Various tilapias	30	30	22	36	Freshwater and seawater systems; red tilapias are esteemed.
Cuba	*O. aureus*	14,675	14,942	15,270	13,268	Largely reservoir production and probably an underestimate as *O. niloticus* is also present in Cuba. Production is expanding.
Dominican Republic	?	3	3	3	3	Tilapia culture is probably now expanding.
Jamaica	*O. niloticus*	1,046	1,442	1,500	2,400	Production is expanding rapidly.
Puerto Rico	Various tilapias	-	-	-	3	Freshwater pond and cage culture are being encouraged.
	O. niloticus	-	-	-	7	
US Virgin Islands	Various tilapias	6	7	7	2	Probably mainly *O. aureus*.
Subtotals for 6 countries		15,760	16,424	16,802	15,719	

[b]Excludes Bangladesh, Cambodia, Laos (and Vietnam for 1987 and 1988).

continued

Table 1 (continued)

Country	Species cultured	Production (t) 1985	1986	1987	1988	Remarks
LATIN AMERICA						
Brazil	Various tilapias	-	-	-	-	Progress towards sustainable aquaculture has been slow.
Colombia	*O. niloticus*	300	300	498	800	Production of *O. niloticus* and red tilapias is expanding.
	T. rendalli	2	2	2	2	
Costa Rica	Various tilapias	50	50	120	142	Probably mainly *O. niloticus*, *O. aureus* and some *T. rendalli*.
El Salvador	*O. niloticus*	15	15	16	15	No recent information available.
Guatemala	Various tilapias	70	70	103	103	Probably *Oreochromis* species and hybrids.
Guyana	*O. mossambicus*	12	11	13	13	-
Mexico	Various tilapias	1,611	2,270	2,395	2,395	As for Guatemala.
Nicaragua	*O. aureus*	2	1	18	14	-
Panama	*O. niloticus*	69	126	166	140	*O. niloticus* culture is most likely to expand in integrated farming systems.
	O. aureus	4	7	9	0	
	Various tilapias	-	-	-	7	
Peru	Various tilapias	52	36	89	230	*O. niloticus* and hybrid tilapia culture may expand in the forest regions isolated from marine fish supplies.
Subtotals for 9 countries[c]		2,187	2,888	3,429	3,861	
MEDITERRANEAN/WEST ASIA						
Cyprus	Various tilapias	2	2	1	1	Limited culture of *Oreochromis* species and hybrids; obvious climatic restrictions.
Egypt	*O. nlloticus*	(47,346)	(50,000)	(51,300)	(57,100)	Egypt cultures *O. niloticus*, *O aureus* and *T. zillii* but statistics are disaggregated.
	O. aureus					
	T. zillii					
Greece	Various tilapias	-	-	16	28	As for Cyprus. Only intensive commercial systems, with seasonal environmental control, are possible.

continued

[c]Lacking data from Brazil.

Table 1 (continued)

Country	Species cultured	Production (t) 1985	1986	1987	1988	Remarks
Israel	Various tilapias, mainly *O. niloticus* x *O. aureus* hybrids	4,114	3,238	4,025	4,536	A dynamic tilapia culture industry and a world in intensive culture technology. Expansion is limited by climatic constraints and aridity
Kuwait	Various tilapias	-	-	-	-	A large research effort into saltwater culture of tilapias showed *O. spilurus* to be a suitable species for commercial aquaculture.
Saudi Arabia	*O. niloticus*	-	6	120	300	Saudi Arabia, in common with some other Gulf States, seeks food security through intensive domestic production. Tilapia culture is just beginning.
	O. spilurus	-	-	18	28	
Syria	Various tilapias	225	357	357	357	Similar climatic constraints to Israel.
Subtotals for 5 countries[d]		4,341	3,603	4,537	5,249	
OTHER REGIONS						
Belgium	*O. niloticus*	120	160	200	230	Heated water culture (power station effluent).
Fiji	*O. niloticus*	3	3	1	1	There is a growing interest in *O. niloticus* culture.
	Various tilapias	-	-	-	-	-
Guam	Various tilapias	66	102	125	125	Mainly *O. niloticus* and hybrids, including red tilapias.
Khazakstan	*O. niloticus* (and other tilapias?)	-	-	-	-	*O. niloticus* culture in a power station in Khazakstan has been reported.

[d]Excludes Egypt; data lacking for Kuwait.

continued

Table 1 (continued)

Country	Species cultured	Production (t) 1985	1986	1987	1988	Remarks
USA	Various tilapias	5	-	-	20	There is some culture of *Oreochromis* species and hybrids in the warm southern states and in geothermal waters. Climatic constraints and bans on introductions/transfers of exotic species may limit expansion, but some commentators forecast otherwise (Davlin 1991).
Subtotals for 4 countries[e]		194	265	326	376	
TOTALS		263,350	262,704	253,009	264,168	
ADJUSTED TOTALS		310,696[f]	312,704[f]	361,809[g]	378,868[g]	

[e]Lacking data from Khazakstan.
[f]Includes freshwater fish data from Egypt as if all is tilapia (1985 [47,346 t], 1986 [50,000 t] and 1988 [57,100 t]).
[g]Includes freshwater fish data from Egypt as if all is tilapia (1987 [51,300 t] and 1988 [57,100 t]) and freshwater fish data from Vietnam as if 50% is tilapia (1987 [57,500 t] and 1988 [57,500 t]).

This enthusiasm may have lessened. The overall rate of increase in tilapia production from 1985 to 1988 was only 7.3%·year^{-1} on the adjusted figures (Table 1). It probably remains slow; for example, production in Taiwan was 47,089 t in 1989 and 53,103 t in 1990 (Fisheries Yearbook, Taiwan Area, 1989-90). Furthermore, there is only one or a few leading countries in each developing region, while the rest produce relatively little. Given the many positive attributes of tilapias as farmed fish (Pullin 1985), what is holding back tilapia farming in most developing countries?

Constraints

Ignorance and Risk

Very few people know how to farm tilapias well. Moreover, tilapia farming systems are very variable. In the Philippines, the biggest producer in the world, tilapias are farmed in fresh- and saltwater ponds, as mixed-sex or monosex male stocks, in mono- or polyculture and also in cages, ricefields, tanks, etc. Progressive farmers are always trying to find ways to improve these systems. Potential *new entrants* to tilapia farming remain ignorant of how to do it and perceive it as a risky enterprise. The solution everywhere is to develop sustainable and environmentally acceptable tilapia farming systems in which new entrants can have confidence. Tilapia farming as a *part-time* enterprise in integrated farming systems has a great appeal because it can help to spread risks (Edwards et al. 1991).

Inappropriate Supportive Research

Pullin and Maclean (1992) reviewed over 2,400 research publications on tilapias, mostly comprising research in a single discipline: 94% biotechnical and 1.4% social science (mainly economics). Only 5% were considered interdisciplinary. Thus, there are large interdisciplinary and social science research gaps in the technical support base for tilapia farming. Moreover, researchers themselves usually conceptualize problems and needs without consultation with farmers.

Anti-tilapia Attitudes

Where tilapias are native fish, they are almost always regarded as an excellent human food and a valued natural resource. Anti-tilapia attitudes are restricted to areas in which tilapias have been or could be introduced as exotic species for aquaculture or enhanced fisheries. This has not been well-documented in the primary scientific literature. Information from Latin America, the Pacific or South Asia (the main regions where anti-tilapia attitudes are prevalent) is largely in reports or in semi-technical and popular serials. Uwate et al. (1984) have reviewed the situation for Pacific Island nations, most of which dislike tilapias, Fiji being a major exception. Nelson and Eldredge (1991) have reviewed the effects of tilapia introductions in the South Pacific and Micronesia.

The basis for anti-tilapia attitudes is twofold: (1) a preference for and an impression of the "superiority" of nontilapiine native species that are traditional foods and (2) bad experiences with tilapia introductions or news of this from elsewhere.

Preference for farming native species is not only understandable, it is sensible. Clearly, aquaculture development in Africa should use tilapias, catfishes and other species, including native carps, rather than following external or local advice to use exotic species. Such an approach, aiming at a valorization of autochtonous species for aquaculture, has been particularly developed in Côte d'Ivoire since the mid-1970s with good results and prospects (Hem et al., 1994). Another example is Malawi, which is now endeavoring to eradicate the "mistake" of inappropriate introduction by destroying

common carp (*Cyprinus carpio*) stocks in the southern region, lest it become transferred to the Lake Malaŵi catchment and establish itself there with unpredictable ecological consequences. Africa has a great wealth of native species to be screened for aquaculture use, not just the tilapias.

In Asia, Latin America and the Pacific, however, the position is more complex. The history of tilapia introductions started there, as in all developing regions, with narrow genetic stocks of *Oreochromis mossambicus*, mostly derived from a tiny feral population. These introductions gave poor stocks for aquaculture. Moreover, escapees sometimes became widely established in inland and coastal waters, outcompeting highly regarded native species and interfering with aquaculture and fisheries operations (e.g., Phillippart and Ruwet 1982; Gillett 1989).

There are no comparable reports of adverse ecological consequences of introductions of *O. niloticus*, which has become the basis for most of the world's tropical freshwater tilapia culture. In many countries, however, *O. mossambicus* has given all tilapias a bad reputation. It is a highly opportunistic, euryhaline and eurythermal species. Paradoxically, it is not necessarily a bad species for all purposes; for example, it provides valuable fisheries in Sri Lankan reservoirs (De Silva et al. 1988; Amarasinghe and De Silva 1992). Its African *native* stocks have never been thoroughly screened for aquaculture potential. Moreover, it provides an important food source for some very poor coastal dwellers (Costa-Pierce 1988).

Attitudes to further development of tilapia culture in which tilapias are exotic as follows:

PERSISTENTLY ANTI-TILAPIA AND LIKELY TO REMAIN SO.

- Countries that seek expansion of aquaculture but that have never imported tilapias and fear the ecological consequences, prefer native species, or both—e.g., Nepal.
- Countries that attempted to develop aquaculture (mainly using *O. mossambicus*) and which experienced failures, ecological disruption, non-acceptance of tilapias or combinations of these—e.g., Kiribati.
- Countries into which tilapias were unofficially or accidentally introduced and escapees became pests—e.g., Australia.

FORMERLY ANTI-TILAPIA BUT NOW CHANGING OR LIKELY TO CHANGE.

- Countries that farm native species and fear disruption by tilapia introductions, but still need good species for low-input, rapid-cycle, small-scale systems or for larger-scale intensive systems—e.g., Bangladesh, India and Pakistan.
- Countries with little aquaculture, with potential for tilapia farming and with fears of ecological disruption diminishing after good experiences with *O. niloticus* and salt-tolerant hybrids—e.g., Puerto Rico and other Caribbean nations.

BECOMING LESS FAVORABLE?

Countries that formerly introduced tilapias for aquaculture but in which aquaculture technology for preferred *native* species is now being developed and/or fears about the ecological disruption persist—e.g., much of South America.

STILL EVOLVING

- Countries in which aquaculture is newly evolving and the relative importance of carps, tilapias, catfishes and other species is not yet determined—e.g., Cambodia, Laos and Vietnam.
- Countries in which climatic factors (cold seasons, aridity or both) limit tilapia culture unless cost-effective solutions are found—e.g., China (parts of), Europe, Japan,

Korean peninsula, Mediterranean, West Asia and USA (mainland).

KEEN TO EXPAND TILAPIA CULTURE.

Countries in which tilapias are popular as farmed fish and there is scope for increased production for domestic consumption, export or both—e.g., southern China, Fiji, Indonesia, Malaysia, the Philippines and Thailand.

With respect to tilapia transfers, past mistakes should not be repeated. International Codes of Practice for transfers of exotic species should be followed. However, some of the private sector and some high officials will probably continue to make transfers without adequate appraisals. Two examples from the past, recounted by Gillett (1989) illustrate this attitude and the possible consequences—usually a permanent legacy:

- In Western Samoa, the following advice was given to the government to start tilapia (*O. mossambicus*) culture:

> "It is evident that it would be preferable to investigate all factors for a whole year, but this would involve great expense and even then, there might be some uncertainty with a few points. It is much cheaper to start with an experimental pond and stock it with tilapia (Van Pel 1954)."

- The view of the Minister for Natural Resources Development in Kiribati, as reported by Iuta (1989), was:

> "The well-known tilapia fish was introduced in our fish ponds and landlocked lagoons by well-meaning developers to increase protein supply. The result was that this highly competitive fish, which rarely grows larger than six inches in Kiribati, has replaced the culturally, commercially and nutritionally important milkfish .... If only applied research on the ecology of tilapia ... had been conducted *before* the project."

Persistent Technical Problems

POPULATION CONTROL

Population control in farmed tilapias has been reviewed by Baroiller and Jalabert (1989) and Mair and Little (1991). They mention a wide range of methods: sex reversal by androgenic hormones, intermittent harvesting, manual sexing, predators, high density stocking, cage culture, delayed sexual maturity, sterilization, hybridization and the "YY" broodstock route for *O. niloticus.* Out of all these options, very few have progressed from use in experimental studies or development trials to widespread adoption by farmers. The exceptions are combinations of hormonal sex reversal and hybridization (widely used in Israel and Taiwan) and hormonal sex reversal of *O. niloticus*, which is becoming more widespread in Asia.

Elsewhere, research proposals and development project plans still select approaches to tilapia population control more or less on guesswork and hardly ever with a thorough assessment of user (farmer and consumer) perspectives.

POOR BREEDS

Tilapia farming, like all warmwater aquaculture, has been very slow in recognizing the scope for improvement of farmed breeds by applied genetics. As reviewed by Pullin and Capili (1988), most farmed tilapias derive from very small founder stocks and little has been done in most countries (exceptions are Israel and Taiwan) to improve farmed breeds, other than occasional attempts at hybridization. Until recently, there were no attempts to apply additive selective breeding to farmed tilapias: an

approach that has been well-proven in livestock but so far only with salmonids in aquaculture (Gjedrem 1985).

Immense opportunities have been lost. For example, a founder stock of *O. niloticus* collected from the wild in Egypt in 1962 was transferred to Japan and its descendants used for transfers to Thailand in 1965 (and from thence to the Philippines in 1972). This "strain" is still used by tilapia farmers: known as "Chitralada" strain (Thailand) and "Thailand" strain (Philippines). After 26 years (certainly more than 50 generations) under "domestication," the performance of the "Thailand" strain in the Philippines, across a range of environments, is inferior or similar to that of a new founder stock of wild Egyptian fish collected in 1988 (Pullin et al. 1991; Eknath et al. 1993).

This neglect of the importance of applied genetics in tilapia breeding and ignorance among farmers about the genetic consequences of stock management means that most farmed tilapias are close to wildtypes *or worse* in their culture performance. Solutions to the problem lie in wise stock management (Eknath 1991), the evolution of national breeding programs (Eknath et al. 1991) and the realization by researchers that the best route to sustainable genetic gain is to marry the "one-step" genetic management techniques to which they are attracted as short-cuts to improvement (e.g., hybridization, polyploidy and possibly transgenic fish) with long-term additive selective breeding.

POOR FARMING SYSTEMS

It is impossible to disaggregate tilapia production statistics into cage, ponds, ricefields, tanks and other farming systems. Indeed, ICLARM proposed the "aquatic chicken" label for tilapias because they can be farmed profitably in a wide range of systems from simple backyard systems to highly intensive "factory farms"—as can poultry.

The success of any tilapia farming system depends upon its sustainability and environmental compatibility, which in turn depend upon cost-effective and wise use of resources (land, water, capital and other inputs) and coexistence with other enterprises and environmental care. At present, most new tilapia farms are experiments. This will persist until tilapia farming systems become better known: more like the routines used for poultry.

The key to a more reliable farming system may be simple. For example, the adoption of hapa nursing techniques has allowed farmers in northeast Thailand to become more confident and successful new entrants to aquaculture on their small-scale rice-based farms (Little et al. 1991). Nursing the fry of tilapia and other fish in a hapa allows the farmer to see them everyday, to watch them grow on whatever feed resources are at hand and ultimately to release larger, more "predator-proof" fingerlings into small ponds and ricefields.

One major unresolved issue in tilapia farming is to what extent hatchery/nursery operations and growout will be under separate management. They are separated in most of aquaculture, and tend to be so in the most advanced tilapia farming areas, such as Israel and Taiwan. Elsewhere there is usually a mixture of public and private sector seed supply to growers, with some growers raising their own seed.

Pullin and Maclean (1992) recommended research "on the dynamics of aquaculture enterprises and of their interactions with other enterprises (agriculture, fisheries, forestry, recreation and waste management). Resource flows and trade-offs in terms of common 'currencies' (land, water, nutrients, energy and cash itself) must be quantified so that the options for balancing and integrating aquaculture development can be evaluated within farms, communities and wider coastal and watershed areas."

Within this general framework, it is clear that the broad categories of tilapia farm-

ing systems have some general problems (Table 2). Solutions will come from closer collaboration between farmers and researchers. Moreover, because of the possibility of genotype x environment interactions, research towards better systems and better breeds must be interactive.

The Future

The comments on inappropriate research above apply not only to small-scale aquaculture in developing countries, but also to large-scale corporate aquaculture. Davlin (1991) forecasts the entry of a growing number of large corporations in aquaculture and believes this will guarantee the success of future intensive systems. He states:

> "Aquaculture as an industry has been led by academia/marine biologists for 30 years. It was their technological breakthroughs that enabled the small farmer to look upon aquaculture as an additional source of income. Eventually, the farmer saw it as a way of life and academia and fish farmers made an early 'odd couple'."

His "odd couple" scenario is still a fair description of many current relationships between academic tilapia research and tilapia farmers. Many companies just get on with the job themselves and learn by their own mistakes.

Researchers generally ignore the fact that technology for food production, whether generated through private- or public-funded research, is not neutral. It will favor either large-scale corporate farming concerns or small-scale farmers—very rarely both.

It is probably cheaper, more profitable and more environmentally acceptable to farm tropical fish in *less* intensive systems in developing countries; for example, value-added white fish products, like tilapia fillets (Pullin 1984). Davlin (1991) mentions new commercial agreements between Colombia and the USA to package and handle tilapia produced in Colombia and that Solar Aquafarms, Inc., California (using intensive recycling technology) will produce over 2,000 t of tilapia in 1992. Will there still be room for small-scale farmers to produce fish for domestic markets and export? Davlin's (1991) punch line and last word is:

> "Tilapia will, during this decade, join catfish as the dominant modest-priced fish in the US, in our view."

The future for tilapia farming remains bright, despite the somewhat disappointing recent statistics. In Africa, wherever inland aquaculture flourishes, tilapias are likely to be a major, if not the major farmed fish commodity. In Asia, there is likely to be significant expansion of tilapia farming in China and Indochina (Cambodia, Laos and Vietnam) and probably also in some of the major producing countries (Philippines and Thailand). In Latin America and the Caribbean, the situation is less clear. Tilapia production may expand in countries where it is already a proven success (e.g., Cuba and Jamaica) but native species may be increasingly preferred for aquaculture in much of these regions. In the Mediterranean/West Asia, climatic constraints and resource limitations will probably prevent much expansion of tilapia farming. This also applies to Europe and northeast Asia. Tilapia farming in the Pacific is unlikely to excite much interest, except in Fiji. In the USA, if the market for tilapia develops as forecast by Davlin (1991), there will undoubtedly be more attempts to supply this from US-based farms as well as from imports.

Pullin (1991) forecasts a doubling of world tilapia production over the next 10 years.

Table 2. Problems associated with tilapia farming systems.

System	Major problems	Farmers' needs
Cages	*Ad hoc* design, guessed at or copied from elsewhere; poor feed conversion; fouling; short operational life.	Systems specifically designed for tilapias in fresh-, brackish- and saltwater.
Pens, acadja-enclos, etc.	Still experimental.	Reliable, sustainable systems that match their resources.
Ponds	Nutrient starvation; *ad hoc* stock management; water availability/quality.	Sustainable systems, well-integrated with other enterprises.
Tanks, raceways and other intensive systems, including recycling	Largely experimental or guesswork at site-specific designs.	Reliable guidelines—as exist for trout culture.
Hatchery/nursery systems	Low and/or seasonal output of fry/fingerlings; no consideration of genetic consequences of broodstock management; low adoption of monosex seed technology.	Reliable seed supply systems that maintain genetic quality and 100% male seed production, where such is appropriate.

This forecast can be met, or exceeded, if research is better directed towards farmers' needs; if better breeds and farming systems are developed together; if anti-tilapia attitudes are changed where they are ill-founded; and if tilapia farming becomes a more sustainable and environmentally compatible enterprise, well-integrated with other development initiatives.

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Tilapia Culture in Francophone SubSaharan Africa: Current Status and Future Prospects

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Abstract

A study conducted in 1989 and 1990 on aquaculture development in subSaharan Africa suggested a typology of the different farming systems using mainly tilapia as cultured species: (1) subsistence fish farming; (2) artisanal aquaculture as a small-scale commercial undertaking; (3) "segmented" aquaculture with specialized operators at each level of the production process; and (4) industrial aquaculture. For each of these types of aquaculture, the following major elements were analyzed: target populations, farming techniques, support/extension structure, financial and economic profiles, land use as well as credit and marketing aspects. This type of analysis was used to study aquaculture activities of relative importance that are part of development projects. The identification of the farming system, farm dynamics, including "post-project" evaluation, were all studied on the basis of concrete cases. This study illustrates, among other things, the constraints limiting the development of types 1 and 4 in terms of economic viability and reproducibility. The study also shows that sustainable aquaculture requires from the different partners professional skills which have often been lacking owing to the insufficient training of the fish farmers. Suggestions are offered for a more harmonious development of aquaculture on the African continent at the level of the governments, the funding agencies and the various operators involved in this activity.

Introduction

Fish culture in Africa is always being restarted and relaunched, like many other agricultural or industrial activities on this continent (Freud 1988; Pisani 1988). In 1989, subSaharan Africa is supposed to have produced 33,000 t of cultured fish with tilapia as the major species (14,500 t) (FAO 1991). Global estimates for the same year were 14 million tonnes with 7,300,000 t of fish making the contribution from subSaharan Africa less than 0.5% of the world production of cultured fish.

Considering the total level of funding to the aquaculture sector in subSaharan Africa (averaging approximately US$15 million per annum between 1980 and 1990), most of

it intended for the implementation of development and R&D projects (62%, FAO 1990), many questions are raised:

- Does the lack of tradition in irrigated agriculture and stabulating livestock farming constitute a handicap to the development of fish culture, an activity still considered at its beginnings?
- Should subsistence and commercial aquaculture be differentiated in terms of project design and strategies for their implementation? Based on the project objectives (charitable, artisanal or industrial production), which aquaculture systems should be promoted and how can they be integrated in the environment and the existing infrastructure (lowlands, hydroagricultural belt) as well as in the existing farming systems?
- Who should be the "actors" of this new activity and, more precisely, which actor for which type of fish culture (Satia et al. 1992)? In other words, can fish farming be "everybody's business" as the widely spread slogan goes (FAO 1984)? Once the actors are identified (farmers, fishers, officials, pensioners, etc.), what is their strategy for fish culture? Does fish farming become a primary or secondary activity? Does it constitute an activity for subsistence, commercial, recreational, prestigious, etc., purposes? Does it give farmers the possibility of diversifying their production and therefore minimize their "risks"?
- What measures should be taken for fish culture to be considered an agricultural activity (or agro-industrial) and therefore fully integrated in the "agriculture-husbandry" sector to which it is naturally related?
- Based on the level of autonomy that is desired and permitted by the chosen aquaculture system, what organizational framework should be promoted to develop aquaculture production: industrial, cooperative (Depelchin and Depelchin 1984; Bérubé 1992), association of producers, self-reliance, etc.?
- What level of technical competence should each aquaculture system require and what type of training, technical, financial and economic management support should be provided to its actors?
- Which market should be aimed at considering the generally high costs of aquaculture production and the specific characteristics of this undertaking (production planning, freshness, grading, monospecificity, etc.), particularly compared with capture fisheries products?
- What are the most significant scientific, technical and socioeconomic elements to be taken into consideration before launching a fish culture development operation? Should these operations be necessarily preceded by full-scale pilot projects to test and validate the research results?
- Which support strategy should be adopted in terms of geographical distribution? Should support be provided to many fish farmers distributed on the whole territory (and producing little individually) or to a limited number of farmers geographically concentrated (and producing a lot individually)? What should be the most efficient approach to the "post-project"?
- What should be the most adequate administrative umbrella for these development projects? Is the Department of Forestry and Water Resources, in charge of this sector of activity in most Francophone countries, the most appropriate service (due to its initial efforts in the protection and conservation, and therefore control, of the natural environment) to promote a new speculation-oriented production activity?
- What structure in terms of support policies (credit, grants, training, support, taxation system, professionals and market organizations, etc.) should be instituted to guarantee the sustainability of aquaculture undertakings after the development projects have ended?
- What funding policy should be adopted to launch this new activity, both at the

investment and operational levels (grants, credits, etc.)?

The fact that these questions remain unanswered today (or is it that these questions were never asked?) attest to the gap existing between 50 years of heavily documented investment made in this sector of activity, on the one hand, and the low level of productive achievement, on the other hand (FAO 1987; Satia 1989).

Today, African aquaculture is at the crossroads. Different diagnoses agree that for the past 20 years, research and development efforts have lacked focus or even the necessary reflection on the possible development options. These attempts have also been characterized by a questionable level of economic efficiency and unresolved problems of integration of the fish farming activity in the African agricultural farming systems or fisheries initiatives in a broad sense. Against this background, any new attempt at aquaculture is a difficult enterprise and its future remains uncertain.

One of the most prominent characteristics of this undertaking, newly "grafted" on traditional activities, is the level of passion it has brought about from all sides which often influence the motivations of the different actors involved: researchers, developers, fish farmers, administrators, consultants. The intensive (battery), artisanal or industrial farming of pigs or chickens in Africa has never stirred so much passion as aquaculture has since its beginnings on the continent; it has nevertheless developed on a much larger scale. While there should not be any doubt that the pig and poultry farmers like their trade, it is clear that the reasons why they practice it are also perfectly clear to them: to earn a living and realize the largest profits.

In aquaculture, things seem neither as clear, nor as simple. If all actors involved in aquaculture agree to expect, in time, a return from the money or the effort they have invested, or even a profit, this is not, far from it, the only reason for them to get involved in this activity. Other motivations involving the operators' view on society and how to behave in such environment are often much stronger. For example, it is not surprising that a handbook on tropical fish culture should also look into poultry and pig farming, irrigated rice culture, water supply and health (waterborne diseases, etc.). In contrast, none of the studies concerned with these subjects cover fish culture. Fish culture is related, even associated with these other activities, but is it not because fish culture seeks points of anchorage and that the "fish farmers" feel they can express their views regarding all these associated activities?

These motivations may partly explain the determination, perhaps even the obstinacy shown by many aquaculture operators. These motivations also contribute to make aquaculture a control activity, which reflects the currents of thoughts and practices underlying agro-industrial and rural development in the different continents, particularly Africa.

Just as African aquaculture, caught between great practical difficulties (which is why fish culture is still practiced at the experimental, pilot or marginal level) and ideals, as shown by the quantity of writing on this subject, is seen vacillating between prolonged birth pains and early death, it seemed to us interesting to take a look at its development. Such is the objective of this study, the main teachings of which are reported hereafter.

Classification of Fish Farming Activities

Between 1989 and 1991, a study (Lazard et al. 1991) was conducted at the request of the French Ministry of Cooperation and Development to analyze the situation of fish culture in subSaharan Africa in order to propose new orientations for the future. This study consisted in a compilation of the

extensive bibliography available on this subject and in field work in Côte d'Ivoire, Guinea, Mali, Burkina Faso, Central African Republic (CAR), Cameroon and Congo. In addition, a mission with the World Bank and several American nongovernmental organizations (NGOs) completed the available information.

In the light of the studies based on concrete cases of development operations implemented in Africa for the past 30 years, four major types of fish culture were identified based on development criteria and not on intensification criteria as generally used (extensive fish culture, semi-intensive, intensive, etc.):

1. subsistence aquaculture;
2. artisanal fish culture as a small-scale commercial undertaking;
3. "segmented" fish culture; and
4. industrial fish culture.

Among these farming systems, subsistence aquaculture is the model that has benefited the most from the support of international organizations and NGOs in areas like fry production, extension, training, support structure, in projects of various sizes except, paradoxically, in the research field. Today, the overall results can be considered globally negative, the major causes for this failure being the following:

- For fish farmers, the satisfaction of their basic needs does not constitute a sufficiently attractive economic motivation considering the technical efforts required by this activity. Moreover, the proposed model did not, in most cases, meet this objective (insufficient work productivity in terms of time and space, excessive dependence *vis-à-vis* the support structures for the management of the fish farm).
- For project initiators, it seems that the implementation of this activity requires a comprehensive approach of the environment (physical, human and economic environments.)

In the end, this form of fish culture at first extolled by colonial administrations, then considered as the essential objective of most development projects, is certainly behind the confusion that has been limiting the development of this activity in the past 50 years.

Artisanal fish culture as a small-scale commercial undertaking is developing, particularly in periurban areas, due to the existence, in this type of environment, of both sources of inputs and a market likely to absorb the production at a more interesting price for the producer (Copin and Oswald 1988). This type of fish culture has yet to be developed in rural environments: integrated in existing farming systems, it should also constitute one of the driving forces of the agricultural dynamics by contributing to additional income, diversification and the integration of agriculture and husbandry.

"Segmented" fish culture is characterized by the structural division of the different farming cycles (fry production, feed production, nursing and production of marketable fish). It is particularly well-adapted to certain environments (lakes, lagoons and rivers) and to certain populations: fishers for whom fish culture can constitute an alternative activity when earnings from capture fisheries become insufficient (depletion of resources); it is also well-suited for city dwellers and entrepreneurs who see opportunities to invest capital and earn profits (Morissens et al. 1986; Parrel et al. 1986). For this type of fish culture to be economically successful, a number of constraints should be considered: technical aspects, credit availability, competence in management, etc.

Industrial fish culture is characterized by large production units which, compared to the previous forms, should be justified by the possibility to engage in economies of scale. The objective in this case is strictly economic or financial. Once the biotechnical parameters are controlled, the objective is to produce fish at the least possible cost.

It seems, at present, that most of the attempts with this type of undertaking have not met their initial objective: cost prices remain substantially higher than market prices. From a more structural perspective, this industrial option, at least for the moment, seems to be poorly suited to the prevailing forms of organization of the socioeconomic context due to its capital-intensive nature. The fact that these units are being privatized may lead one to believe in a certain economic efficiency. In reality, the lack of transparency in the farm accounts and the multiplication of grants to this sector can be misleading (Anon. 1987; Lazard 1987) and in most cases, privatization efforts consist in the mere institutional (but not economic) disengagement of the State. In addition, this industrial fish culture is likely to compete with artisanal or small-scale aquaculture enterprises. Great caution is therefore required in the development of industrial aquaculture as well as a rigorous approach in the identification of projects, particularly in the study of their economic feasibility.

However, an industrial farm could also be the driving force of a development dynamics by participating, for example, in the research process or by supporting upstream sectors (inputs, training, etc.) around which other aquaculture undertakings could be organized following other principles.

From Experiment to Operating a Farm; Trained and Organized Farmers

From the biotechnical perspective, a number of farming systems (ponds, cages, pens, raceways, etc.) using indigenous species (tilapia, siluriforms: *Clarias*, *Chrysichthys*, etc.) or introduced species (common carp, mainly) have been developed on-station and validated on a full-scale basis in R&D pilot projects. In Africa, there is a variety of species of demonstrated or potential aquacultural importance (Hem et al. 1994) that warrant the pursuit and the amplification of ongoing research studies, and require the increase of the knowledge-base of African genetic resources. However, in economic terms, particularly microeconomics, few data are available on fish farming outside pilot operations. The experimental nature of these pilot projects limits the reproducibility and the extrapolation of the results due to the limited scale of the undertaking, the extensive support structure and the specificity of the various sources of funding (grants, credit, etc.). Whatever the case, it seems that the operators of fish culture development projects should aim, first and foremost, at the economic efficiency of the target fish farmers before that of the projects themselves that have promoted and supported them.

At present, it seems that the greatest prospects lie in the entrepreneurial or artisanal fish culture systems. This type of undertaking is both a production and a development instrument (Lazard 1975 and 1977). If the periurban model has already proven successful, an efficient model using inputs other than the agro-industrial by-products must be developed (composts, integrated farming, acadjas and polyculture). In the current context of crisis facing African agriculture where diversification is strongly needed, there is hope that the concerned populations will respond quickly as soon as an efficient, "rural" fish farming model proves successful. In this context, and in the light of past experiences, caution is warranted as the level of intensification is not necessarily related to the fish farmers' technical competence.[a]

[a]It can prove more difficult to manage a farm considered a "semi-intensive" operation based on the use of farm effluents, composts, etc., that requires a "sense" for farming, than it may be for an intensive fish farm using agricultural by-products of known composition applied according to available feeding tables.

Technical competence, professionalism and the organization of the African fish farmers seem to be the key to future fish farming development on the continent. In this context, a number of actions taken so far without real social objective, i.e., without clearly defining target populations, may acquire a new meaning and increase in efficiency. Among these actions, training is of primary importance as it enables a rapid increase in productivity. The target populations of the future projects will have to be selected carefully based on technical, social and economic objectives and on the chosen fish farming model (Koffi 1992). The training efforts will have to be aimed, first and foremost, at the fish farmers and for quality rather than quantity, as opposed to what has been done so far in most development operations. Aside from the strictly biotechnical aspects of training, it will be advisable to include management training in a broad sense (general farm management, bookkeeping, cashflow aspects, etc.). Such training initiatives, however, have often been used as a lure presented by many projects to secure their extension or to conceal a faulty development policy, such as the generalized use of an expensive compound feed, or the systematic use of fry from nursing station at a high production cost (Galbreath and Ziehi 1992). Fish culture must certainly be integrated in the environment of the target population: it should therefore make a more efficient use of the existing production factors such as land, water, labor, agricultural inputs, etc. The organization of production and the development of professional associations adapted to this new sector of activity should constitute a priority area.

These professional organizations, besides acting as "pressure groups" for the protection of their members' interests, should gradually be responsible for the training and technical diffusion aspects. If (or when) a technical model proves successful, the first role of the formal or informal professional organizations will be to ensure the quantitative expansion of the model (number of fish farmers, ponds, etc.) conferring a social dimension to it. Today, the first objective of these organizations should be the reproduction as "spontaneous" as possible of the model, i.e., with minimal participation by the government: it is only in these conditions that fish farming models will really be the reflection of a rural dynamics.

The technical competence and expertise of the fish farmers must be accompanied by the corresponding technical and scientific quality in the research aspects, i.e., researchers trained at the highest level, highly rigorous and able to work in a stimulating scientific environment. This objective will require that temporary linkages be established with researchers and laboratories from the North and the constitution, in the South, of critical masses of researchers and resources, to be sought first at the regional transnational level.

While professionalism and technical competence is being pursued, the fish farmer's profession must acquire a social dimension with actors able to define their specific constraints by themselves.

Controlling the Aquaculture Space (Land); Borrowing in order to Produce; Producing in order to Sell

Controlling Land Constraints

Land constraints are differently appreciated, depending on the various farming systems, for example, depending on whether the farming model entails the appropriation of lowlands or of lagoon or lake areas. In general, in this field like in others, it seems that development operators simplify the problem of control and use of land.

The analysis of the project designs indicates that operators, while denying the

problems of access to land tenure, reproduce explicitly or implicitly foreign legal concepts in the societies concerned. In Francophone Africa particularly, operators can even refer to a legislation establishing State ownership of aquatic spaces, contradicting traditional practices. In some cases, the control and the use of terrestrial or aquatic areas (lagoons, rivers, lowlands, estuaries, etc.) rely on traditional practices. Ignoring these traditional practices can lead to serious conflicts: in agricultural societies or communities of fishers, both the control of land and land tenure (including aquatic spaces) reflect bonds of traditional dependence, closely related to classic or lineal relationships and to social relationships between individuals of a common lineage or different lineages.

In the end, it is essential that the operations of aquaculture development integrate preliminary investigations on the control and use of the terrestrial or aquatic areas concerned. For the owner or the user of these spaces, the choice to allocate these spaces depends on the opportunity cost of this resource. Preliminary negotiations not only with potential fish farmers, but also with traditional authorities (which differs from the authoritarian allocation by administrations) should help reduce potential conflicts likely to hinder ongoing operations of aquaculture development.

Borrowing in order to Produce

As a rule, in Africa, the current situation in the sphere of credit is characterized by an important crisis affecting large official structures of agricultural credit. Consequently, projects should manage credit operations themselves, as past experiences have shown that this option encourages a better followup and reduce the costs of financial management. Obviously, once aquaculture operations have reached a certain volume, credit can only be arranged with professional organizations: in this case, while drawing on the lessons from past experiences, it is important that credit arrangements be negotiated with potential fish farmers, and that there be no confusion between investment and operation credit in order to ensure economic efficiency of aquaculture operations.

Noninstitutional credit can participate in the establishment of aquaculture development operations. Traditional informal credit is adapted to the cycle of aquaculture production as its flexibility tends to lessen the risks involved in this activity, and to balance the production-consumption cycle over the year. Because they are integrated in a socioeconomic system characterized by a high personalization of social relationships through lineal, ethnic or village ties, informal moneylenders approve loans at very short notice, ask for little collateral and tend to impose few restrictions regarding use of funds. For this informal credit arrangements to be considered, it is necessary to conduct preliminary studies using a methodology that is adapted and already tested. The use of this informal credit arrangement would be the sign of an integration of aquaculture projects into socioeconomic systems.

Producing in order to Sell

In subSaharan Africa and in all the countries considered under this study, farmed tilapia (assimilated to tilapias fished in inland waters) is a relatively expensive fish outside the immediate production zones, but it also meets the specific demand of the African consumer who values "inland" fish highly.

However, the market situation for farmed tilapia varies from country to country in the subSaharan regions. The study already referred to (Lazard et al. 1991) has shown that African fish markets belong to three main categories, described as follows, and which also apply to cultured tilapia. The

following situations offer all opportunities for a market for farmed tilapia to develop.

- The first situation occurs in countries and regions where there is a very high demand for animal proteins due to high demographic pressure and a high urban or periurban concentration, but also where there is a category of buyers with a relatively high purchasing power. In these countries (Côte d'Ivoire, Nigeria, Togo, Benin and Congo), although popular demand for fish is satisfied by the mass importation of inexpensive fish, there is a high demand for "inland" fish, satisfied almost exclusively by the fresh/brackishwater fisheries, as the quantities of farmed fish are generally negligible (Weigel 1989). Potentially, farmed tilapia could fill this niche as long as it is competitive with the capture fisheries and does not radically modify the quantities offered.
- The second situation occurs in countries that are large producers in comparison with their own demand and therefore where, globally, supply is greater than demand, and mass importation of fish is not necessary. Whether we are speaking of marine or inland production, the price of fish is lower than in the previous situation, but the demand is sustained by ancient, widespread eating habits. In these countries (Ghana, Senegal, Mali and Chad), fish farming is hardly competitive with the fish from capture fisheries. However, the trend towards overexploitation coupled with a potentially high demand related to eating habits may lead to a potential market for farmed fish where production costs would be strictly controlled.
- The third situation occurs in areas with low fish consumption, possibly due to a limited production not compensated by mass importation of fish, a low purchasing power or a rejection of fish as a regular food item. Countries like Burkina Faso, Niger, the CAR, Rwanda, Burundi or Madagascar, fall, to various extent, under this category. In these conditions, the potential market for farmed tilapia is inevitably limited, particularly as the low purchasing power corresponds frequently to a very loose economic environment that increases the costs of aquaculture production (lack of agro-industrial by-products, need to import material, etc.)

Where such conditions prevail, the emergence of tilapia aquaculture as a large-scale sector of production having its own dynamics is difficult to achieve. Still, the alternative solution is to encourage fish farming operations in the context of agricultural or husbandry development projects. This option guarantees the supply of necessary inputs and reduces infrastructure and operating costs.

For aquaculture to remain competitive with traditional fisheries, marketing unit costs and margins must be reduced. For this, it is necessary to compress the segmentation of this sector of activity or engage in economies of scale, or both. These commercial options are obviously closely related to the farming systems in place. Therefore, a limited and decentralized aquaculture production unit should move closer to the centers of consumption (for example, periurban aquaculture). A high-yielding, centralized aquaculture production will be able to engage in economies of scale by creating an efficient marketing structure.

Conclusion

Today, it seems difficult to justify, based on financial criteria alone, the implementation of fish culture development operations on the African continent outside highly suitable conditions. Other parameters must be considered such as daily wage levels (in terms of opportunity cost, for example), use of land, water and inputs, etc.

Therefore, given current trends, we are dealing here with investment for the future, which is difficult to imagine without aquaculture, considering the stagnation of fisheries production (and even its decline in some

inland environments) and the increase of demand for fish. This "bet" seems all the more worthwhile as the growing number of research and R&D operations conducted for the past 15 years are beginning to bear fruit. Furthermore, the inexpensive fish imported in great quantities by some countries with important trade deficits may well become more expensive, owing to the recent shift in the economic system of Eastern European countries (currently the major suppliers of marine fish to African countries), the impact of energy costs (fishing vessels, transportation, etc.) and the devaluation of the CFA Franc in 1994.

In view of this, fish culture can constitute, in time, an instrument for the regulation of the production of living aquatic resources, in comparison with an environment that has demonstrated its fluctuating nature. The very existence and development of fish culture raise various questions regarding a number of economic assumptions on the use of aquatic environment and the extensive utilization of fish resources (particularly to assess the value of water and land, the cost of pollution, etc.). In addition, a comparative analysis of fish culture and other animal productions should be done in terms, for example, of the use of agricultural by-products and the quality of the proteins produced. From now on, fish culture must be considered as one of the elements of agricultural production systems.

Today, it is possible to establish productive fish culture development projects using farming techniques that have been tested and are integrated in a suitable socioeconomic context. Simultaneously, and at a more global level, aquaculture development on the African continent will require in some areas (for some time still) experimental development studies, even research efforts in the strictest sense. At this point, researchers should not be afraid to clearly state these priorities vis-à-vis all operators and partners concerned who would rather rapidly develop production-oriented projects.[b]

This experimental dimension must not prevent the preparation of the delicate transition to the post-project conditions. Experience has shown that there is no successful post-project example in African fish culture yet. The preparation and the implementation of this phase (that will condition the successful takeoff of this new activity) must necessarily associate, in equal measures, its major four actors: national administrations (in general, the "umbrellas" and the agents directly reporting to these administrations), target populations (fish farmers and the different parties involved in the production of farmed fish: feed producers, fry producers, etc.), operators (consultancy firms, technical assistance, etc.) and funding agencies.

Furthermore, in view of the current situation in Africa, one should not be afraid to banish the word "aquaculture" from the vocabulary of some regions where constraints of implantation and development (sites, competition with fish from the capture fisheries, target populations, etc.) make its success unlikely.

Thus, funding agencies (international agencies, cooperation funds, NGOs, development banks, etc.) and national administrations play a determining role in giving African aquaculture a second wind.

During the elaboration of aquaculture development projects, funding agencies must demonstrate the necessary competence and objectivity in the analysis of all project components: project objectives, available biotechnical data, identification of target populations, market evaluation, marketing, available sites for the implantation of farm infrastructures, post-project anticipatory

[b]In Africa, most of the fish culture development projects appear, *a posteriori*, to have been used as a more or less confessed justification for conducting research either within the project or in the form of a research component.

study, etc. Once this comprehensive identification is completed, funding agencies must see to it that the project becomes rapidly operational. In doing this, both funding agencies and operators must refrain from outbidding each other by having the operators compete excessively. Instead, projects need to be carefully evaluated, and adjusted if necessary.

Project evaluations should emphasize quality rather than quantity: the relevance of the project within a global policy of rural development, its reproducibility and viability in the "post-project" should be more important than the catch or the number of development operations. This is, currently, only rarely the case.

On the other hand, African administrations should loosen their administrative procedures, and strengthen their technical participation and capacity to make proposals during elaboration and implementation of these projects. National administrations need to be more constructive and increase their involvement. Furthermore, these need to acknowledge that fish culture is a production activity pertaining to both agriculture and animal husbandry, even, in some cases, to the industrial sector, and not an activity undertaken in the context of conservation of natural resources, which generally and traditionally fall under the Department of Forestry and Water Resources. In essence, the aim of an administration is neither to produce nor to sell: it is therefore in its best interest to remain outside these processes and to adopt a benevolent attitude, at best becoming the catalyst of all private initiatives, even when these question what the administration has always considered as being part of its own prerogatives.

In this view, the administration should not take the place of economic agents who are involved in aquaculture, but support them by:

– defining an aquaculture sectoral policy (based on the agricultural policy) regarding taxation systems, grants, credit, land tenure, pricing policy, marketing, promotion of professional associations, etc.; and

– implementing this policy through the different necessary companion actions: statistics of production, training, research, follow-up, control, etc.

In the current context, privatization, a growing concern of the States (which are seeking to devolve authority), and of the funding agencies, need to preserve its true meaning. Fish culture cannot simply be integrated in the market economy by transferring the State responsibilities to a private company (corporation, mixed economy, etc.), without first demonstrating its economic efficiency. Privatization also means:

– the organization of aquaculture activity to be developed with all the existing private operators: feed producers, entrepreneurs, farmers, fishers, etc.;

– the creation of a suitable economic environment for the development of all operators involved in aquaculture operations;

– the disengagement of the State from undertakings that have proven profitable so it can fully act as a driving force and catalyst for this new activity by engaging in training and research; and

– to stimulate a rural fish culture dynamics which, for the State, constitutes a development with great potentialities. For this, only units of aquaculture production which can really be integrated in their farming systems should be proposed to the farmers.

All these elements, added to the already existing positive elements, constitute basic conditions to meet in order to finally ensure a flourishing African aquaculture.

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B. PAPERS PRESENTED

SESSION I. CULTURE SYSTEMS, MANAGEMENT AND PRODUCTION

Comparative Growth of *Oreochromis niloticus* and *Sarotherodon galilaeus* in Small Artificial Lakes in Burkina Faso and the Larger Lakes of Africa

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BAIJOT, E., I. BARRY, S. BOUDA, K.H. KABORE and A.C. TRAORE. 1996. Comparative growth of *Oreochromis niloticus* and *Sarotherodon galilaeus* in small artificial lakes in Burkina Faso and the larger lakes of Africa, p. 28-32. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

The growth of *Sarotherodon galilaeus* and *Oreochromis niloticus*, dominant species in small reservoirs in Burkina Faso, was estimated by scale reading and length-frequency analysis. The use of the growth index $\phi' = \log(K)+2\cdot\log(L_\infty)$ developed by Pauly and Munro (1984) shows that the growth potential of both species is similar and that their growth is inferior to that recorded in other larger waterbodies in Africa.

The ecological significance of these results is analyzed and discussed.

Introduction

Burkina Faso has approximately 350 artificial lakes ranging from 10 to 1,500 ha in area, all characterized by high area variations between the high and low water level cycles. Created essentially for water supply purposes from the 1940s onwards, these lakes have been, for the past 15 years, used for fish farming, an activity that is now being rationalized by the project "Valorisation du potentiel halieutique au Burkina Faso." These reservoirs are populated by several natural species, including *Oreochromis niloticus* and *Sarotherodon galilaeus*. The attention of the project leaders was rapidly drawn to the small maximal sizes observed in these populations of tilapia, which prompted the undertaking of the present work, with the following objectives:

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- to assess the growth and longevity of these populations; and
- to compare the results with similar information from other parts of Africa.

Materials and Methods

This study was conducted in the lakes listed in Table 1. These reservoirs were chosen because they are easily accessible and are representative of the entire range of waterbodies in Burkina Faso.

Fish growth was determined by counting the annuli on the scales using traditional techniques (Merona et al. 1988) or by analyzing the length-frequency histograms using the method developed by Pauly and David (1981). The samples used came from experimental fisheries using beach seine during the low water level cycle.

The age-length relationship was determined by adjustments to the equation developed by von Bertalanffy (1957):

$$L_t=L_\infty(1-\exp(-K(t-t_0))) \quad \text{... 1)}$$

where:

L_t is the length at age t;

L_∞ is the asymptotic length reached at an infinite age;

K is the growth factor measuring the speed at which the curve approaches its asymptote; and

t_0 is the x-axis of the point of origin with the age axis for which $L_t=0$.

The adjustment was made using the method developed by Gaschütz et al. (1980) summarized by Pauly (1982). Lengths are total lengths in centimeters.

The linear growth comparison between several populations of fish cannot be made by using only K or L_∞: both parameters must be considered simultaneously. Pauly (1979) and Pauly and Munro (1984) have developed an index which takes these two parameters into account. This index is now commonly used and is expressed as follows:

$$\phi' = \log(K)+2.\log(L_\infty) \quad \text{... 2)}$$

which was actually used for the time first in tilapia research by Moreau et al.

Table 1. General characteristics of the artificial lakes included in the study.

Lake	Year	Areas (ha)		Temperature (°C)		Populations
		Min	Max	Min	Max	(Observations)
Boromo	1978	100	300	18	32	*Sarotherodon galilaeus* (dominant, tree stumps)
Boulmigou	1948	3	45	18	34	*Oreochromis niloticus* (dominant, turbid waters)
Kokolo	-	2	20	19	35	Idem
Manga	1962	10	90	18	33	*S. galilaeus* (dominant, turbid waters)
Ramitenga	1985	3	25	18	35	Idem
Sourou	1985	2,000	10,000	20	32	*S. galilaeus* and *O. niloticus* (found together)
Tanguiga	1984	2	30	19	35	*O. niloticus* (dominant, stockings)
Tapoa	1950	300	800	18	30	*O. niloticus* (dominant, abounding aquatic vegetation)

(1986) and also used in the present study.

The age-length keys generated by scale reading and the length-frequency distributions estimated with the method of Pauly and David (1981) are available from the authors.

Results

Tables 2 and 3 call for the following remarks:

In Burkina Faso, the mean values of ϕ' (2.14 and 2.15) are the same for both species. Individual values in both cases are within the same range (1.90 and 2.32).

The values of ϕ' are lower than in other African environments cited by Moreau et al. (1986) (see Table 3). Note that in the other parts of SubSaharan Africa, both species show the same growth performances as in Burkina Faso.

The poor growth of *S. galilaeus* and *O. niloticus* in Burkina Faso can be explained as follows:

The environments in the present study are relatively poor and are subject to severe ecological conditions during the low water level cycles (high turbidity and lack of dissolved oxygen), particularly where the area is limited. Note that it is in Lakes Taoa and Sourou, the largest lakes with the largest water volumes,

Table 2. Growth parameters and indices ϕ' in *Oreochromis niloticus* and *Sarotherodon galilaeus* from artificial lakes in Burkina Faso. (Source: Baijot et al. 1994.)

Lake	L_∞ (cm; LT)	K (year^{-1})	ϕ'	Methodology
O. niloticus				
Sourou	22	0.7	2.53	LFA[a] (ELEFAN I)
Tapoa	36	0.39	2.32	LFA (ELEFAN I)
Ramitenga	18.22	0.58	2.28	Scale measurement
Boromo	21.3	0.522	2.37	Scale measurement
Tanguiga	17.6	0.462	2.16	Scale measurement
Boulmigou	23.7	0.286	2.21	Scale measurement
Kokolo	23.3	0.196	2.03	Scale measurement
			2.33	
S. galilaeus				
Sourou	30	0.36	2.51	LFA (ELEFAN I)
Tapoa	30	0.38	2.53	LFA (ELEFAN I)
Ramitenga	17.36	0.448	2.13	Scale measurement
Boromo	36.2	0.222	2.46	Scale measurement
Tanguiga	17.8	0.52	2.22	Scale measurement
Manga	20.0	0.21	1.92	Scale measurement
			2.30	

[a]LFA: length-frequency analysis.

Table 3. Comparison of growth performance of *Oreochomis niloticus* and *Sarotherodon galilaeus* in Burkina Faso and other parts of Africa.

Species	Burkina Faso			Africa		
	ϕ' min	ϕ' mean	ϕ' max	ϕ' min	ϕ' mean	ϕ' max
O. niloticus	2.03	2.33	2.7	2.41	2.65	3.11
S. galilaeus	1.92	2.3	2.53	2.33	2.65	3.04

that growth is optimal. Two explanations, not mutually exclusive, can be offered:

- these populations, genetically isolated for several years and confined in smaller waterbodies, suffer from a degeneration due to inbreeding which could be responsible for certain forms of stunting; and
- the small sizes are due to stress caused by hostile conditions in the environment (Pauly 1979).

These two hypotheses are compatible with the small sizes observed at first maturity (Pauly 1984).

It is impossible here to assess the relative importance of either phenomenon. It should simply be noted that in rivers located in forested areas in the Sudanian zone in Sierra Leone, where there are no adjacent flood plains, Payne and McCarton (1985) have also recorded very low growths in native tilapias. These observations support the hypothesis of ecological problems affecting tilapias in Burkina Faso.

Discussion

Two methodological points must be discussed:

1. It relates to the validity of scale reading. Annuli were clearly seen in most cases and correspond to one episode of stunted growth per year, as is the case in the Sahel region (Merona et al. 1988). However, in some populations, double annuli comparable to those identified in some populations of Lake Chad were observed (Merona et al. 1988). These annuli are most probably due to the halt in the normal growth occurring during the low temperature season (December to February) followed by an additional growth check related to the ecological conditions during the low water level cycle (May and June). Tilapias, planktivorous fish, are found in very turbid waters where photosynthesis is practically interrupted and where food is lacking.

2. Certain values of L_∞ are high and are clearly higher than the maximal length observed in the fishes under study. This may be due to low longevity. Except in Lake Tapoa, it is impossible to find individual fish more than four years old for which the growth in length slows down significantly, allowing estimation of L_∞ values corresponding to the maximum observed lengths. Massive mortalities of old fish may be due to intensive fisheries in the reservoirs.

To conclude, in the reservoirs of Burkina Faso, the growth of tilapias is poor compared to other areas of Africa. The major reason seems to be of ecological origin. However, a phenomenon of genetic stunting cannot be discounted.

Acknowledgements

The authors wish to thank J. Moreau (INP, Toulouse, France) for his assistance in the preparation of this manuscript.

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The Integration of Extensive Aquaculture (Acadja-enclos) into the Lagoon Village Environment in Côte d'Ivoire

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Abstract

With a production of 150 to 200 $kg \cdot ha^{-1} \cdot an^{-1}$, capture fisheries in West African coastal lagoons no longer meet the local demand for fish. This situation is aggravated by the increasing pressures of urban expansion in the lagoon areas. The Ebrié lagoon in Côte d'Ivoire is affected by the development of the city of Abidjan and provides a good example of this expansion. Improved production for lagoon areas is consequently drawing increasing interest. The adoption of acadja-enclos and pen production systems in the Ivorian lagoons reflect this development. Pen culture is essentially based on the intensive culture of catfish and requires private investment, but acadja-enclos systems are extensive, village-based farming systems. The problems of competition (for space and resources) between the acadja-enclos culture system and capture fisheries are discussed, as well as the introduction of acadja-enclos into villages and their economic efficiency.

Introduction

The integration of a new production system always affects an entire farming system. This article investigates and discusses the prospects of acadja development in Côte d'Ivoire, based on the acadja systems of Benin. Although there are differences in terms of environment and technical adaptation between the lagoons of Benin and Côte d'Ivoire, competition with capture fisheries and the appropriation aquatic space are problems common to both (Durand and Verdeaux 1991; Verdeaux 1994).

Towards Aquaculture Systems

Natural living aquatic resources are potentially renewable resources. Their quantity and rate of renewal depend on the efficiency of the successive transformations within the various food webs, leading to final production, and upon their level of exploitation, this process implies that resources are variable. Resource variability is affected by intrinsic factors (competition among species and success in the reproduction) and climate fluctuations. This combination of factors introduces various degrees of variability,

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both in seasonal and interannual terms. Final production also fluctuates and so do the catches, as they are based on a complex resource management system.

The lagoons in the northern parts of the Gulf of Guinea are among the world's most productive environments, yielding annually 150-200 $kg \cdot ha^{-1}$ under optimum conditions. However, demand for animal protein and the difficulty of controlling fisheries operations have generally resulted in excessive pressures on the stocks and in frequent conflicts between fisheries operators. This suggests the development of aquaculture which theoretically should free the fishing communities from natural conditions or, at least, minimize their effects while providing additional yields.

Although an extreme enthusiasm for aquaculture has resulted in many frustrations, this analysis concerning Africa is made within the framework of this symposium (see especially Lazard et al. 1990; Lazard, this vol.) and our intention is not to deny all development prospects. On the contrary, we encourage the adaptation of techniques to develop extensive aquaculture in lagoon environments. It is clear that any new project must take into account the analysis of previous failures, the major causes of which are:

- the lack of basic knowledge;
- the absence of a long-term reflection;
- the weakness of the economic analyses; and
- the underestimation of sociocultural aspects.

Finally, it may be useful to specify the type of aquaculture that concerns us here. The principle of farming is clearly understood in highly productive, controlled systems, but this may not be the case for extensive farming which depends on the natural environment. The following general definition can be suggested: one can speak of aquaculture when the controlled modifications of the natural conditions generate an increase in productivity. Such a definition applies to the "acadja" farming system.

Acadjas and Pens

Acadjas and pens have in common that they are part of natural environments, on shallow, soft bottoms. The pen system is not a native concept; it evolved from Southeast Asian traditions (Hem 1982). Implanted on shallows or along banks, pens are made of commercial, small mesh nets (14 mm) stretched on poles that are imbedded in sediments. The pens can be used alone, as for example in the semi-intensive farming of catfish (*Heterobranchus longifilis* and *Chrysichthys nigrodigitatus*: Hem et al. 1994) or in combination with the acadja system (Hem and Avit, this vol.).

According to Pliya (1980), the term "acadja" is of Goun (Oueme) origin. Acadja construction can vary, but the principle consists in imbedding branches in 1-1.5 m-deep soft bottoms. The type of acadja is determined by the nature, density and coverage of the branches. Although this system can be found in many regions of the world (Kapetsky 1981), it is in Benin that it has known its greatest development: 245 ha in Lake Aheme in 1969 (Pliya 1980). The acadja, a unique form of "artificial reef," provides a refuge against predation, can play a role in the reproduction of some species and is particularly useful in food production. Dead branches promote natural productivity in providing opportunities for the rapid, spontaneous growth of periphyton and associated fauna for grazers such as the Cichlidae.

For the past 30 years, scientists and developers have been increasingly interested in the use of acadjas in the lakes and coastal lagoons of Benin (Buffe

1958; Pélissier 1963; CTFT 1965; Hurault 1965; FAO/UNDP 1971; Bourgoignie 1972). Particular attention should be given to Welcomme (1971, 1972) who attempted a fisheries approach to farming and to Pliya (1980) for his comprehensive description of fish farming in the waters of southwestern Benin and of the acadja crisis.

The socioeconomic origins of the conflicts arising from the use of acadjas must be emphasized, but their potential impact on the environment must not be overlooked (Rabier et al. 1979; Tixier et al. 1979). Increased silting in the lagoons as a result of erosion is closely related to:

- the presence of accumulated branches imbedded in the water that slow down runoffs and accelerate the sedimentation of materials in suspension; and especially,
- the lack of vegetative cover in the catchment areas which accelerates land erosion.

The use of branches (raw material to construct acadjas) has led to the deforestation of surrounding areas.

The rapid decay of the branches also plays an important role (70% have to be replaced annually, representing some 30 $t \cdot ha^{-1}$ [Welcomme 1972]). This is a source of organic matter which can pollute the environment.

Natural Environments and Farming Systems

"Fisheries require at least three elements to exist: fishing operations, fish and traders. Each of these elements influences the two others... either directly or indirectly" (Quensière 1990). In a given environment, the management of living aquatic resources through fisheries therefore constitutes a system. The modification of any element of a system can have an effect on the whole. Any new system of production has therefore, directly or indirectly, important repercussions because it implies modifications in the sharing of and/or access to the resources. Generally, what is at play is the "combination of forces and social interests...which define the rules and practices for the use of such environment" (Verdeaux 1986).

In environments with high productivity, old fisheries traditions and high demand, such as in West Africa, as well as the lack of consideration of the potential effect of a new production system often result in conflicts as can be shown in several coastal environments in the Gulf of Guinea (Durand and Verdeaux 1991).

Along the Ebrié lagoon (Verdeaux 1981, 1986), the use by some villagers of a small beach seine designed for particular species has led to conflicting interpretations as to the fishing grounds where it can be used. Villagers using passive gears refused to use seines on their traditional fishing grounds. The ensuing inter-community conflicts forced the administration to intercede by asking the villages concerned to set territorial limits within which each village would be free to use the techniques of its choice. The old system, which consisted in seasonally alternating techniques in the entire lagoon area, was now split in as many subsystems as there were villages.

The major crisis which occurred some 10 years ago should be recalled. First accepted and restricted to some lagoon areas, beach seines had such impact on the resources that they were totally banned under the pressure of local fishers (Ecoutin et al. 1994).

In Lake Aheme (Pliya 1980), the acadjas introduced by the fisheries administration were not only very much appreciated by some of the riverine populations, but also by outside entrepreneurs who were attracted by the high returns guaranteed by this production system. Other fishers opposed this technique objecting that multiplying the acadjas reduced the availability of fish in

open waters. The impossibility for both the local and central authorities to stop this phenomenon and to solve the conflicts caused by the acadjas compelled the State to have the branches removed by force and to ban their use indefinitely, allowing only the use of traditional fishing techniques. A similar process led to the same restrictions in Lake Togo in 1975 (Weigel et al. 1989).

In the Abi lagoon (Charles-Dominique 1988; Verdeaux 1989), purse seines were introduced by the fisheries administration, a development bank and an organization for rural support, in a context of competition between two social groups: the owners of beach seines, on the one hand, and the direct producers brought together around the "unionized" net, on the other. The latter group, in the process of being marginalized, took the opportunity of the funding programs offered to them to acquire these units which were more efficient than their old nets. The number of these units grew rapidly; production was over 10,000 tons in 1979-1980 but dropped to less than 1,000 tons the following year, forcing the authorities to order the temporary suspension of the fishing activities.

All innovations, each according to a particular combination, are a new challenge in terms of access to the environment.

It is with these examples in mind that the prospects of integration of the acadja system in the Ivorian lagoons should be considered. The proposed innovation will certainly have an impact on the farming system and, in order to succeed, it must be accepted by the fishers and villagers. Problems can be analyzed in terms of potential competition for resources, on the one hand, and for space, on the other.

Competition for Resources

If the installation of pens in shallow lagoons poses the problem of space appropriation, in contrast, its effects on the resources are marginal. These are limited to the passing of the fry (predominantly cichlids) through the nets (14 mm). The resulting stock modification does not constitute so much of a problem in itself, but traditional aquaculture may suffer from the proliferation of fish in the acadjas and, consequently, from stunted growth or from the presence of undesirable species.

The implantation of traditional acadjas must be considered from different angles since the function of this production system varies according to its age. Three periods can be distinguished:

- At first, the acadja plays a role of *refuge*, and, at this stage the young acadja functions as a simple trap which competes with other artisanal gears in open waters. The initial stock in the acadja is equal to a biomass produced in the lagoon open waters. Note here that the stock in the acadja is dominated by usually one or a few particular species. In mixohaline areas close to Abidjan, *Lutjanus goreensis* is the dominant species (Hem and Avit 1991; A. Bert, pers. comm.); in oligohaline waters, *Sarotherodon melanotheron* is dominant with 70-90% of the biomass. The same phenomenon has been observed in Ebrié lagoon (Hem and Avit 1991) and in Lake Nokoue (Welcomme 1972).
- Later, the acadja plays the role of an "artificial reef" where branches contribute to the development of algae, protists and zooplankton on which the fish feed. The entire system benefits from this considerable increase of natural productivity.
- Finally, reproduction and growth contribute to the development of a balanced biomass in relation to the volume of water

and its trophic potential. Under these conditions, the acadja may even contribute to the export of resources to open waters.

Regarding competition between acadjas and other artisanal fisheries, the following must be clarified:

- the stocks in the acadjas are clearly different from those found in open waters; therefore, competition, if it occurs, will concern only the species that can colonize the acadja; and
- for these species, competition depends on the use of the acadja. If the acadja serves as a trap that is harvested frequently (every two to three months, for example), the fish trapped inside could have been caught by other traditional techniques. In contrast, if the acadja is harvested less frequently, production (reproduction and growth) wins out, and the catches do not depend on the external stocks.

Provided that harvesting is sufficiently spread out in time, acadjas can generate additional productivity and do not at all obstruct access to resources by other artisanal fisheries. In fact, the yields of other fisheries are even likely to increase due to the export of part of this new production from the acadjas. Note also that the yields from the acadjas are much higher than those from open water fisheries. Based on branch densities, acadja size and production cycles, Welcomme (1972) reported yields of 2-9 $t \cdot ha^{-1} \cdot year^{-1}$. In Lake Aheme, the 35 hectares of acadjas gave, in 1969, mean yields of 5-6 $t \cdot ha^{-1} \cdot year^{-1}$.

However, while overall acadja production increased without detriment to traditional fishers, conflicts developed very rapidly because of the poor organization of the acadja implantation: "the lack of a sound management system, of a rigorous administrative organization and of sufficient understanding of the sociological environment would contribute to the failure of the otherwise technically sound acadja system..." (Pliya 1980).

Space Appropriation

Permanent Appropriation

Acadja and pen production systems monopolize entire areas permanently, discouraging any other activity. To this problem of space control is added the problem of fishing limits imposed in the proximity of acadjas. As the grounds that are suitable for the implantation of acadjas are unevenly distributed, a concentration of fishing activities in these areas is likely to occur, particularly as these grounds can be accessed freely. Lake Aheme provides a good example of this situation: at the end of the experiment, entire areas were off-limits for other fishing activities. Permanent appropriation of space can only lead to conflicts if such practice is not governed by principles agreed upon by all and respected. In Benin, the theoretical, complementary existence of fisheries and extensive aquaculture finally turned out to be a source of conflicts mainly because of the transformation of the lake into a pioneer front, conquering and confiscating the environment (space and resources) for the benefit of a socially heterogeneous fishing population that eluded control. The misuse of the acadja as a simple fish trap is another manifestation of this problem: if the traditional authorities cannot channel and limit the spatial expansion of this phenomenon, neither will the fisheries administration be able to enforce the resources management regulations it has established. In general, the permanent appropriation of aquatic space to accommodate such an exclusive technique constitutes a bias against the most common fisheries activities. The justifications

offered and the precautions taken, in the context of this traditional (or more recent) production systems, show the extent to which these forms of environment may have negative social impacts. They should therefore be closely controlled.

Strategic Spaces

The type of space used for aquaculture, i.e., the shallows, may, like the Ivorian lagoons, fulfill an important symbolic function (Verdeaux 1981, 1986). Even if, at first, these spaces appear not to be utilized, they are collectively appropriated for the establishment of "fixed fishing gears," the constructions which consolidate the entire system of social relationships. The proximity of shallows has always encouraged the establishment of traditional community settlements. Each given a name, the shallows are included in the community's aquatic territories and are only accessible to mobile fishing gears.

The reintroduction of permanent appropriation of these spaces for aquaculture is not without creating conflicts. It is indeed difficult to accept that outside entrepreneurs should be given these grounds without the preliminary agreement of the family and village authorities. The above, concerning the village territorial waters in the Ebrié lagoon, which includes fishing grounds (and shallows) formerly managed by villages, limits at least the risks of dispute to each of these villages. This being said, the villagers should not be made to suffer from such arrangements; they should, on the contrary, benefit from them. If research efforts do not focus on the role that these new techniques can play within the local social systems (and the situation can change from one village to the next), projects for the extension of fish farming techniques may well face unmanageable conflicts.

From Appropriation to Privatization

Ultimately, the implantation of aquaculture systems in the natural environment transforms the status of the spaces thus used. By being treated almost as are agricultural lands, these grounds acquire commercial value and encourage privatization. If fish farming produces "n" tons of fish in one hectare of lagoon area, the value of this hectare can be established since the productivity and the profitability of this area depend only on the fish farmer's management system and no longer on the overall intensity of the fisheries as in capture fisheries.

However, in terms of gross margins, one hectare covered with pens is equivalent to several tens of hectares of palm trees (Lirola 1986). This comparison is also valid for acadjas. Regardless of the particular use of the environment, these techniques may create social problems with unexpected consequences. In the context of agricultural land saturation, for example, these farming techniques may be a palliative to the lack of land and lead to the appearance of unexpected claimants for tenure of lagoon areas.

Discussion

In view of the difficulties encountered in Benin and the analysis of the causes of the conflicts, the prospects of exporting the acadja technique to other African contexts appear at first to be bound to fail. This is not necessarily the case, however, if the specific characteristics of the Beninese and Ivorian coastal environments are taken into account.

Concerning the effects on the environment in Benin, several major inconveniences have been pointed out, including deforestation, rapid silting and organic pollution. None of these problems should affect the Ivorian environment. The hydroclimatic conditions contribute to the

growth of a thick, lush ground cover (annual rainfall = about 2,000 mm on the coast) and the use of wood would not lead to increased erosion and sedimentation in the lagoons. In addition, wood could be substituted with bamboo which presents several advantages (Hem and Avit 1991) and especially releases less organic matter. It should also be noted that Ivorian lagoons are much larger and deeper (average of 5 m in the Ebrié Lagoon). Assuming the shallows and coastal zones to be covered with acadjas in the western oligohaline areas which are the only truly suitable areas would still only represent 1-2% of the total surface area. Competition for resources with the artisanal fisheries does not constitute a rational objection either: due to the specific composition of the catches, acadjas do not significantly affect the other forms of fishing activities. Moreover, the use of this technique increases total production. Still, the permanent and visible appropriation of part of the lagoon territory may be resented by the fishers who would be excluded from the acadja production system. Finally, the full-scale analysis of the technical feasibility and economic efficiency of the acadja-enclos production system in various hypothetical instances of implementation show very encouraging results (Hem and Avit 1991).

It is therefore at the sociocultural level that the major knowledge gaps are to be found. Assuming that aquaculture takes an important place in the lagoon, the two potentially most destabilizing effects would be a change in the challenges and stakes involved, on the one hand, and the transfer of the management of resources to new categories of participants, on the other. The social role that these methods occupy in production systems is in fact the main issue. The potentially ensuing divide between fishers and aquaculturists would imply the formation of two unequal social groups. This situation would be similar to that which prevailed in the Ivorian lagoon capture fisheries. The opposition between direct producers and seine owners has resulted either in conflicts or in increased activities and overfishing. In contrast, the long-term appropriation of space and the returns guaranteed by pen and acadja farming techniques form a system that is similar to that found in plantations. The latter, an extensive production system, reaches land saturation in these areas. In this context, the acadjas could be an extension or timely substitute to the plantation crops, as world commodity market prices are declining. However, at this point, it is still not clear whether an important contribution of cultured fish in the domestic market would affect production costs.

Conclusion

The conception of a new production system in the Ivorian lagoons is the fruit of comparative and multidisciplinary approaches on the environment, resources and societies. It takes into account the lessons learnt from the many failures of aquaculture projects and the conflicts related to the management of aquatic resources. It finally draws on a particular farming concept which is extensive-oriented and favors minimized inputs, hence encouraging the use of native species and an implantation in the natural environment. These considerations have led us to suggest the following recommendations:

- biological and economic "monitoring" of the performance of the acadjas and implementation of the necessary adaptations (combination with pens or not, forms and size of acadjas, materials, etc.);
- anthropo-historical analyses of the changing relationship between societies and the environment: representation and appropriation of space, role of the fisheries in the farming systems, etc.; and

- ecological mapping of the physically suitable sites with a description of their potential use and users.

Regardless of the choice of aquaculture production systems and beyond the immediate satisfaction of the needs of the local populations, the impact of cultured fish on the *market* is yet to be assessed: consumers' attitude, prices and marketing potential.

Important research and development efforts will be necessary for the sustainable integration of this new production system. We believe that the adaptation of the acadja technique can constitute a new, appropriate activity in the lagoon context which, combined with traditional fisheries, will improve the management of species and contribute to better control and increased production.

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Philippine Tilapia Farming Technologies and Their Relevance to Africa

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Abstract

To solve the immediate problems of inadequate seed supply and stunted growth of tilapias in ponds due to reproduction, low-cost technologies for seed production and control of reproduction were developed by Philippine government institutions in the 1970s and were commercialized by the private sector. With the many similarities in the geographic, economic and cultural conditions obtaining in Southeast Asia and Africa, the appropriate technologies developed in the Philippines for tilapia seed production and population control might be adapted for application in Africa. Methods for fry production and sex reversal of Nile tilapia (*Oreochromis niloticus*) appropriate for small-scale farms are presented and their possible applications in Africa discussed.

Introduction

Culture of tilapia was reported in Kenya, Africa, in 1924 by Balarin and Hatton (1979). Due to development constraints such as the shortage of qualified personnel for extension and lack of fisheries organizations, tilapia farming in Africa has remained largely at the subsistence level, with the exception of a few commercial farms in some countries. Aquaculture production in Africa was estimated to be 45,000 t in 1988 mainly from freshwater pond culture of tilapia (Balarin 1988).

There are many similarities in the geographic, climatic and economic conditions in Southeast Asia, where tilapia culture is of importance (such as the Philippines, Table 1), and African countries. The majority of freshwater fish farms in Southeast Asia and Africa are operated by small-scale farmers with less than 2.5 ha of land. Fish farming is also closely related to or integrated with agriculture in both regions. The Nile tilapia (*Oreochromis niloticus*) is the most important cultured freshwater fish in the Philippines (Guerrero 1987) and in Africa (Balarin and Hatton 1979).

This paper discusses some Philippine technologies for tilapia farming of relevance to Africa, with emphasis on freshwater pond culture. It is hoped that the Philippine experience will be of benefit in promoting further development of tilapia culture in Africa.

Philippine Tilapia Farming Technologies Relevant to Africa

Like many other countries farming or seeking to farm tilapia, the Philippines has had to overcome two major constraints: shortages of fish seed and the problem of stunted growth in pond-cultured tilapias.

Table 1. Production distribution of tilapia cultured in the Philippines in 1991 (Source: BFAR 1992).

Culture system	Production (t)	% of total
Freshwater ponds	37,358	49
Brackishwater ponds	14,072	18
Fish cages (inland waters)	21,048	28
Fishpens (in lakes)	4,092	5
Total	76,046	100

Low-cost technologies for the mass production of Nile tilapia fry and fingerlings were developed by Philippine government institutions in the latter half of the 1970s. Freshwater ponds and hapas-in-ponds for fingerling production were extensively adopted for commercialization by the private sector in the 1980s (Escover et al. 1987). The poor growth of tilapias in ponds and their prolific breeding were also the subject of in-depth studies by government researchers in the 1970s. The sex reversal technique for the production of 95-98% male tilapia fry was found to be the most practical and economical method for increasing yields of harvest-size tilapia (Guerrero 1979).

Seed Production Techniques

There are at present thousands of freshwater tilapia hatcheries being operated in the Philippines by the private sector. Most of the hatcheries are small-scale, land-based operations with areas of 0.1 to 0.5 ha. The two most common methods of producing tilapia seed (fry and fingerlings) in the country are the use of open earthen ponds and hapas-in-ponds.

Open Earthen Ponds

Manually constructed earthen ponds with sizes ranging from 200 to 400 m^2 are used in suitable areas with clay soil and abundant water supply (e.g., irrigated riceland). The ponds are rectangular and 0.5 to 1 m deep.

Breeding ponds are fertilized with chicken manure at the rate of 250 $kg \cdot ha^{-1} \cdot week^{-1}$ and stocked with up to 4 $breeders \cdot m^{-2}$. The breeders weigh 50 to 100 g each and have a sex ratio of one male to three females. At stocking density of $4 \cdot m^{-2}$, fine rice bran is given to the fish as supplemental feed at 5% of biomass per day.

Two weeks after stocking of the breeders, schooling fry are scooped daily in the morning and transferred to net enclosures (hapas) for holding or rearing prior to stocking in nursery ponds. The fry are stocked at densities of 200 to $400 \cdot m^{-2}$ in nursery ponds which are fertilized with chicken manure. Feeding of fine rice bran at 10% of biomass is applied. Fingerlings are harvested from the nursery ponds after about four weeks of rearing by seining and draining the ponds. Survival is usually 60-80%.

This method of tilapia seed production in small ponds, often constructed in ricelands, is directly applicable in African countries with similar land and water resources.

Hapa-in-Pond Seed Production

Fine-mesh hapas (net cages) provide a simple yet very efficient method for producing tilapia seed. Hapas were first used in India for the breeding of carps and their application for tilapia production was first reported in the Philippines (Guerrero 1977). For breeding Nile tilapia, hapas of 1.5x1x

1 m, 3x3x2 m and 12x4x2.5 m have been used for commercial tilapia seed production. The hapas are constructed by sewing portions of the fine-mesh netting by machine, using nylon thread. The hapas are installed in earthen ponds or shallow lakes by hitching them to bamboo poles or wooden stakes driven into the bottom. At least 0.25 m of the top side of the hapa (if covered) is above the waterline. For uncovered hapas, a freeboard of 0.5 m is necessary to prevent the escape of fish.

The stocking density of breeders usually applied in cages is $4 \cdot m^{-2}$ with a sex ratio of one male to three females. The breeders range in size from 60 to 80 g at stocking. A feed consisting of 75% of fine rice bran and 25% fish meal is given at 3% of biomass in two feedings (once in the morning and once in the afternoon) per day. The breeding cycle in cages usually lasts for four weeks, as in ponds. Breeders are segregated by sex after each cycle and conditioned for at least a week between cycles. They are replaced when they reach 250 to 350 g.

The collection of fry from hapas is facilitated by the installation of footwalks made of wooden planks or bamboo. Fry and fingerlings are collected by using dip nets or by lifting up the cages and scooping the fish out. Daily collection of fry 10 to 12 days after stocking of breeders yields more fry than periodic harvesting (i.e., every 15 days) because of the higher incidence of cannibalism with the latter operation.

From the breeding hapas, fry are transferred to nursery cages, stocked at $1{,}000 \cdot m^{-2}$ for the first week of rearing and fed a high-protein feed (35% crude protein) at 15 to 20% of biomass in four feedings per day at 2-hour intervals. In the second week, their density is reduced to $500 \cdot m^{-2}$ and feeding rates are 12-15% biomass; in the third week, the density is $250 \cdot m^{-2}$ and feeding remains at 10-12%.

To reduce mortality due to poor water quality, regular cleaning and/or replacement of the hapas is done. Grading of the fingerlings is necessary to minimize cannibalism and ensure uniform growth.

The use of hapas for fry production may be a problem in Africa with the unavailability of the fine-mesh polyethylene netting required for hapa construction. Old mosquito nets may serve the purpose as long as nylon instead of cotton thread is used for stitching the material for durability. Of late, experimental work on the use of hapas for the breeding and rearing of tilapia was introduced in Malaŵi, Africa by the International Center for Living Aquatic Resources Management (R.S.V. Pullin, pers. comm.).

Sex reversal Technology

To solve the problem of stunted growth of tilapia and of their prolific reproduction in ponds, various techniques including use of predators, hybridization, manual sexing and sex reversal have been tested in the Philippines. Of these techniques, the sex reversal method was found to be the most feasible for commercial application (Guerrero and Guerrero 1988). Production of sex reversed tilapia fingerlings is now being done by at least four commercial fish farms in the country.

The simplest way of applying the sex reversal technique for producing as much as 99% male Nile tilapia fingerlings is through giving feed containing $30\ mg \cdot kg^{-1}$ diet of 17α-methyltestosterone (hormone feed) to sexually undifferentiated fry in hapas for three weeks. The treatment process is easily incorporated in the fry rearing stage using available facilities. The cost of applying the sex reversal technique is low and affordable, even by poor small-scale fish farmers.

The application of the sex reversal technology in small tilapia farms in the Philippines has required many years of development work. An efficient hatchery system is needed to produce the right age and quantity of fry for treatment. Access to the

hormone feed by small-scale farmers was made possible by the private sector.

Where there is a preference for large-sized tilapia (300 g per fish or more) by consumers in African countries, some sort of population control for Nile tilapia in ponds will be necessary for effective management. The use of the sex reversal technology may be considered wherever feasible.

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Acadja-enclos Used in Côte d'Ivoire as an Extensive Aquaculture System

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HEM, S. and J.L.B. AVIT. 1996. Acadja-enclos used in Côte d'Ivoire as an extensive aquaculture system, p. 46-53. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Acadja is a fishing method widely practiced in the coastal lagoons of Benin. The principle of this traditional fishery is to set dense masses of branches in shallow water, which attract wild fish in large numbers. The yearly production is very high: 7 to 20 $t \cdot ha^{-1}$. Exploited within a short time (two to three months), the acadja system could be considered simply as a fish trap or aggregating device. If harvested after a longer period (six to 12 months), it may be considered as a culture system (retention, breeding, propagation, natural feeding and growth). This contribution explores the latter. Two production systems were used: an acadja-enclos (enclosure with acadja) and an enclosure without acadja used as a control. After 12 months, a biomass equivalent to 8 $t \cdot ha^{-1}$ was harvested from the acadja, eight times higher than the control system. Among the 18 species of fish harvested, *Sarotherodon melanotheron* represented 79% of the biomass. Analysis of the fish population showed that the young fry had entered through the net at the beginning of the experiment and grown in the acadja-enclos. There were no differences in condition factor between the *S. melanotheron* from the acadja-enclos and from open water. Further experiments have shown that acadja-enclos, which can be considered as "bamboo reefs," are very suitable for fish production in rural Côte d'Ivoire. The acadja-enclos system appears to increase productivity greatly in lagoon fish culture. The same principle could be applied in extensive aquaculture or in various aquatic management programs. Further research is needed to understand the basis of this high productivity.

Introduction

Coastal lagoons are a vast area, rich and favorable for a potential aquaculture development in West Africa (Pauly 1976), but lagoon aquaculture in brackishwater has started only recently. Plans and projects have been proposed (Dada 1976), but few actions have taken place. In Côte d'Ivoire, experimental lagoon aquaculture began in 1978, with a catfish species (*Chrysichthys nigrodigitatus*) (Hem 1987), but the costs of feeding these fish is a real budget constraint (about 50% of the production cost) and hardly affordable in the rural context. For this reason, a new aquaculture research program was set up by the Centre de recherches océanologiques (CRO) d'Abidjan to develop extensive aquaculture systems for rural conditions. For this, production

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methods must be technically simple, with minimum external input (such as energy and food pellets, etc.) and using the available local resources and village labor.

Acadja is a traditional fishery system in Benin (West Africa). Usually implemented in shallow water of about 1 m depth, an acadja is a brush park made from wood and branches. These attract fish. This kind of fishery is found in many countries (Kapetsky 1981), but it is particularly developed and well-designed in the lagoons of Benin (Welcomme 1972). The high productivity of the acadja system has a dual basis: (1) attraction and migration of wild fish into the acadja, (2) reproduction and growth of fish inside the acadja system (Fig. 1, I). Harvests of 4 to 20 $t \cdot ha^{-1} \cdot year^{-1}$ have been recorded from acadjas and their prolific spread within the lagoons of Benin have caused serious social conflicts (Pliya 1980): conflict between the acadja owners and lagoon fishermen who complain that the acadja attracts all the fishes from the wild stock (natural resource competition); and conflict between acadja operations and navigation (lagoon space competition). Hence, acadjas are no longer allowed in some regions, e.g., lakes Aheme in Benin and Togo in Togo.

Therefore, our research was designed to avoid such conflicts and to turn the acadja into a culture system. Our new fish production system has been called an "acadja-enclos" (Fig. 1, II). For this, the traditional acadja is modified by surrounding the densely packed tree as other material with a net.

Materials and Methods

The experiment was made in the Ebrié Lagoon in brackishwater (0-5 ppt salinity) at the Layo Aquaculture Research Station. Three 25x25-m enclosures surrounded by net no. 210/60 (mesh 14 mm) (knot to knot) were used to enclose the devices. They were built on sandy bottom with the same technique already used for catfish enclosures (Hem 1982). At the start, the three enclosures were cleared of all fishes, using a small mesh seine net (8 mm) (knot to knot). The first enclosure was kept empty and used as a control structure (Fig. 2). The second enclosure was filled with *Sclera* sp., a kind of floating aquatic grass which thrives along the lagoon border, spread over 100 m^2, surrounded by bamboo sticks embedded in the sand (Fig. 2, Section AB). This aquatic grass is known as one of the natural habitats of *Sarotherodon melanotheron*, the main species which colonizes acadjas. This experiment was designed to investigate other sources and kinds of substrates rather than using wood, the depletion of which in the surrounding area may have undesirable ecological impact.

The third enclosure was an acadja-enclos: a brush park, set up like the traditional acadja in Benin. One hundred packages of dry branches were spread over 100 m^2, and also surrounded by embedded bamboo sticks (Fig. 2, Section CD).

There was no stocking with fingerlings. The first recruitment began with small wild fish (1 to 2 g body weight) being attracted into the acadja-enclos through the meshes (net no. 210/60, meshes 14 mm). Once inside the acadja system, they feed and grow larger so that they are trapped within the acadja-enclos.

The three systems were left for 12 months without any intervention, except to inspect the nets every three weeks. After this, the three structures were harvested and the total length and body weight of every fish collected were recorded.

Results and Discussion

The total production of each device is presented in Table 1: 11.7 kg and 18.2 kg were respectively collected from the control enclosure and the acadja-enclos with aquatic grass. On the other hand, 80.5 kg

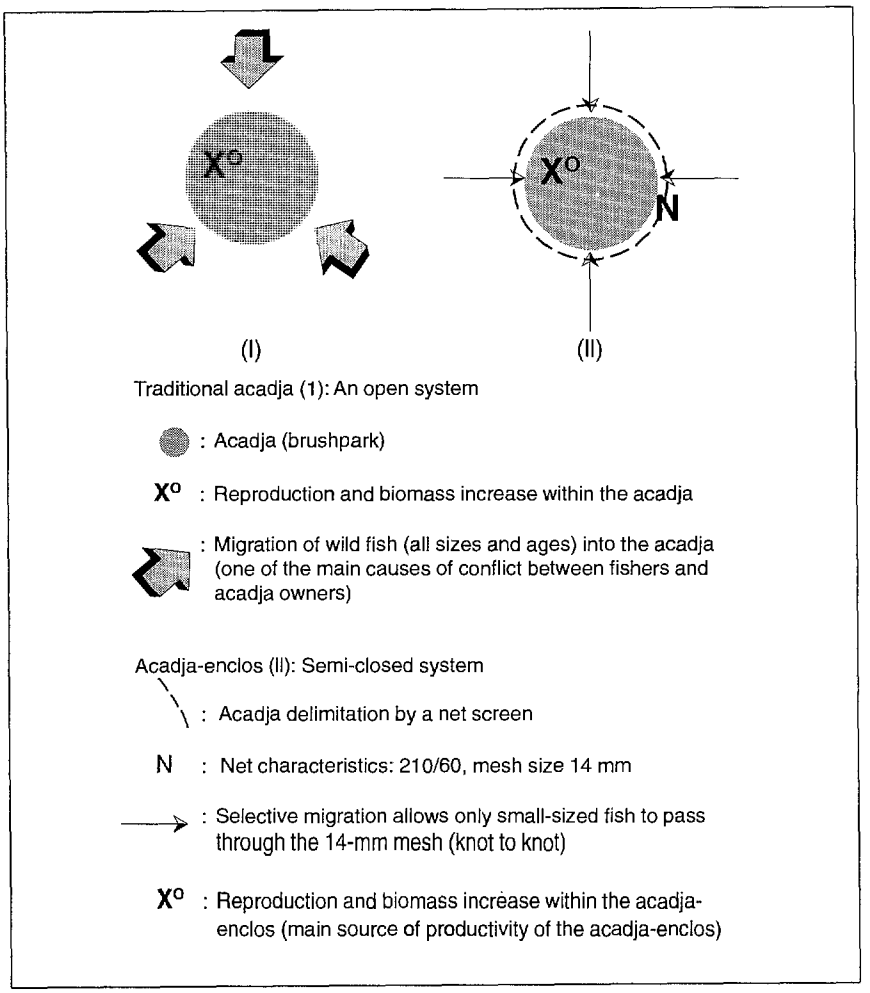

Fig. 1. Diagram of traditional acadja (I) and acadja-enclos (II).

were recorded from the acadja-enclos with a brush park. Thus, it is clear that acadja-enclos produced about seven times more than the control enclosure.

The acadja-enclos was strongly colonized by *S. melanotheron* (79% of the biomass). The acadja device appears to be well-adapted to *S. melanotheron* behavior, enhancing their colonization and population development. The other species listed in Table 1 had little or no influence on the biomass in the acadja-enclos. However, the presence of other catfish, *Heterobranchus longifilis*, a very fast growing species and predator of *S. melanotheron* and other species could lower the biomass.

The high percentage of *S. melanotheron* in the biomass requires a more detailed analysis. As young *S. melanotheron* entered the acadja-enclos through the mesh net, it was noticed that their growth corresponded probably to about the age of one year with a 210-g average body weight for females and 160 g for males.

Comparing the condition factor index, there were no differences between the *S. melanotheron* in the acadja-enclos and those growing in the open waters.

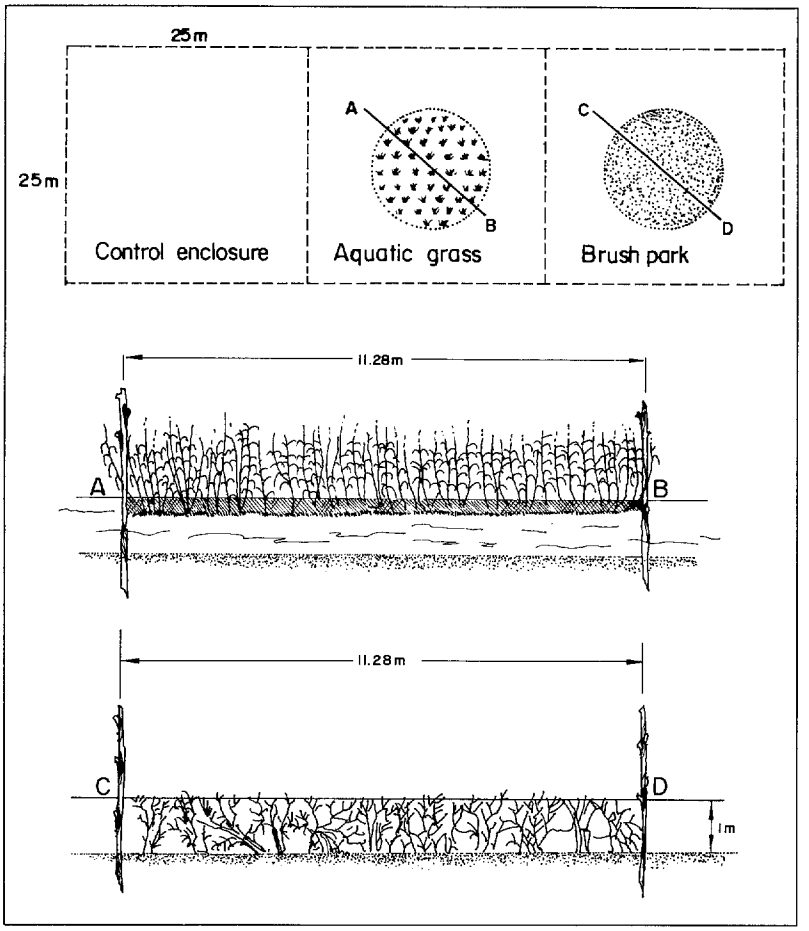

Fig. 2. Experimental design of an acadja-enclos used as a culture system. Device surrounded by net no. 210/60, mesh 14 mm. Section AB: acadja-enclos made from aquatic grasses; and Section CD: acadja-enclos made from brush park.

Acadja-enclos with brush park. After this first experimentation, the replications of the acadja-enclos were performed with larger surfaces (200, 400 and 2,500 m^2). The harvests from two 200-m^2 acadja-enclos were 109.1 kg and 195.9 kg (equivalent, respectively, in yield per hectare: 5.4 t and 9.8 $t \cdot ha^{-1} \cdot year^{-1}$). Only 131.9 kg (equivalent in yield per hectare: 3.3 $t \cdot ha^{-1} \cdot year^{-1}$) was recorded from the 400-m^2 acadja-enclos. Harvests of 241.1, 358.8, 337.1, and 877.5 kg were recorded from four larger acadja-enclos (2,500 m^2). By extrapolating the data to yield per hectare, an average of 1.8 $t \cdot ha^{-1} \cdot year^{-1}$ was recorded (Hem and Avit 1991). There was no proportional relationship between the size of acadja-enclos and their respective yields: curiously, yields became lower with the larger sizes of acadja-enclos. These results show that large acadja-enclos units (over 500 m^2 per unit) give apparently lower yielding profit than small ones. But the main problem related to acadja-enclos using brush park is more of its environmental rather than economic impact.

The building of larger acadja-enclos, however, can lead to ecological and social problems. The quantity of wood and branches, required for a 2,500-m^2 acadja-enclos is 18 to 20 t and the impact of wood

Table 1. Biomass (g) of different species harvested from three acadja-enclos; for details, see text.

Species	Control	With aquatic grass	With a brush park
Elops lacerta	75	16	51
Ethmalosa fimbriata	22		
Hepsetus odoe		34	
Chrysichthys nigrodigitatus			91
Chrysichthys maurus	2,212	831	2,936
Synodontis spp.	176		888
Heterobranchus longifilis	7,104	2,108	8,311
Gerres spp.			32
Hemichromis fasciatus	240	710	1,514
Tylochromis jentinki jentinki	26		24
Tilapia guineensis	313	3,058	762
Sarotherodon melanotheron	1,491	10,437	63,697
Tilapia mariae			355
Ctenopoma kingsleyae		54	464
Eleotris senegalensis		377	1,316
Citharichthys stampflii		134	
Synaptura lusitanica			
Cynoglossus senegalensis	18	347	
Penaeus notialis	72	72	7
Macrobrachium spp.		69	74
Callinectes spp.		48	
	11,749	18,295	80,522

cutting on the surrounding forest is highly visible. Moreover, the yearly destruction of brush parks (60 to 70% in this case), and accumulation of organic matter in the lagoons, would have undesirable long-term consequences for the environment. Therefore, the idea of making acadja-enclos out of brush parks has been abandoned, in favor of using only bamboo.

Acadja-enclos with bamboo. The search for new substrates for acadja-enclos led us to try bamboo (Fig. 2). Trials were first made in small acadja-enclos, followed by replications of larger dimensions (800, 1,250 and 2,500 m^2). The density of bamboo sticks was 10 per m^2. The results were very promising. An average of 8.3 $t \cdot ha^{-1} \cdot year^{-1}$ of biomass has been recorded (see Table 2) showing also the advantage of bamboo substrates which can last a longer time (five to six years). No accumulation of organic matter has been found with acadja-enclos made with bamboo. Moreover, vertical bamboo sticks are an ideal substrate for the proliferation of natural fish feeds: periphyton and aufwuchs (Plates 1 and 2).

In order to evaluate the total productivity of such acadja-enclos, we harvested by removing all the bamboos. This was necessary for the experiment, but not realistic under commercial conditions. Such complete harvesting includes the small fishes which are not marketable and would mean starting the next cycle with uncertain recruitment (Fig. 3). An alternative harvesting strategy is shown in Fig. 4.

Further research programs will focus on appropriate harvesting techniques without removing the bamboos. The design of a bamboo acadja-enclos for selective harvesting is shown in Fig. 5.

Undoubtedly, acadja-enclos can contribute to enhancing the productivity of West African lagoons. Technically simple, using materials and labor from local sources, acadja-enclos can be considered as using appropriate technologies for rural aquaculture in Africa. The present lack of bamboo in some

Table 2. Results of four trials with acadja-enclos using bamboo.

Item	1988	1989	1990	1991
Surface of acadja-enclos using bamboo (m^2)	800	2,500	800	800
Biomass harvested after 12 months ($kg \cdot year^{-1}$)	878.4	1,530	771	518
Productivity extrapolated ($t \cdot ha^{-1} \cdot year^{-1}$)	11.0	6.1	9.6	6.5

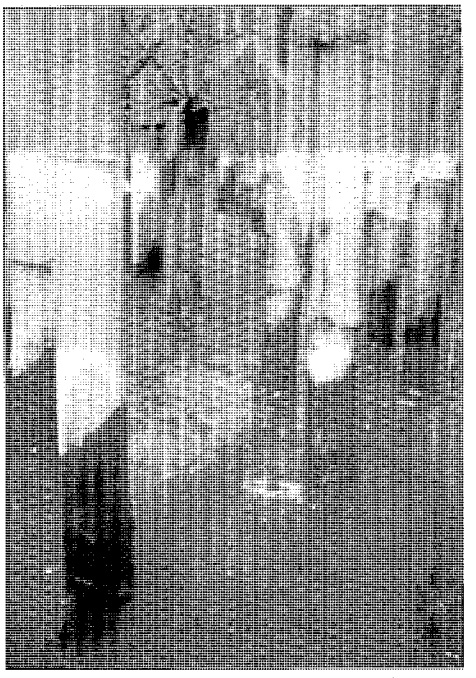

Plate 1. Close view of the bamboo sticks.

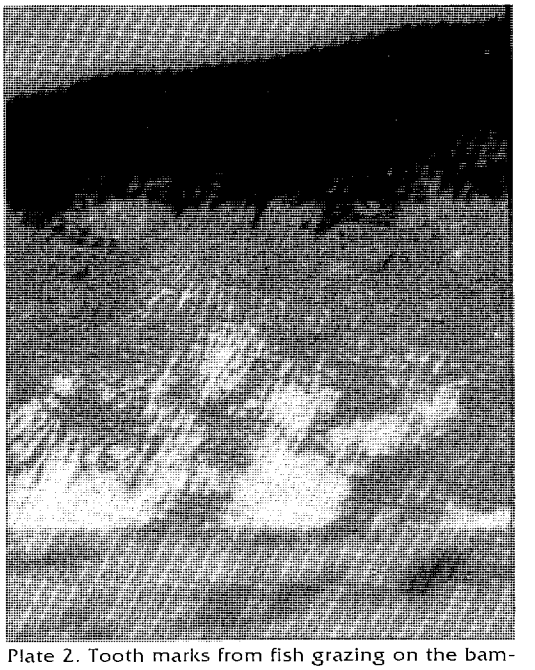

Plate 2. Tooth marks from fish grazing on the bamboo.

regions should not be considered as a serious constraint. The creation of bamboo plantations could solve this rapidly. Bamboo, considered in Asia as a serious "wonder grass," also protects soil against erosion and can be used for many other purposes.

These few preliminary trials have shown promising results. However, before application within the rural context, preliminary economic and social considerations must be examined. This is the only way to ensure a long-term successful development.

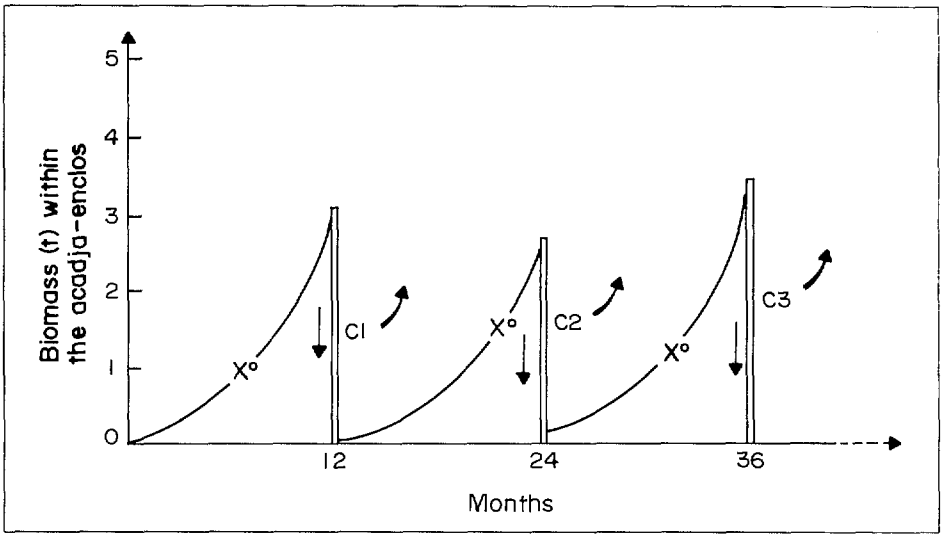

Fig. 3. Fish biomass within an acadja-enclos made with bamboo, based on annual total harvesting.

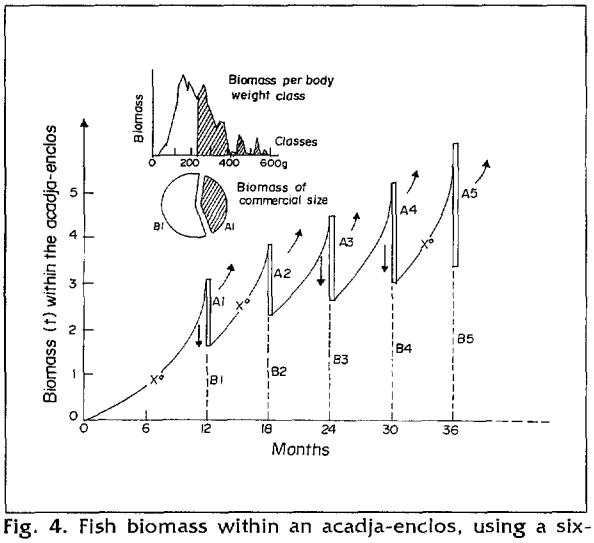

Fig. 4. Fish biomass within an acadja-enclos, using a six-monthly selective harvesting strategy where small-sized and young fishes are saved, and only fish of commercial size are exported from the system.

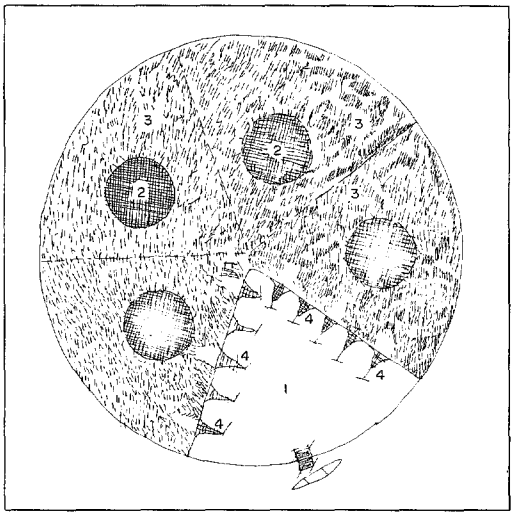

Fig. 5. Design for an acadja-enclos to allow selective harvesting, without removing the bamboo.

1. Fish attraction area (by feeding) used also as harvesting zone
2. Breeding areas arranged with dry branches
3. Bamboos
4. Size-selective access to open area

Acknowledgement

This research program was supported financially by the International Development Research Centre (IDRC), Ottawa, Canada.

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Liming of Fishponds in Malaŵi: A Comparative Study of Limed and Unlimed Ponds[a]

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Abstract

Experiments are summarized on the use of firewood ash and agricultural lime, and the diagnosis of lime requirements in experimental fishponds situated on ferruginous and weathered soils lacking soluble bases in Southern Malaŵi. The nutrient status of soils on land available for pond construction at the National Aquaculture Centre, Zomba, Malaŵi was determined, based on their %C, %N, P, K^+, Na^+, Mg^{2+}, Ca^{2+} contents and relating these to recommended levels for soil fertility in Malaŵi. Lime requirements for existing old ponds were calculated using Boyd's (1979) procedure. *Oreochromis shiranus* and *Tilapia rendalli* (1:1 polyculture) were stocked (13-18 g) in 200-m^2 ponds at 1.5 fish·m^{-2}. Triplicate treatments consisted of diammonium phosphate applied at 100 kg·ha^{-1}·$month^{-1}$, maize stover compost at 5% mean fish body weight·day^{-1}. One set of ponds was limed at 2.5 t·ha^{-1} using a 60:40 ash/agricultural lime mixture and another set was not limed. Total alkalinity, chlorophyll *a*, pH, nitrate, nitrite and Secchi disk visibilities were compared among treatments. Nutrient status of excavated soils at the study site was generally low and soils strongly acidic (pH = 4.10-4.50). Old ponds had higher soil pH values (5.11-6.22) but values were highly variable. Lime requirements for old ponds ranged from 0 to 3,970 kg·ha^{-1}. Boyd's (1979) procedure could not be used for strongly acidic ponds. Total fish production ranged from 276-631 (unlimed ponds) to 434-661 kg·ha^{-1}·$year^{-1}$ (limed ponds). There were no significant differences ($P>0.05$) among treatments except for maize stover compost as the sole input where fish production was significantly lower ($P<0.05$). Ash/lime applications at 2.5 t·ha^{-1} failed to maintain alkalinity within the "optimal" range of 15-20 mg·l^{-1} as $CaCO_3$, attributed to seepage and evaporation (10-150 mm·day^{-1}) and possible depletion of alkalinity in ponds where diammonium fertilizer was applied.

Introduction

One of the principal reasons why fish farmers have become disillusioned in Africa is poor water quality which adversely affected fish yields. In Malaŵi, the benefits of liming tilapia ponds were demonstrated in the early years of fish culture. Although the liming processes are well-understood in agriculture (for example, Adams and Evans 1962), they have only recently been elucidated in aquaculture (Boyd 1979). There are differences between fishpond muds and crop soils in the relationships between base unsaturation and pH. Therefore, determination of pond lime requirements based on crop soils and their needs are likely to be erroneous.

[a]ICLARM Contribution No. 974.

In tropical Africa, most calculations of the lime requirements for fishponds have followed recommendations by Maar et al. (1966) and Miller (1975), who utilized the following scale ($t \cdot ha^{-1}$): new ponds - clay soils (1,680-2,240), sandy soils (1,120-1,680); old ponds - clay soils (1,120), sandy soils (560-1,120).

Not only is such a scale simplistic, its use might provide insufficient lime to effect desired pH changes.

Boyd (1979) derived a simple method for quantifying lime requirements which depends on mud pH and that of a nitrophenol buffered mud solution. The method assumes that soils with mud pH of above 5.75 will not need lime. However, the sunfish populations on which Boyd's recommendations were based are less dependent on primary production than are the tilapias. Environmental tolerances among tilapias are species-specific (Philippart and Ruwet 1982). In Malaŵi, for example, *Oreochromis shiranus chilwae* tolerates a wide range of conditions and inhabits Lake Chilwa which undergoes salinity changes ranging from 0.30 to 16.7 ppt (Morgan 1972; Cantrell 1988). Water quality parameters in fishponds in Malaŵi, especially pH and alkalinity, could be important determinants of fish yields (Msiska 1988).

Although the value of liming fishponds in Malaŵi is recognized, the low purchasing power of the farmers and uncertainty of profits from aquaculture mean that a liming program solely dependent on agricultural lime is unlikely to be implemented. Recent studies by Jamu (1990) focused on alternative and cheaper forms of neutralizing agents such as wood ash from cooking fires in rural areas. This paper summarizes liming reports in organically- and inorganically- fertilized ponds and the implications of using general recommendations on lime requirements for a wide range of soil conditions in fishponds.

Materials and Methods

Determining the Lime Requirements of Fishponds

Soils from ponds with various histories at the National Aquaculture Centre (NAC), Zomba, since their construction in 1960, were sampled at the ponds' deepest and shallowest points. The samples were dried, pulverized and passed through a 0.85 mm sieve. The modified p-nitrophenol buffer (pH 8.0 + 0.1) procedure (Boyd 1979) was employed on soil samples of 20 g to estimate their lime requirements. Original mud pH was determined on a 1:1 mixture of distilled water and soil. After adding the nitrophenol buffer, the mixture was shaken until a stable pH reading was obtained, usually 20-30 minutes of shaking. A Hach pH meter (Model 43800-00) was used for pH measurements.

Samples from adjacent (control) fields (without ponds) were sent for pH estimations and chemical analysis to the Bvumbwe Agricultural Research Station. Carbon (%C, HCO_3^-, CO_3^-) and nitrogen (%N, NO_3^-) fractions, phosphorus, magnesium, calcium, sodium and potassium contents of soils were analyzed following standard methods (APHA 1989).

Comparing Organically and Inorganically Fertilized Ponds With and Without Liming

FISH STOCKING AND SAMPLING

Oreochromis shiranus and *Tilapia rendalli* were stocked in 200-m^2 ponds as a 1:1 polyculture, at a total density of 1.5 $fish \cdot m^{-2}$. Initial individual stocking weights ranged from 11.2 to 20.0 g for *O. shiranus* and 9.0 to 20.4 g for *T. rendalli*. Ten per cent of the total fish stocked for each species in each pond were sampled every two weeks

and their total lengths, standard lengths, and weights were measured. Specific growth rates, % mortality, and yield parameters were calculated.

FERTILIZATION AND LIMING TREATMENTS

Three fertilization treatments were used: (1) diammonium phosphate (DAP) at 50 $kg \cdot ha^{-1}$ applied in solution every two weeks; (2) maize stover compost (MSC) prepared anaerobically (Little and Muir 1987) applied at 3% estimated fish biomass·day^{-1}; and (3) DAP+MSC with or without liming [basal application of a 60:40 combination of agricultural limestone and cooking fire ash (Jamu 1990) hand-mixed in a plastic container and broadcast]. These six treatments were arranged in triplicate ponds in a completely randomized design.

WATER QUALITY

Minimum and maximum temperatures were measured daily between 0800 and 0900 hours. Dissolved oxygen (DO), pH, Secchi disk visibility (SDV), total alkalinity ($mg \cdot l^{-1}$ as $CaCO_3$), NH_4, PO_4, conductivity, and 48 hours water loss (mm) were determined weekly. Chlorophyll *a* was determined every two weeks. All measurements were done using standard methods and procedures (APHA 1989).

STATISTICAL ANALYSES

One-way ANOVA was used to determine differences between treatments. Duncan's multiple range test (Zar 1984) was used to isolate differences between treatment means.

Results

Lime Requirements and Nutrient Status of Pond Soils

The nutrient status of unexcavated soils at the National Aquaculture Centre was very low: soils were strongly acidic (Table 1).

Table 1. Chemical characteristics of new pond soils at the National Aquaculture Centre, Domasi, Malaŵi.

Parameter	Mean	Range	N	Nutrient status*
pH	4.23	4.1-4.5	4	strongly acidic
HCO_3^-($meq \cdot l^{-1}$)	1.38	1.05-1.70	4	NA
CO_3^{2-} ($meq \cdot l^{-1}$)	<1.00	<1.00	4	NA
NO_3^- ($mg \cdot l^{-1}$)	24	22-27	4	NA
%C	2.6	1.8-8.3	4	medium
%N	0.04	0.03-0.04	4	very low
PO_4^{3-}-P($\mu g \cdot l^{-1}$)	1.00	1.00	4	very low
Mg^{2+}($mg \cdot l^{-1}$)	0.03	0.03	4	very low
Ca^{2+}($mg \cdot l^{-1}$)	<1.00	<1.00	4	very low
K^+($mg \cdot l^{-1}$)	0.028	0.027-0.030	4	very low

*Based on threshold values for chemical data used for fertilizer recommendations in Malaŵi (Lupwayi 1990).

Soil pH values for new pond soils were low (4.1-4.5) compared to those of old ponds (5.1-6.2, Table 2). Lime requirements for new pond soils could not be determined using the lime requirement procedure of Boyd (1979) because soil pH values were below the minimum value of 4.7 for Boyd's procedure.

Pond mud pHs were very variable (Table 2) and this was reflected in the calculated lime requirements: zero to 3,970 $kg \cdot ha^{-1}$.

Effects of Liming in Organically and Inorganically Fertilized Ponds

WATER QUALITY

Water quality parameters measured were within acceptable ranges for *O. shiranus* and *T. rendalli* pond culture except for total alkalinity which remained below 20 $mg \cdot l^{-1}$ despite liming (Fig. 1). Total alkalinity declined in all treatments, especially DAP-lime and DAP-MSC-lime treatments. Water

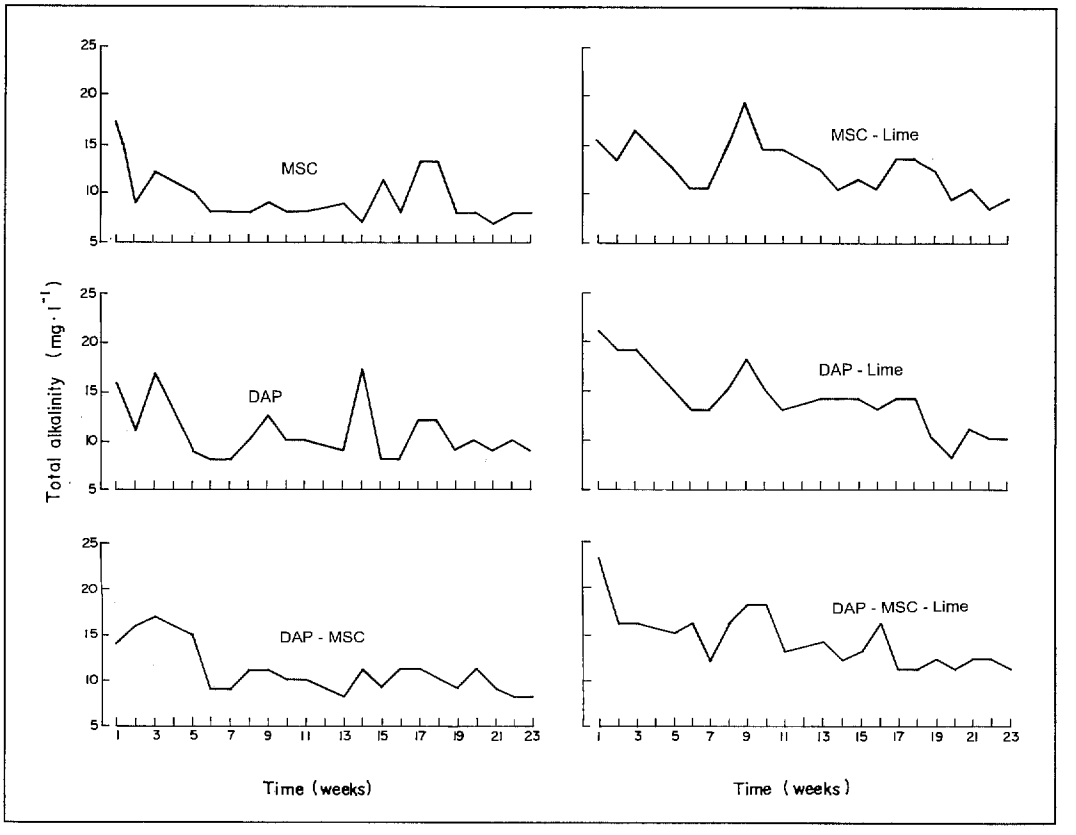

Fig. 1. Total alkalinity ($mg \cdot l^{-1}$ as $CaCO_3$) in limed (60:40 ash/lime combination) and unlimed 200-m^2 ponds fertilized with maize stover compost (MSC) at 3% mean body weight·day^{-1} and diammonium fertilizer (DAP) at 50 $kg \cdot ha^{-1} \cdot 2$ $weeks^{-1}$.

Table 2. Calculated lime requirements for soils from old ponds with various histories at the National Aquaculture Centre, Domasi, Malaŵi. Samples were taken from the shallowest point at the pond inlet(s) and the deepest point at pond outlet(s).

Pond history (inputs used)	Pond no.	Mud pH	Mud pH in nitrophenol buffer solution	Lime requirement ($kg \cdot ha^{-1}$)
CM/MB	1	D 6.1	7.9	Nil
		S 6.2	7.7	Nil
CLM	13	D 5.6	7.3	880
		S 5.6	7.3	880
MB	14	D 5.4	7.2	2,320
		S 5.7	7.3	640
MB	15	D 5.1	7.1	3,970
		S 5.2	7.1	3,520
MB/RB	16	D 6.0	7.1	Nil
		S 5.8	7.4	Nil
MB/RB	20	D 6.1	7.5	Nil
		S 6.1	7.9	390
MB/RB	23	D 5.4	7.9	290
		S 6.1	8.0	Nil
MB/RB	11	D 5.8	7.8	270
		S 6.4	7.9	Nil

D = Deepest part of pond near outlet
S = Shallow part of pond near pond inlet
CLM = Chicken layer mash
CM = Chicken manure
MB = Maize bran
RB = Rice bran

loss due to seepage and evaporation was high in all treatments (10-150 $mm \cdot day^{-1}$).

FISH PRODUCTION

The lowest extrapolated fish production (276 $kg \cdot ha^{-1} \cdot year^{-1}$) was achieved in ponds where only MSC was applied (Table 3) and this was significantly different ($P<0.05$) from other treatments. Lime applications significantly ($P<0.05$) increased extrapolated fish production to 434 $kg \cdot ha^{-1} \cdot year^{-1}$. There were no significant differences among other treatments. The specific growth rate (SGR) of *O. shiranus* was significantly higher ($P<0.05$) in the DAP-compost-lime treatment. No significant differences were observed among the mean weights and SGRs of *T. rendalli* after 174 days.

Discussion

There is need to derive calibration curves and liming tables for the soils in Malaŵi that are too acidic for the application of

Table 3. Stocking and harvesting results from liming and fertilization treatments in 200-m^2 ponds stocked with a 50:50 polyculture of *Oreochromis shiranus* (OS) and *Tilapia rendalli* (TR) after growout for 174 days (DAP = diammonic phosphate; MSC = maize stover compost; lime is mixed agricultural limestone and wood ash; for further details, see text). Data in rows bearing the same suffix letter are not siginificantly different ($P<0.05$).

Yield parameters	DAP		DAP-MSC		DAP-LIME		MSC-LIME		DAP-MSC-LIME		COMPOST	
	OS	TR	OS	TR	OS	TR	OS	TR	OS	TR	OS	TR
Individual stocking wt (g)	13.8a	13.4a	15.3a	19a	16.8a	14.4a	16.6a	14.7a	15.1a	14.4a	18.9a	15.7a
Individual harvest wt (g)	31.9b	23.4a	30.4b	23.2a	34.1b	26.0a	24.6b	21.9a	44.9c	25.8a	19.5a	21.7a
Total stocking wt (kg·pond)	1.7a	1.6a	1.7a	2.7a	2.7a	2.8a	1.4a	1.9a	1.9a	1.7a	2.5a	2.0a
Total harvest wt (kg·pond)	2.2a	1.1a	3.2ab	2.3a	3.5ab	1.9a	2.5a	1.4a	3.4b	1.9a	1.8a	1.1a
% Mortality	52a	68a	30b	34a	28a	52a	23b	52a	39a	46a	50a	26a
Specific growth rate (%·day^{-1})	0.45	0.30	0.38	0.11	0.39	0.32	0.21	0.22	0.60	0.33	0.22	0.19
Total number of fry from breeding (OS + TR)	1,738a		2,045a		2,061ab		1,105a		1,736ab		476c	
Total wt of fry (OS + TR)	2.0a		1.9a		2.5a		1.5a		2.1ab		0.7b	
Total wt of fry + fish (kg)	4.2ab		6.4a		6.7a		4.4a		6.2a		2.8b	
Total extrapolated production (OS + TR) (kg·ha^{-1}·$year^{-1}$)	416.6ab		631a		661a		434a		612a		276b	

Boyd's (1979) procedure. Malaŵian soils include those that are freely draining, acidic and/or leached of bases (latosols) and shallow stony soils (lithosols) (Anon. 1979). The wide range of lime requirements shown here for soils at the NAC may reflect similar situations elsewhere in the country. High seepage likely accounted for the inability to increase alkalinity levels, which never exceeded 20 $mg \cdot l^{-1}$ as $CaCO_3$. Again here, computations based on methods developed using soils of the southeastern USA were not applicable. Teichert-Coddington and Phelps (1989) also found that the lime requirement tables of Boyd (1979) may be inappropriate for tropical countries.

The need for liming was demonstrated by increases in fish production in MSC-lime ponds vs. MSC alone. Since the MSC treatments gave just as much fish production, liming may not be the only way of raising fish yields in low fertility pond waters. Other carbon sources may be important. Mandel and Boyd (1980) have shown that carbon could be limiting in certain aquaculture systems. T.M. Williams (unpubl. data) has found that the C:N ratio of most pond soils in Malaŵi is low (4.0-14.5).

Low fish production in DAP treatments here may have been compounded by the possible neutralization of alkalinity by acidity resulting from the fertilizer, as shown by Hunt and Boyd (1981). This suggests that applications of ammonium fertilizers in waters with marginal pHs and alkalinity should be done with caution.

Acknowledgements

We would like to thank Dr. Barry Costa-Pierce who helped in the design of the liming study and comments on the manuscript. Staff of the National Aquaculture Centre are thanked for their assistance. Studies were funded by the Malaŵi Government and the International Center for Living Aquatic Resources Management (ICLARM) through funds made available by the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH. Funds to attend the conference were made available by The Technical Center for Agricultural and Rural Cooperation (CTA) for D.M. Jamu and the International Foundation for Science (IFS) for O.V. Msiska.

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Stock Manipulation in Farmed Tilapias in Malaŵi

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KAUNDA, E.K.W.H. 1996. Stock manipulation in farmed tilapias in Malaŵi, p. 62-69. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Stock manipulation experiments were carried out at the National Aquaculture Centre in Malaŵi to study the effects of biomass on stunting in two species of tilapia, *Oreochromis shiranus* and *Tilapia rendalli* (Cichlidae).

There were no significant differences in the individual growth performance of fish due to different stock manipulation procedures (ANOVA; $P>0.05$). However, significantly different yields (ANOVA; $P<0.05$) were obtained from different stock manipulation procedures: ponds left without removing any fish produced significantly lower yields than all treatments which involved removal of fish. The optimum procedure, considering cost and revenue implications, was to remove half of the recruits caught every 37 days. The implications of these results for small-holder farms are discussed.

Introduction

Tilapias (*Oreochromis shiranus* and *Tilapia rendalli*, Cichlidae) contribute most of the total biomass of fish harvested from the ponds of small-scale farmers in Malaŵi (Mandeng 1988). A mixed-sex culture system is practiced, and hence the fish breed prolifically and stunting often occurs (Balarin 1984; Pullin 1985; Msiska 1987; Maluwa 1990).

There are a number of techniques which can be used to manage tilapia population size and structure: monosex culture, stocking at high density and use of predators (Guerrero 1982; Mair and Little 1991). In monosex culture, monosex fry can be obtained by hand sexing, hybridization or through steroid hormones. Use of predators involves stocking ponds with predatory fish that eat surplus tilapia fry (Guerrero 1982). These techniques are usually expensive and/or require high management inputs and may therefore not be practicable for small-scale farmers in Malaŵi. Other methods for controlling overpopulation are: stock manipulation techniques, irradiation, chemosterilants and reproduction inhibitors. These methods require further research to determine their potential for routine use (Guerrero 1982).

Stock manipulation, usually in the form of sequential harvesting, may be less expensive (where labor is cheap) and require less management inputs than the other above-mentioned methods, and may thus be useful to small-scale fish farmers in Malaŵi.

Sequential harvesting of tilapia was reported by van Someren and Whitehead (1961). Two ponds were stocked with "*Tilapia nigra*" (*Oreochromis spilurus niger*) at the same stocking density. When the fish started breeding, the parent fish from pond A and the fry or

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fingerlings in pond B were removed by means of one seine haul per month. This resulted in pond A having lower yields than pond B and "the larger of the fry in pond B never reached the size of the stocked fish due to overpopulation." Van Someren and Whitehead (1961) therefore suggested a "skimming off" procedure for removing fry.

This study follows up on van Someren's study, by considering the impact of alternative harvesting schemes on: individual fish growth performance; overall fish biomass at harvest; and monetary returns.

Materials and Methods

Studies were carried out in ten 200-m^2 ponds. The treatments were randomly assigned to the ponds (Fig. 1). *O. shiranus* and *T. rendalli* of 5.5±0.4 g mean body weight (MBW±1 SD; range=4.8-6.22) and 3.2±0.3 g (MBW±1 SD; range = 3.2-4.2, respectively) were stocked at 2 fish·m^2 at 1:1 ratio. Stocking was done on 22 and 23 May 1990.

The fish were fed with maize bran at 2.5% of mean body weight (Kadongola 1990) adjusted to account for growth every two weeks to 9 September 1990 and every month thereafter. Feed adjustment was stopped on 23 October 1990 when the biomass became virtually constant because of the balance between growth and mortality.

Fish sampling was done using a nylon seine net (15.5 m and 10 mm mesh size) every two weeks up to 4 September 1990; thereafter, it was conducted every month to reduce fish mortalities caused by handling stress.

Initially, one to three hauls were made in each pond: from 21 August 1990 on to the end of the experiment, a constant sampling effort of three monthly hauls was applied. Caught fish were designated as "adults" (stocked fish) or "juveniles" (fish produced in the pond); the latter were further separated into species *(T. rendalli, O. shiranus)* when their development allowed this.

Approximately 94% (range = 64-100) of the stocked fish that were caught were weighed and measured (total and standard lengths). For "juveniles", too numerous to be measured individually, standard procedures (Okpanefe 1982; Sparre et al. 1989) were used to obtain size frequency distributions. All fish were returned to the ponds after weighing and measuring. At the end of the experiment, the ponds were drained with a 50-hp water pump and all remaining fish collected by hand.

Stock manipulations, combined with sampling, started on 7 November 1990, 132 days after stocking. For treatments which involved returning fish to the ponds, the following procedure was followed: recruits caught in three hauls were weighed; the largest recruits from all three hauls were selected, put on a balance in decreasing order of size until half of the total sample weight was reached and returned to the pond after their individual weights and lengths were noted. The smaller fish were discarded.

Growth in length was determined by estimating the parameters L_∞ and K of the von Bertanlanffy growth equation, of the form:

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right) \qquad \text{...1)}$$

where:

L_t = predicted length at age t;

L_∞ = asymptotic length, i.e., the mean length the fish would reach if they were allowed to grow indefinitely;

K = rate (here in years) at which L_∞ is approached; and

t_0 = theoretical (and usually negative) "age" at length zero (the parameter t_0 is not further discussed here).

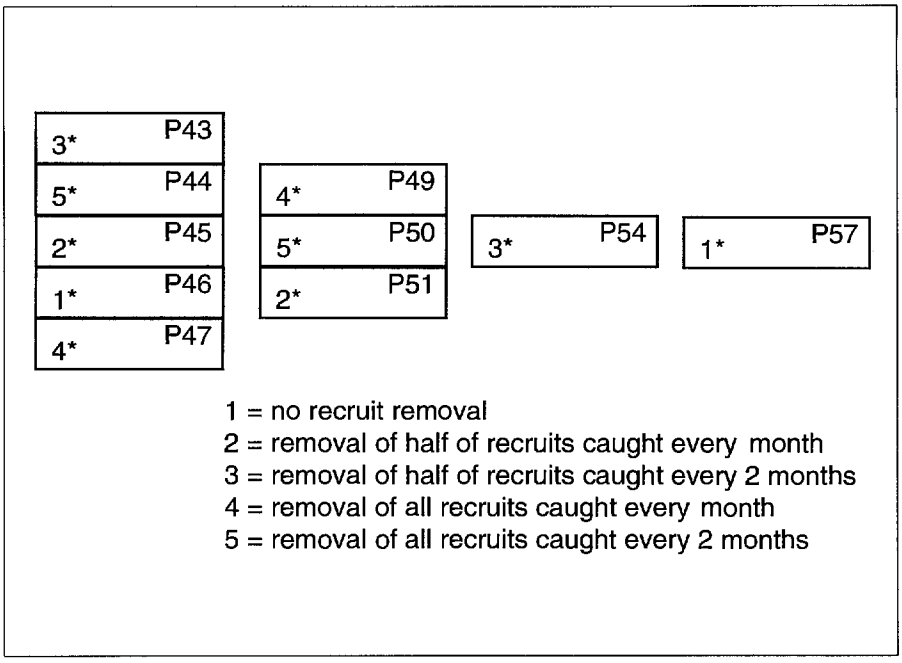

Fig. 1. Experimental layout (n* = treatment no.; P = pond no.).

The estimation of L_∞ and K was performed using the ELEFAN I program (Pauly and David 1981; Pauly 1987), for fitting equation (1) to the length frequency (L/F) data, derived from the above-mentioned sequential samples.

Given the inverse relationship between L_∞ and K, comparisons of growth performance cannot be based solely on either L_∞ or K. Therefore the index:

$$\phi' = \log_{10}K + 2\log_{10}L_\infty \qquad ...2)$$

of Pauly and Munro (1984), computed using the software of Vakily (1988), was used for all comparisons of growth performance; the comparisons relied on randomized block analysis of variance (ANOVA).

Length-weight relationships of the form:

$$W = a \cdot L^b \qquad ...3)$$

were estimated for both species investigated here, by taking logarithms on both sides, and fitting linear regressions to the log (weight) - log (length) data pairs (Pauly 1984).

The parameters a and b of equation (3) were then used to turn the observed L/F distribution into weight frequency distribution, and after adjusting for changing fish numbers, to estimate fish biomasses in each pond.

Total yields were obtained from the summation of total fish weight at harvest and total weight of recruits discarded over the experimental period. Recruitment was defined as the percentage of total weight of recruits (over the experimental period) to total yield. Total yields and recruitment were analyzed by two-way ANOVA.

To determine optimum stock manipulation procedure, the "marginal" analysis concept was used (Panayotou 1982). Each procedure was given a score: removal of all recruits caught by three seine hauls every month, with seven removals from November 1990 to May 1991 was considered the standard treatment with a score of 1. To determine

scores for other stock manipulation procedures, the following formula was used:

$$S = G^{*}H/T \qquad ...4)$$

where:

S = score;
G = number of recruit removals in the treatment under consideration;
H = fraction of recruits removed at each removal in the treatment under consideration; and
T = number of removals in the standard treatment.

The different stock manipulation procedures implied different costs. The cost of operation was the only variable cost considered when deriving that total cost curve. Another cost included was of labor used to operate the seine net, set at the government rate of K0.28/hour.[b] The marginal cost (MC) curve is the slope of the total cost curve (Panayotou 1982):

$$MC = \frac{\text{change in total cost}}{\text{change in score}} \qquad ...5)$$

The selling price for *O. shiranus* was estimated from eight farmers' harvests at K3.94/kg (range = 1.63-4.40), while that for *T. rendalli* was calculated from harvests at K2.21/kg (range = 1.66-2.40); for fingerlings (<10 g), the price was estimated from harvests at K1.11/kg. Total revenue for each treatment was estimated following Panayotou (1982), from:

$$\text{Total revenue} = P_1Y_1 + P_2Y_2 + P_3Y_3 \qquad ...6)$$

where:

P_1 = selling price of *O. shiranus*;
Y_1 = total yield of *O. shiranus*;
P_2 = selling price of *T. rendalli*;
Y_2 = total yield of *T. rendalli*;
P_3 = selling price of mixture of *T. rendalli* and *O. shiranus* fingerlings (<10 g); and
Y_3 = total yield of fingerlings.

The marginal revenue (MR) curve was obtained from the slope of the total revenue curve (Panayotou 1982), i.e.,

$$MR = \frac{\text{change in total revenue}}{\text{change in score}} \qquad ...7)$$

Results and Discussion

The estimated growth parameters are given in Tables 1 and 2, along with published values. Results showed that growth performance was not affected by the stock manipulation procedures (ANOVA; $P<0.05$). The growth performance index of *T. rendalli* (mean ± 1 SD= 2.46±0.14; n=10) did not differ significantly from those compiled in Pauly et al. (1988) (mean ± 1=2.59±0.41; n=4). The growth performance index of *O. shiranus* was lower (mean ± 1=2.52± 0.07; n=10) than reported (3.08; n=1) by Pauly et al. (1988), but the differences may be due to the sample of n=1 for the latter value.

The following length-weight relationships were obtained:

$$W = 0.03(TL)^{2.82} \text{ for } T. \text{ rendalli}; \qquad ...8)$$

$$W = 0.02(TL)^{2.83} \text{ for } O. \text{ shiranus}; \qquad ...9)$$

$$W = 0.025(TL)^{2.83} \text{ for "unidentified recruits"} \qquad ...10)$$

Fig. 2 shows the increase of estimated fish biomass. Biomass increase stabilized after about 240 days either due to limited space or reduced reproduction due to high fish density. This

[b]Kwacha 1 = US$0.22 (August 1993 rate).

Table 1. Growth parameters for mixed sex *Tilapia rendalli*.

Treatment no.[a]	Pond no.	Culture period (days)	L_∞ (TL, cm)	K (year^{-1})	ϕ'	r	DF	F	Country[b]
1	46	303	30.9	0.45	2.63	0.027	11	0.31	Malaŵi
	57	358	23.3	0.51	2.44	0.056	13	0.77	Malaŵi
2	45	358	18.8	0.86	2.48	0.040	13	0.55	Malaŵi
	51	358	13.1	1.21	2.32	0.224	12	3.47	Malaŵi
3	43	358	16.5	1.03	2.45	0.114	14	1.81	Malaŵi
	54	358	43.9	0.20	2.59	0.006	13	0.07	Malaŵi
4	47	357	13.8	1.00	2.28	0.044	14	0.65	Malaŵi
	49	359	21.5	0.48	2.35	0.018	13	0.24	Malaŵi
5	44	358	58.8	0.15	2.71	0.003	13	0.04	Malaŵi
	50	358	13.8	1.05	2.30	0.110	13	1.60	Malaŵi
-	-	>60	33.9	0.185	2.33	-	-	-	Zambia
-	-	>60	21.7	0.615	2.46	-	-	-	Zambia
-	-	>60	13.8	3.19	2.78	-	-	-	Zambia
-	-	>60	13.1	3.785	2.81	-	-	-	Uganda

[a]See Fig. 1.
[b]Results from Malaŵi are from this study and were estimated according to Vakily (1988); data from Zambia and Uganda are from Pauly et al. (1988).

Table 2. Growth parameters for mixed sex *Oreochromis shiranus* in Malaŵi[a].

Treatment no.[b]	Pond no.	Culture period (days)	L_∞ (TL, cm)	K (year^{-1})	ϕ'	r	DF	F
1	46	267	27.3	0.51	2.58	0.019	10	0.19
	57	358	18.0	0.91	2.47	0.104	13	1.51
2	45	358	15.3	1.71	2.60	0.124	13	1.84
	51	358	15.3	1.11	2.42	0.120	12	1.64
3	43	265	18.4	1.12	2.58	0.023	11	0.26
	54	-	-	-	-	-	-	-
4	47	357	14.2	1.26	2.40	0.056	14	0.83
	49	304	13.9	1.78	2.54	0.112	11	1.39
5	44	302	25.8	0.56	2.57	0.035	11	0.42
	50	358	13.3	1.72	2.48	0.134	13	2.55
-	-	>60	11.0	9.87	3.08	-	-	-

[a]As estimated using the approach and software of Vakily (1988); except for lowest row, taken from Pauly et al. (1988).
[b]1 = no recruit removal
2 = removal of half of recruits caught every month
3 = removal of half of recruits caught every 2 months
4 = removal of all recruits caught every month
5 = removal of all recruits caught every 2 months

supports Moav et al. (1977) who suggested that lower yields should be expected from the ponds of farmers who harvest fish after a year than from the ponds harvested intermittently. Maluwa (1990) found that most of the 30 Malawian farmers interviewed harvested fish after a year. The present study suggests that these farmers could increase their yields by intermittent harvesting.

Total yields showed significant variation due to location of ponds (block effect) and treatment (stock manipulation procedure). Ponds 43 to 47, representing one replicate for each treatment (Fig. 1), had higher yields than ponds 49 to 54 (ANOVA; $P<0.05$). Similar results were obtained for recruitment. The 10 ponds in which the experiment was conducted had not been used before; ponds 43 to 47 (Fig. 1) were ready earlier than ponds 49 to 54 because the latter had seepage problems. Age and seepage of the ponds resulted in the two blocks having different levels of nutrition, which may have affected the results.

Table 3 gives the mean yields from different treatments. The mean yields from all ponds with Treatment 1 (no recruit removal) were lower than for all other treatments ($P<0.05$), whereas removal of half of recruits caught every two months (Treatment 3) produced the highest yields.

The intersection of the marginal cost and revenue curves (Fig. 3) suggest that the optimum "score" is located between treatments 3 and 2, i.e., at a "score" of 0.42. From equation (4), it was estimated that the optimum manipulation procedure would be removal of half of recruits caught every 37 days. This result

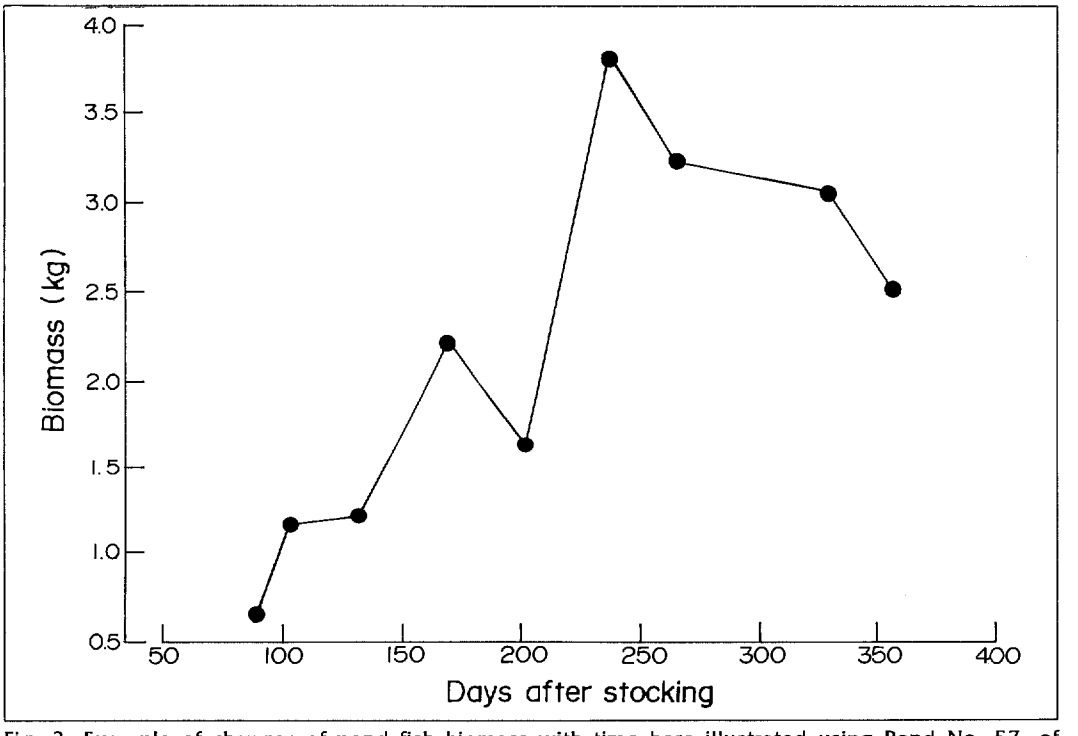

Fig. 2. Example of changes of pond fish biomass with time here illustrated using Pond No. 57, of 200 m² (Treatment 1, see Fig. 1).

Table 3. Differences in means of percent recruitment for tilapias in different ponds (for further details, see text).

Treatment no.* (in order of increasing percent recruitment)	1	4	2	5	3
Percent recruitment	45[a]	46[a]	56[b]	57[b]	64[b]
Yield (g)	3,870	7,540[c]	6,860[c]	7,450	8,225

*See Fig. 1.
Values with the same letter are not significantly different (differences tested by standard errors, Bailey 1981).

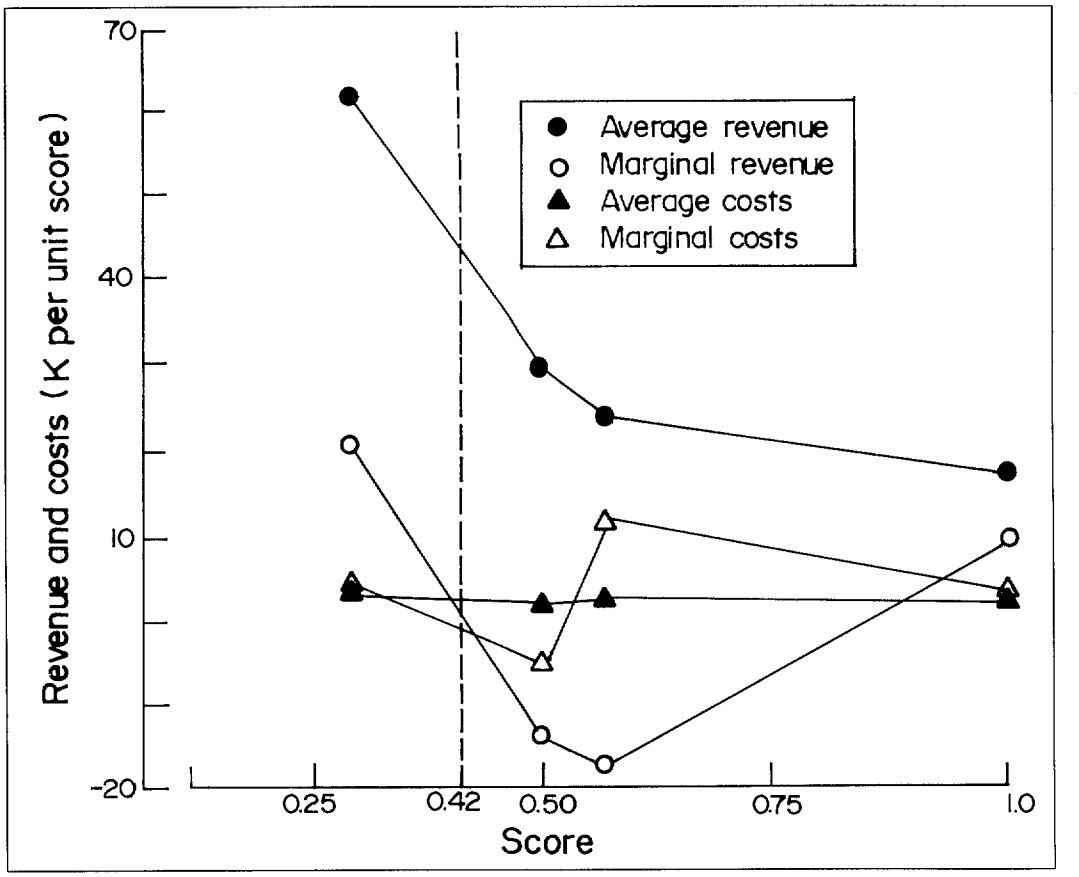

Fig. 3. Marginal cost (MC) and marginal revenue (MR) curves showing location of optimum score (0.42).

does not indicate exactly what yields can be obtained using the suggested optimum harvesting procedure. It can be expected, however, that this proposed procedure would increase farmers' yields and incomes.

Acknowledgements

The author gratefully acknowledges the help of Dr. Daniel Pauly in the preparation of this paper. Support for this work was provided by the Deutsche

Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH.

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Strategies for Stocking Nile Tilapia (*Oreochromis niloticus*) in Fertilized Ponds

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Abstract

In a 149-day grow-out experiment, we tested the effects of stocking density, partial harvesting and intermediate stocking on net fish yield (NFY) and harvest size of Nile tilapia (*Oreochromis niloticus*). Sex reversed male tilapia were raised in 280-m^2 earthen ponds, which received 8 kg dry weight chicken manure·ha^{-1}·day^{-1} with urea and triple superphosphate supplement to give a total fertilization rate of 4.0 kg N·ha^{-1}·day^{-1} and 1.0 kg P·ha^{-1}·day^{-1}. The five treatments were three stocking densities of 0.8, 1.6 and 2.4 fish·m^{-2}, fish stocked at 0.8 fish·m^{-2} with an additional 0.8 fish·m^{-2} added after 2.5 months, and fish stocked at 1.6 fish·m^{-2} with 50% of fish removed after 2.5 months.

Stocking density significantly affected fish yield (r^2=0.57, P<0.02); extrapolated mean NFY in ponds stocked at 0.8, 1.6 and 2.4 fish·m^{-2} were 14.2, 19.2 and 25.7 kg·ha^{-1}·day^{-1}, respectively; mean weights were 335, 230 and 214 g·$fish^{-1}$, respectively. Mean NFY for the first 2.5 months exceeded 39.0 kg·ha^{-1}·day^{-1} in ponds stocked at 2.4 fish·m^{-2}. Partial stocking gave slightly higher total NFYs than partial harvesting, or 21.7 kg·ha^{-1}·day^{-1} compared to 18.0 kg·ha^{-1}·day^{-1}. Additional stocking did not significantly affect fish growth of the originally stocked fish. Mean harvest weights of fish stocked at 0.8 fish·m^{-2} were similar to the first stocked fish in the treatment receiving an additional 0.8 fish·m^{-2} after 2.5 months. Results suggest a partial intermediate stocking and partial harvesting strategy may produce annual tilapia yields of 30 kg·ha^{-1}·day^{-1}, with mean weights over 300 g·$fish^{-1}$. Implications for managing ponds for higher yields at desired mean fish weights are discussed.

Introduction

Manipulation of stocking densities is an established management consideration in pond aquaculture. Net fish yield (NFY) tends to increase with increasing stocking density (Milstein et al. 1988; Colman et al. 1990). Competition for natural food (Diana et al. 1991) and increased aggressive territorial behavior (Balarin and Haller 1982; Fishelson 1983; Owusu-Frimpong 1987) set limits to this positive relationship. If either of these two factors are related to fish size, then yields may be increased by reducing competitive pressures by raising fish of two different size classes in the same pond.

The following experiment was designed to test whether partial harvesting and/or intermittent stocking may affect NFY and/or size at final harvest of Nile tilapia (*Oreochromis niloticus*).

The objective was to optimize NFY and desired fish size through stocking and harvesting management strategies.

Materials and Methods

Research presented here was conducted at the Ayutthaya Freshwater Fisheries Center of the Royal Thai Government Department of Fisheries at Bang Sai (14°11' N, 100°30' W), located approximately 60 km northwest of Bangkok, Thailand. The area is characterized as a tropical lowland, with average annual air temperature of 28°C and rainfall of 1,370 mm (Egna et al. 1987). The experiment was conducted in fifteen 280-m^2 earthen ponds maintained at a depth of about 0.95 m. Duration of grow-out was five months, from 24 April to 20 September 1990.

The experiment was designed to (1) determine effects of initial stocking density of Nile tilapia on NFY and fish size at harvest under conditions of high fertilizer input and (2) determine effects of additional stocking or partial harvesting on NFY and fish growth. There were five treatments (Fig. 1). Three consisted of initial stocking densities of 0.8, 1.6 and 2.4 fish·m^{-2}, addressing the first question. To answer the second question, a fourth treatment with initial stocking of 0.8 fish·m^{-2} was stocked with an additional 0.8 fish·m^{-2} after 2.5 months, while a fifth treatment began with 1.6 fish·m^{-2} with half the fish randomly harvested after the same period. These last two treatments together with the 0.8 and 1.6 fish·m^{-2} treatments gave a 2x2 factorially designed experiment (Fig. 1).

There were three replicates per treatment; treatment allocation to ponds was completely random. Male Nile tilapia with individual weight of about 10 g, sex reversed using 17α-methyltestosterone (Buddle 1984), were used for all stockings. All ponds were fertilized at a rate of 70 kg chicken manure dry wt·ha^{-1}·$week^{-1}$, with urea and phosphorous (P) as TSP (45% P_2O_5) added to give a nitrogen (N) input of 0.4 g N·m^{-2}·day^{-1} and a N:P ratio of 4:1 by weight.

Analysis of variance (ANOVA) and regression analyses presented here were done according to Steele and Torrie (1980) using the Statgraphics 4 statistical software package. Means are given with ± standard error (SE) in parentheses.

Results

Response to Initial Stocking Density

The relationship between NFY and initial stocking density changed from the first 2.5 months of grow-out when compared to the last 2.5 months (Fig. 2). Mean NFY in ponds stocked at 2.4 fish·m^{-2} was 39.4 (±5.8) kg·ha^{-1}·day^{-1} during the first 2.5 months, which was significantly greater ($P<0.05$) than 16.2 (±1.2) kg·ha^{-1}·day^{-1} and 20.9 (±3.1) kg·ha^{-1}·day^{-1} observed in ponds stocked at 0.8 and 1.6 fish·m^{-2}, respectively. NFYs for both 0.8 and 1.6 fish·m^{-2} stocking densities varied very little between the first and second 2.5-month intervals, whereas in the 2.4 fish·m^{-2} treatment, NFY decreased by more than half to an average rate of 17.7 (±3.5) kg·ha^{-1}·day^{-1}. Over the entire 5-month grow-out period, NFY sharply increased with initial stocking density (Fig. 3).

Fish weight after 2.5-month grow-out demonstrated no relationship with initial stocking density; treatment mean individual weights averaged about 150 g (Fig. 4). Differences were more noticeable after 5-month grow-out. Fish initially stocked at 0.8 fish·m^{-2} had mean harvest weights of 315.9 (±41.6) g,

whereas mean weights for fish stocked at 1.6 and 2.4 fish·m^{-2} were quite similar at 209.1 (±43.9) and 203.8 (±24.9) g, respectively.

Response to Change in Density

Of all five treatments, the additional stocking treatment gave the highest mean NFY during the second 2.5-month growth interval (Fig. 2). The average 5-month NFY was 23.6 (±6.4) kg·ha^{-1}·day^{-1}. Mean fish weights at harvest were 339.3 (±39.9) g for the initially stocked fish and 129.7 (±18.2) g for the additionally stocked fish at 2.5 months (Fig. 4).

The partial harvest treatment gave NFYs similar to 1.6 fish·m^{-2} during the first 2.5 months and similar to 0.8 fish·m^{-2} during the second 2.5 months. The average 5-month NFY was 17.9 (±3.5) kg·ha^{-1}·day^{-1}, in between the NFYs

Experimental design

Fish density (2.5-5 months)	Fish density (0-2.5 months): 0.8 fish·m^{-2}	1.6 fish·m^{-2}	2.4 fish·m^{-2}
0.8 fish·m^{-2}	Constant 0.8 fish·m^{-2}	Partial harvest	
1.6 fish·m^{-2}	Additional stocking	Constant 1.6 fish·m^{-2}	
2.4 fish·m^{-2}			Constant 2.4 fish·m^{-2}

Fig. 1. Experimental design for examining the effects of initial stocking density, partial harvesting and intermittent stocking on growth and yield of Nile tilapia (*Oreochromis niloticus*).

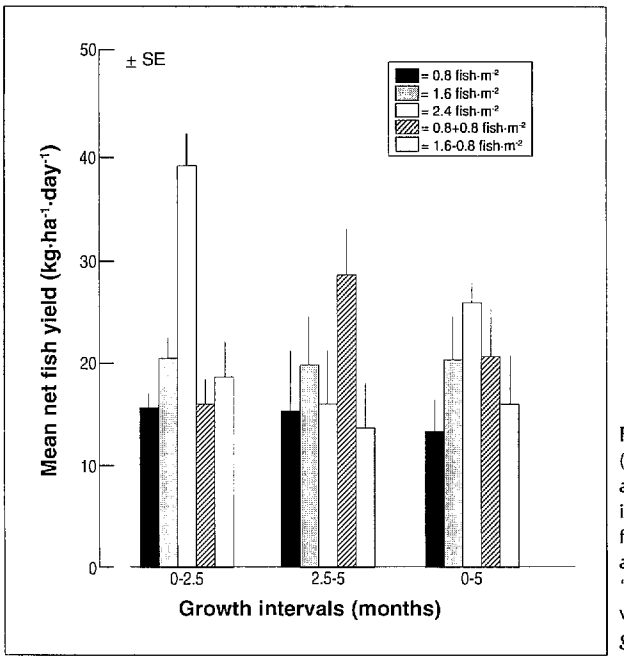

Fig. 2. Relationship between mean net yield (±SE) of Nile tilapia (*Oreochromis niloticus*) and different densities. Treatment "0.8+0.8" indicates initial stocking density of 0.8 fish·m^{-2}, with an additional 0.8 fish·m^{-2} added after 2.5-month grow-out. Treatment "1.6-0.8" indicates stocking at 1.6 fish·m^{-2}, with 0.8 fish·m^{-2} removed after 2.5-month grow-out.

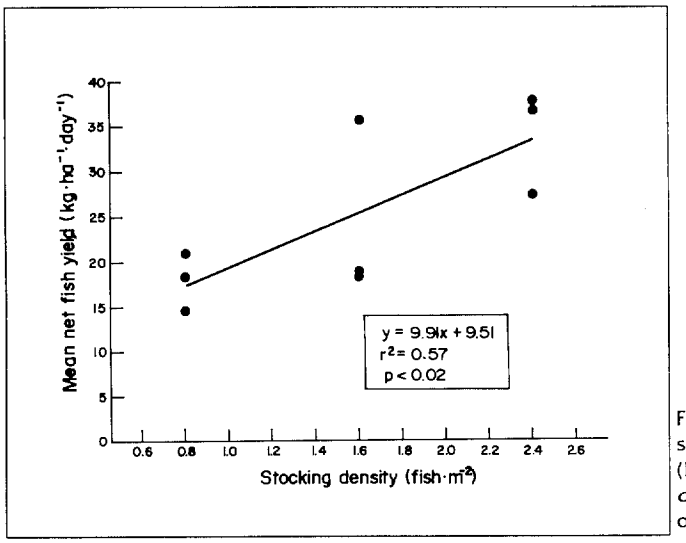

Fig. 3. Relationship between initial stocking density and NFY ($kg \cdot ha^{-1} \cdot day^{-1}$) of Nile tilapia (*Oreochromis niloticus*) after 5-month grow-out.

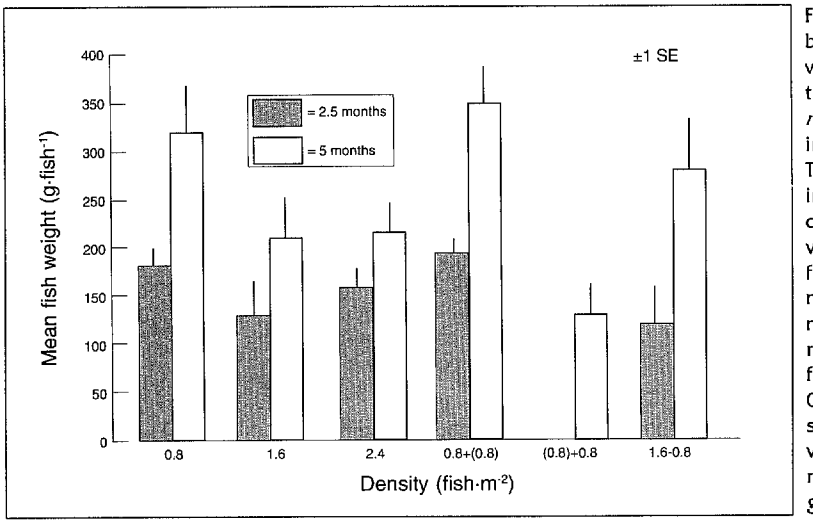

Fig. 4. Relationship between mean final weight (±SE) of Nile tilapia (*Oreochromis niloticus*) and different initial stocking densities. Treatment "0.8+0.8" indicates initial stocking density of 0.8 $fish \cdot m^{-2}$, with an additional 0.8 $fish \cdot m^{-2}$ added after 2.5-month grow-out. Designation "0.8+(0.8)" represents initially stocked fish only. Treatment "1.6-0.8" indicates initial stocking at 1.6 $fish \cdot m^{-2}$, with 0.8 $fish \cdot m^{-2}$ removed after 2.5-month grow-out.

observed for 0.8 and 1.6 $fish \cdot m^{-2}$ initial stocking densities. Fish weight also reflected growth intermediate between the two stocking densities, with final mean weight of 286.8 (+61.3) g (Fig. 4).

By comparing fish data from the 2x2 factorial treatments (Fig. 1), it is possible to make several conclusions regarding the effects of density changes on growth and yield of tilapia:

1. Adult tilapia grew better when density was reduced. Fish remaining in the partial harvest treatment (i.e., density of 0.8 $fish \cdot m^{-2}$) grew during the second 2.5-month interval at a mean rate of 15.2 (±3.5) $kg \cdot ha^{-1} \cdot day^{-1}$. This rate compares to mean NFYs of 15.5 (+2.6) $kg \cdot ha^{-1} \cdot day^{-1}$ and 10.3 (±2.7) $kg \cdot ha^{-1} \cdot day^{-1}$ for fish with constant densities of 0.8 and 1.6 $fish \cdot m^{-2}$, respectively. Relatively slower individual fish growth when initially stocked at 1.6 $fish \cdot m^{-2}$ had no apparent effect on the ability of fish to increase their growth rate when fish density was halved after 2.5 months.

2. Fingerlings raised with other fingerlings grew at similar rates when raised

with adults (individual weight about 150 g or more) at same total fish density. Fingerlings additionally stocked (0.8 fish·m^{-2}) at 2.5 months grew at 12.2 (±1.3) kg·ha^{-1}·day^{-1} and after 2.5 months had an average weight of 129.7 (±18.2) g. These data are similar to growth measurements for fingerlings stocked at 0.8 fish·m^{-2} with other fingerlings also stocked at 0.8 fish·m^{-2} (i.e., stocking density of 1.6 fish·m^{-2}). During the first 2.5-month growth interval, fingerlings stocked with fingerlings gave a mean NFY of 10.5±1.6 kg·ha^{-1}·day^{-1} and a mean weight of 124.6±16.1 g. Therefore, the size of coexisting tilapia did not influence the observed density effect on the growth of tilapia fingerlings.

3. Adults (individual weight about 150 g or more) grow better with fingerlings than with other adults at the same total fish density. The first stocked fish in the additional stocking treatment gave a mean NFY of 17.9 (±5.1) kg·ha^{-1}·day^{-1} during the second 2.5-month interval and an average harvest weight of 339.3 (±39.9) g. Even though there was an additional 0.8 fish·m^{-2} in the ponds, fish grew very similarly to those in ponds with only 0.8 fish·m^{-2} (NFY=15.5±2.6 kg·ha^{-1}·day^{-1} and harvest weight of 315.9±41.6 g). Adults stocked at 0.8 fish·m^{-2} and grown with other adults at 0.8 fish·m^{-2} (i.e., 1.6 fish·m^{-2}) grew at an average rate of 10.3 (±2.7) kg·ha^{-1}·day^{-1} with mean harvest weight of 209.1 (±43.9) g.

Survival averaged 86.4% (range, 75.9-94.1) for all treatments and stockings (n=15).

Discussion

The progressive increase in NFY with increasing initial stocking density found here was in agreement to results reported by Milstein et al. (1988) for tilapia stocked between 0.15 and 0.9 fish·m^{-2}. This was extended to initial stocking densities up to 5 fish·m^{-2} in a short-term tank investigation (Colman et al. 1990). In contrast, Diana et al. (1991) reported decreasing NFY of Nile tilapia as stocking densities increased from 1 to 3 fish·m^{-2}. They fertilized ponds with only chicken manure at a nitrogen loading rate of about 20% of that used in our investigation. As tilapia derive most of their nutrition from algae (Colman and Edwards 1987; Schroeder et al. 1990), food limitation at higher stocking densities suggested by Diana et al. (1991) was likely. In our study, high inorganic nitrogen and phosphorous fertilization often resulted in afternoon dissolved oxygen concentrations above 25 mg·l^{-1} and chlorophyll *a* concentrations above 300 mg·m^{-3}. With greater primary productivity, food constraints at higher stocking densities were not indicated. A subsequent study using the same high inputs and a stocking density of 3 fish·m^{-2}, however, gave NFYs which were not significantly different from those obtained at 2.4 fish·m^{-2} in the first or second 2.5-month growth intervals (C.F. Knud-Hansen, unpubl. data).

It is unclear why fingerlings did not induce a density effect on adult growth, whereas increased adult and fingerling densities affected the growth and yield of other fingerlings. Two explanations may be competition for food and/or competition for space. As fertilization inputs were relatively high and ponds were quite green, it seems likely that adult fish limited fingerling growth by outcompeting fingerlings for phytoplankton. Differences in size selection for zooplankton may explain why adult fish were unaffected by coexisting fingerlings, but does not answer why fingerling growth was negatively affected by adults. It is more probable that territorial aggressive behavior noted among male tilapia (Balarin and Haller 1982; Owusu-Frimpong 1987) occurs only between fish of similar size or greater. Fishelson (1983) observed

among male tilapia a strong correlation between social rank and aggressive activity. Fish high up on the hierarchy needed only to swim by lesser fish to maintain dominance. The great size differential between newly stocked and 2.5-month-old fish (10 g versus 150 g) should establish clear dominance by larger fish. Fingerlings would therefore be affected by adults, but not the reverse.

Although intermittent harvesting alone did not increase fish yield, a conclusion supported by Moav et al. (1977), partial harvesting coupled with intermittent stocking may be a way to balance the trade-off between high yield and smaller fish at higher stocking densities, and smaller yields but larger fish at lower stocking densities. The observation that adult growth was unaffected by additional stocking of fry suggests a stocking and harvesting strategy where fingerlings are stocked every 10 weeks, and after 20 weeks adults are harvested every 10 weeks (Table 1). By stocking 0.8 fish·m^{-2} every 10 weeks in ponds fertilized at approximately 4 kg N·ha^{-1}·day^{-1}, hypothetical yields of about 10,000 kg·ha^{-1}·year^{-1} of tilapia with mean weights exceeding 300 g may be sustainable.

Table 2 summarizes different stocking strategies depending on the desired size of tilapia at harvest. Estimated fish sizes and yields were extrapolated from data from this study. Estimated grow-out periods assume stocking of fish of approximately 5 to 10 g in ponds fertilized with 4 kg N·ha^{-1}·day^{-1} with sufficient phosphorous and carbon added to maintain nitrogen limitation (Knud-Hansen et al.

Table 1. Theoretical net fish yields of Nile tilapia (*Oreochromis niloticus*) with estimated mean weights of 325 g·fish^{-1} using an intermittent stocking and harvesting strategy and high fertilizer input.

Treatment/yield	Weeks 0	10	20	30	40	50
Stocking density (fish·m^{-2})	0.8	0.8	0.8	0.8	0.8	0.8
Net fish yield (kg·ha^{-1}·day^{-1})		12.2	12.2	12.2	12.2	12.2
			17.8	17.8	17.8	17.8
Harvest yield			2,100	2,100	2,100	2,100

Table 2. Summary of stocking strategies of Nile tilapia (*Oreochromis niloticus*) for obtaining desired fish sizes and NFYs (ponds fertilized with 4 kg N·ha^{-1}·day^{-1} and 1 kg P·ha^{-1}·day^{-1}).

Mean fish weight (g·fish^{-1})	Stocking density (fish·m^{-2})	Grow-out period (weeks)	Estimated mean NFY (kg·ha^{-1}·day^{-1})	Estimated mean NFY (kg·ha^{-1}·year^{-1})
125	0.8	8	15	5,500
	1.6	8	26	9,500
	2.4	8	39	14,000
225	0.8	14	15	5,500
	1.6	26	18	6,500
	2.4	26	27	9,500
325	0.8	21	15	5,500
	1.6	40	13	5,000
	2.4	50	20	7,000
	PH/IS[a]	20	30	11,000

[a]Partial harvest/intermittent stocking (see Table 1).

1991; Knud-Hansen and Batterson 1994). To obtain average fish of 125 g or 225 g, the highest stocking density gave the greatest extrapolated NFYs of 14,000 $kg \cdot ha^{-1} \cdot year^{-1}$ and 9,500 $kg \cdot ha^{-1} \cdot year^{-1}$, respectively. Although not tested, it is likely that intermittent stocking and partial harvesting would give greater yields for the larger fish size. To obtain fish of about 325 g, the partial harvesting and additional stocking strategy described in Table 1 should give the highest NFY of about 11,000 $kg \cdot ha^{-1} \cdot year^{-1}$.

Acknowledgements

Research presented here was conducted jointly by the Michigan State University (USA), the University of Michigan (USA), National Inland Fisheries Institute (AIT, Thailand), and the Asian Institute of Technology (Thailand) as a part of the Pond Dynamics/Aquaculture Collaborative Research Support Program (CRSP) supported by the Agency for International Development, Grant No. DAN-4023-G-SS-7066-00 and by contributions from the participating institutions. This is Pond Dynamics/Aquaculture CRSP contribution no. 1064. Acknowledgement is also made to the Michigan Agricultural Experiment Station for its support of this research. The AIT aquaculture laboratory staff is gratefully acknowledged for their technical assistance.

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The Spontaneous Reproduction of Tilapia: an Opportunity or a Handicap for the Development of African Aquaculture?

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Abstract

The year-round, spontaneous and continued reproduction of tilapia in captivity was initially regarded as an unexpected asset for African aquaculture development, but it was quickly found to be a serious handicap when farming the fish in ponds due to the resulting population. Various techniques have overcome this obstacle.

The reproductive efficiency of tilapia has advantages and disadvantages for the supply of fry to fish farmers. Among the advantages is the possibility for fish farmers to produce their own fry; among the disadvantages is the difficulty to control the broodstock quality. The low number of eggs in each spawning requires the use of a large number of broodstock and the asynchrony of spawnings makes the simultaneous production of large quantities of graded fry difficult. Finally, the control of undesirable reproduction in farming involves the use of monosex populations.

These issues are analyzed and discussed in relation to development strategies, microeconomics and the identification of research themes that can be developed to improve the different options. The data for three species of tilapia (*Oreochromis niloticus*, *Sarotherodon melanotheron* and *Tilapia guineensis*) are compared to the data for two species of African catfish of the family Clariidae (*Clarias gariepinus* and *Heterobranchus longifilis*) that require induced reproduction techniques, but are far more fertile.

Introduction

African aquaculture began approximately 50 years ago with tilapia as the basic species. The essential advantages of this fish were its spontaneous reproduction in captivity, easy nursing and diet that is both located at the beginning of the food chain and very flexible. The major disadvantage of tilapia was its very early sexual maturity in captivity at a size when it was often smaller than its marketable size. This early maturity very rapidly induces overpopulation and stunting during culture in ponds (the infrastructure mainly used in African aquaculture).

Various studies in the 1970s, on the African continent and elsewhere, contributed to the improved control of the disadvantages of farming tilapia in closed structures (monosexing and mixed-sex farming with predators) and the development of farming in open structures (cages and pens). Furthermore, control of the biological cycle of other species with aquaculture potential (particularly catfish) has been achieved.

The major species of tilapia studied in this paper are *Oreochromis niloticus*, *Sarotherodon melanotheron*, and *Tilapia guineensis*—covering the three genera of the tilapia group—and *Clarias gariepinus* and *Heterobranchus longifilis*, two African catfish of the family Clariidae, whose aquaculture potential was demonstrated more recently. First, the reproductive biology and spawning induction methods in captivity for the two groups of species will be compared. The economic and social impact of these different technical and biological characteristics on the development of tilapia and clariid farming is then analyzed.

Biological Data Relating to the Reproduction of Some Tilapia and Catfish

The comparative status of tilapia and catfish reproduction is given in Table 1.

Age at First Sexual Maturation

Age and size at first sexual maturation vary depending on the environment of the fish. Among the five species studied, sexual maturity occurs earlier and at a smaller size in culture conditions than in the natural environment.

For *O. niloticus*, first sexual maturation generally occurs at about age two or three years in optimal conditions in the natural environment (lakes), whereas in unfavorable conditions—like captivity in small ponds—reproduction can occur from the age of three months (McBay 1961; Ruwet et al. 1976). For *S. melanotheron* and *T. guineensis* reared in lagoon pens, first sexual maturation occurs at six to eight months (50 g) and at seven to nine months (60 g), respectively. In the natural environment (Ebrié Lagoon, Côte d'Ivoire), the weight at first maturation of these two species is higher (130 and 80 g, respectively) for fish of undetermined age (Legendre et al. 1990).

For *C. gariepinus*, sexual maturity occurs at about two years of age in the natural environment (Richter 1976). In culture conditions, maturity is reached at about eight to 10 months (Pham 1975) or five to seven months (Legendre et al. 1992), at a weight of 100-200 g.

For *H. longifilis*, sexual maturity occurs at the age of 12-14 months and at a weight varying from 300 to 1,500 g

Table 1. Comparative reproductive characteristics of tilapia and catfish: biological data and constraints (ONI, *Oreochromis niloticus*; SME, *Sarotherodon melanotheron*; CGA, *Clarias gariepinus*; and HLO, *Heterobranchus longifilis*).

	Tilapias	Catfish
Spawning; fecundity	Spontaneous spawning in captivity; 350-3,500 eggs/♀ (ONI)	Spawning induction required; 60,000-80,000 eggs/kg of ♀
Spawning frequency	2 (SME) to 6 (ONI) weeks	6-8 weeks (CGA) 4-8 weeks (HLO)
Control of reproduction (constraints)	Sex ratio management Stocking density Individual weights of ♀ and ♂ Duration of spawning/ replacement of broodstock	Choice of mature broodstock (biopsy and examination of the genital papilla) Hormonal induction of ovulation Artificial fertilization incubation of the eggs
Renewal of broodstock	Approx. every 18 months	CGA (?), HLO (> 6 years)
Relationship to marketable size	Size at final spawning < marketable size	Size at final spawning ≥ marketable size (CGA, 200 g; HLO, 300-1,500 g)
Sexual dimorphism in growth	♂>♀	CGA: ♂>♀ HLO: ♂ = ♀
Hybrids	Quality of strains Hybrid fertility < intraspecific fertility	Hybridization CGA x HLO is possible
Larval culture requirements	No larval culture for *Oreochromis* and *Sarotherodon* spp.; brief larval phase for *Tilapia* spp.	Larval culture required for CGA and HLO
Constraints	Production planning Large number of broodstock Asynchronous spawning (hence large areas required)	Induction of ovulation Artificial fertilization and larval culture are needed

depending on culture conditions (Legendre et al. 1992). In the natural environment, first sexual maturation is thought to occur at two years of age (Motwani 1970).

Fertility

In tilapias, as in other fish, absolute fecundity increases with the size of the females. In *O. niloticus*, the minimal absolute fecundity observed is 340 ova for a 26-g female and the maximum fecundity is 3,500 ova for a 550-g female (Mélard 1986). In *S. melanotheron*, the mean absolute fecundity (in pens) is 450 eggs for a 100-g female, 700 eggs for a 200-g female and 1,000 eggs for a 300-g female (Legendre et al. 1990). For similar weights, fecundity is 4,000,

8,000 and 12,000 eggs, respectively, per female of *T. guineensis* reared in pens (Legendre et al. 1990).

In contrast, relative fecundity (expressed as the number of fertilized eggs or fry produced per kilogram of female) varies inversely with the mean body weight of the female tilapia. This implies that in *O. niloticus*, large numbers of small females of approximately 100-150 g must be kept in order to ensure a maximal fry production with the same biomass of broodstock (Mélard 1986).

In the clariid species studied here, the average relative fecundity recorded per kilogram of female varied little with individual weight. In *C. gariepinus*, absolute fecundity was 39,000 eggs for a 500-g female, 81,000 eggs for a 1-kg female and 132,600 eggs for a 1.5-kg female, which is approximately 80,000 eggs per kg of female (Hogendoorn 1983).

In *H. longifilis*, fecundity is maximal in the rainy season. It averages 130,000 eggs for a 2-kg female and 344,000 eggs for a 4-kg female (Legendre 1986). High seasonal variations in fecundity are observed: 28,000 eggs per kg of female in the dry season and 68,000 eggs per kg of female in the rainy season.

Spawning Frequency

In tilapias, spawning frequency and the quantity of fry produced are directly correlated, and it seems more logical to consider mean values during a given spawning period than the results for single spawnings. In optimal conditions and at a temperature of 25-28°C, a female of *O. niloticus* can spawn every 30-40 days (Ruwet et al. 1976), but a very high individual variability is observed (Mires 1982). Thus, according to this author, the spawning frequency in aquarium of a female of *O. niloticus* of 400-500 g mean body weight varied from 23 to 50 days with very great variability in the total number of spawnings obtained (from two to seven) during the observation period (11 months).

In *S. melanotheron* and *T. guineensis*, the mean spawning frequency observed in 4-m^2 concrete tanks (sex ratio 1:1) is significantly higher, with an interval between two spawnings of two weeks and three weeks, respectively (Legendre et al. 1990).

In general, spawning within a population of tilapia broodstock (of the same age, size and stage of sexual maturity) is irregular and asynchronous for the various females.

In optimal farming conditions of *C. gariepinus* (correct feed, temperature, quality and water renewal), the minimal frequency of repeated induction of ovulation by hormone treatment of the same female is approximately six to eight weeks without loss of fecundity per single spawning (Hogendoorn 1983).

In *H. longifilis*, the spawning frequency of females treated with HCG is two months without loss of fecundity (Legendre 1986) and could certainly be higher (one month; Z. Otémé, pers. comm.).

Control of Reproduction

Hormonal induction of spawning is not practiced in tilapias, but rigorous management of broodstock is necessary for the mass production of eggs and fry. Thus, in *O. niloticus*, the best results can be obtained with the following culture conditions and techniques:

- separation of the sexes before placing broodstock in reproduction structures (ponds, hapas and concrete tanks) for more rapid and regular fry production (Guerrero 1987; Parrel et al. 1990);
- more frequent replacements of broodstock: every 21 days in hapas ac-

cording to Lovshin and Ibrahim (1987), which increases egg and fry production by approximately 16%; and

- stocking densities of 0.7 broodfish·m^{-2} in 400-m^2 ponds (Lazard 1984) or 4-5 broodstock·m^{-2} in hapas (Bautista 1987; Guerrero 1987) and optimal sex ratios (♀:♂) generally of about 3:1.

It should be noted that for *S. melanotheron*, in which mouthbrooding of the eggs is done by the male, the optimal sex ratio is 1:1.

Reproduction with no hormonal induction of ovulation is possible in *C. gariepinus* and *H. longifilis*, but gives poor results in terms of the number of eggs and fry produced. Thus, in *C. gariepinus*, natural reproduction in ponds where a flood is simulated (by filling the pond in one day) leads to a production of approximately 145 fry per 100 m^2, 1.5 months after stocking with sexually mature broodstock.

The best results for *C. gariepinus* are obtained by induction of ovulation with different types of hormones: HCG, pituitary carp extracts and LH-RHa with pimozide. In *H. longifilis*, HCG gives a 100% induction response.

Renewal of Broodstock

The most productive period for tilapia broodstock is the first 18 months of sexual activity (Rana 1988), after which it is recommended that the broodstock be renewed.

In contrast, no decrease in fecundity seems to have been found among the catfish in the experiments conducted so far on each species studied here. For *C. gariepinus*, no recommendation concerning the appropriate age at which to renew the broodstock seems to have been made and for *H. longifilis*, no decrease in relative fecundity has been observed up to the age of six years (6 kg mean body weight).

Maturation and Marketable Size; Sexual Dimorphism in Growth

Among the main species of tilapia used in aquaculture, sexual maturity occurs well before marketable size is attained. In addition, sexual dimorphism in growth dimorphism in favor of males (the precise cause of which is still unknown) is observed in all tilapias: hence, the different methods used to produce all-male populations (mainly hormone treatments and interspecific hybridization).

For *H. longifilis*, no significant difference between male and female growth has been shown (Legendre et al. 1992). In contrast, a significant growth dimorphism in favor of males was observed in *C. gariepinus* (Henken et al. 1987). After 307 days of culture in concrete tanks, Legendre et al. (1992) reported a mean body weight for *H. longifilis* of 700 g for females and 680 g for males, and for *C. gariepinus*, of 260 g for females and 450 g for males.

Hybridization

The production of *Oreochromis* hybrids has been going on for 30 years and by the 1970s, it was produced commercially (initially in Israel, then subsequently in many other countries). The goal of hybridization is generally twofold: inheriting suitable parental qualities (growth, cold resistance, better catchability, mesentery coloration, etc.) and obtaining a high percentage of males.

The use of these interspecific crosses has always been faced with problems of low fertility compared to intraspecific crosses (Rothbard et al. 1983) and the need to maintain pure broodstock lines to produce progenies that are near 100% male. Increased fry production has been achieved by selecting parental strains (Hulata et al. 1985) or by using F_1 hybrids as broodstock, then applying a

masculinizing hormone treatment to progenies (Rothbard et al. 1983).

Hybridization is also possible in the Clariidae using in vitro fertilization (Legendre et al. 1992). The hybrid *H. longifilis* x *C. gariepinus* shows growth equal to or greater than (depending on environmental conditions) that of the best performing parent (*H. longifilis*). The sex ratio of the hybrids is balanced; they are fertile, but gametogenesis is relatively poor, and male and female growth is equivalent. In contrast to the use of *H. longifilis*, the aquaculture potential of such hybrids remains to be seen (resistance to certain environmental conditions and pathogens, etc.).

Size of Eggs and Larval Culture

The eggs of *Oreochromis* and *Sarotherodon* are clearly larger (2.5-4.5 mm) than those of *Tilapia* (1.5-2.0 mm) and Clariidae, *C. gariepinus* and *H. longifilis* (1.5 mm).

This has a direct impact on the nursing period: following resorption of the yolksac, at the start of their trophic phase (20 mg approximately), *Oreochromis* and *Sarotherodon* already exhibit the definitive adult morphology; they are robust and their food requirements are easily met.

Conversely, clariid larvae, whose weight is approximately 1.8 mg at the start of the trophic phase, require a specific feed and particular culture conditions.

Tilapias of the genus *Tilapia* that have a brief larval period represent an intermediate situation, with the possibility of high mortality during first feeding (observations on *T. guineensis*; Legendre et al. 1990).

Biotechnical Data on Fry and Fingerling Production in Tilapia and Catfish

Infrastructure Used: Technical and Biological Constraints

TILAPIAS

At the start of African aquaculture development, the same ponds were used for all phases of production: reproduction, nursing and grow-out (farming in mixed-age classes). Results were poor (overpopulation, competition for food and cannibalism) for all three phases, and the number of fry harvested was low—between 0.5 and 1.2 individuals$\cdot m^{-2} \cdot month^{-1}$—with a great heterogeneity in size. Furthermore, with this system, it was not possible to distinguish between recently hatched fry and small-sized fish afflicted by stunting and reproducing early (neoteny).

Two techniques that are more recent and more efficient will be briefly described here: first, the production of fry in ponds, which is the most widespread technique and apparently the method best adapted to Africa, and second, the production of fry in hapas or small cages, a technique widespread in Asia and potentially suitable for many African situations since it requires little capital and surface area.

In Ponds. The numerous attempts these last few years to produce *O. niloticus* fry have led to the recommendation for pond production of the major methods summarized in Table 2.

Concerning broodstock conditioning and management, broodstock are given a break from sexual activity and reconditioned for their next spawning by

Table 2. Biotechnical data on the production of *Oreochromis niloticus* fry in ponds of various sizes (RB, rice bran; PC, peanut cake; and FM, fishmeal).

Pond surface area (m^2)	4,500	350	200
Broodstock density ($ind \cdot m^{-2}$)	0.16	0.7	4
Mean weight of broodstock (g)	62-356	100 (♀) 240 (♂)	80-100
Sex ratio (♂:♀)	3:1	3:1	3:1
Duration of culture (days)	250	120	45-60
First harvest of fry (days after stocking)	60	35	14
Harvest intervals (days)	30*	15*	6 times/day at 2-hour intervals starting at 0700**
Feed/Fertilization	organic and mineral fertilization	50% RB + 50% PC	organic fertilization + RB (75%) + FM (25%)
No. of fry produced ($ind \cdot m^{-2} \cdot month^{-1}$)	8.0	45.4	200-250
Mean weight of fry produced	4.3 g	0.7 g	a few mg to 0.1 g
Reference	Broussard et al. (1983)	Lazard (1984)	Guerrero (1987)

*Using seine nets.
**Using small-mesh handnets.

separation of the sexes and the use of a high-protein feed at the rate of 4% of body weight·day^{-1}. This reconditioning lasts for one to two weeks (after three to four weeks of reproductive sexual activity in ponds of 300-500 m^2 according to Guerrero [1985]), or for a period equal to the period of reproduction [four months in 350-m^2 ponds according to P. Parrel (pers. comm.)]. According to Guerrero (1987), the female broodstock must be slightly larger than the male (by 20-30 g) for broodstock weighing from 100 to 200 g. Lazard (1984) uses males that are larger than the females (240 versus 100 g) and Parrel et al. (1990) recommend mean broodstock body weights greater than 200 g. For broodstocks of 50-100 g reproducing in small ponds, Guerrero (1987) recommends replacement when 250-350 g mean body weight is reached.

Nursing in ponds, starting with fry of 0.5-1 g, is done up to a weight of 5 g at a density of 25 individuals·m^{-2} over 60 days with a compound feed that is 20% fishmeal.

The nursing of fry with a body weight lower than 0.1 g is done in hapas at a density of 500-1,000·m^{-2} over one to two weeks, then in ponds at a density of 200-400·m^{-2} over two to four weeks, depending on the final size desired.

In Hapas. The production of fry with a mean body weight ranging from 60 to 80 g in small cages or hapas is generally done at a density of 4 broodstock·m^{-2}, with a sex ratio of 3:1. The frequency of fry harvests varies: daily, with the first beginning 10-12 days after the stocking of broodstocks (Guerrero 1987), or at intervals of 15-21 days with all fry harvested (Bautista 1987). Production in hapas has ranged from 10

to 15 fry·m^{-2}·day^{-1}, regardless of harvest frequency. The duration of the production cycle in hapas can vary from 30 days (Guerrero 1987) to 11 months (Bautista 1987). The feed is 75% rice bran and 25% fish meal, distributed at a rate of 3% of broodstock biomass per day in two distributions.

The first nursing phase is done in hapas over three weeks (a density of 1,000·m^{-2} for the first week and up to 250·m^{-2} for the third week), with a feed containing 10% fishmeal and 40% copra cake, up to a size of 0.5-1 g (Guerrero 1987). The grow-out phase that follows is generally done in ponds.

CATFISH

Captive breeding methods for *C. gariepinus* have already been substantially described (Hogendoorn 1983).

H. longifilis spawns year-round in lagoons, based on broodstock fish in pens (1 individual·m^{-2}) (Legendre 1986). The weight of the broodstock used is from 3 to 5 kg. After hormonal injection (HCG), artificial fertilization takes place in a hatchery and the average egg production is 68,000·kg^{-1} of female in the rainy season and 28,000·kg^{-1} in the dry season. A male, killed for the purpose, can fertilize approximately 600,000 eggs. An average 75% of normal larvae are recovered after hatching, with a mean body weight of 1.8 mg after yolksac resorption.

In the hatchery, larvae receiving an Artemia-based feed develop after two weeks into fry with a mean body weight ranging from 150 to 300 mg and an average 65% survival rate (Legendre et al. 1991).

In 1-m^3 small-mesh cages (0.6 mm) placed in limed ponds (Legendre et al. 1991), 2-mg larvae reach between 100 and 300 mg after two weeks (natural feed the first week, followed by a troutlet feed the second week). Stocking densities per cage range from 2,000 to 15,000 larvae, or from 2,000 to 15,000·m^{-2}. After two weeks, the survival rate in cages is approximately 25% (between 1 and 50%). Fry are then placed in a nursing pond at a density of 15·m^{-2}. Using an artificial feed of 35-40% protein, fry reach a mean body weight of 30-50 g after 2.5 months with a mean survival rate of approximately 60%.

Extent of Facilities Required for Fry and Fingerling Production

If the annual production target is 1 million fry or fingerlings, what are the necessary resources in terms of infrastructure or broodstock for each species studied?

TILAPIAS

In Ponds. Production of fry of 0.5-1 g. Using the "semi-intensive" method to produce *O. niloticus* fry described in Table 1 (Lazard 1984), the annual production of 1 million fry of 0.5-1 g requires 0.35 ha of ponds of 300-400 m^2 and 1,550 broodstock (based on a 10-month culture period per year); but, to allow for periods of sexual inactivity, approximately double their number of broodstock (100-300 g) are required to ensure continued fry production.

Production of 5-g fingerlings. To produce 5-g fingerlings, the required additional pond surface area is approximately 0.4 ha to account for rotations, based on a stocking density of 60 individuals·m^{-2} and a 70% survival rate. That rate corresponds to the nursing of 1.4 million fry.

Production of 30-g fingerlings. In this case, the required additional pond surface area is approximately 1.2 ha to

account for rotations, based on 260 individuals·m^{-2}, a 90% survival rate and an average cycle duration of 60 days.

In Hapas. In hapas, the production of 1 million *O. niloticus* fry of 0.5-1 g requires:

- 350 m^2 of reproduction hapas and 100 m^2 for conditioning the broodstock and
- 200 m^2 of nursing hapas (500·m^{-2}, 70% survival rate).

This production requires the permanent maintenance of 1,500 reproductive broodfishes, ranging in mean body weight from 60 to 80 g and the maintenance of a resting broodstock (50% of reproduction time, i.e., 700 broodstock). A total of 2,200 broodstock must be maintained.

CATFISH

The methods necessary to produce 1 million fingerlings of *H. longifilis* are as follows[a].

A 3-kg female produces an average of 144,000 eggs per spawning per year, considering the seasonal variations in fertility. This results in the hatching of approximately 100,000 larvae.

In one scenario (Case 1) of nursing in a hatchery with an Artemia-based feed, 65,000 fry are obtained after two weeks in a trough (approximately 10,000 larvae per 2x0.5-m trough).

In a second scenario (Case 2) of nursing in cages immersed in ponds with natural feed and the distribution of a compound feed, 25,000 fry are obtained after two weeks with an initial stock of approximately 6,000-8,000 larvae per 1-m^3 cage.

The nursing in ponds of fry weighing 150-300 mg at a density of 15·m^{-2} leads, after 2.5 months, to the production of 39,000 fingerlings (mean body weight: 30-50 g) in Case 1 and 15,000 fingerlings in Case 2.

Assuming a frequency of 10 reproductions per year:

- Case 1 requires the use of three females and one male per cycle, 30 larval culture troughs and 1.3 ha of ponds to produce the fingerlings; and
- Case 2 requires the use of seven females and one male per cycle, 70 larval culture cages immersed in seven 500-m^2 ponds and 1.3 ha of ponds to produce the fingerlings.

Whichever alternative is chosen, it is recommended to keep a permanent minimum stock of approximately 50 broodfish (mixed sexes), at a density of 1 individual·m^{-2} in pens (requiring a penned surface area of 50 m^2), to sustain the genetic variability of the species.

An experiment on *C. gariepinus* fry production at the La Landjia Station in the Central African Republic (CAR) (Janssen 1984) suggested, in addition to the use of a hatchery, the need for 17 ponds of 400 m^2 to produce 1,200,000 fry of 1-2 g per year in six cycles. The number of 500 g broodstock permanently in the ponds would be 400 per 1·m^{-2}.

Economic Data

For comparability, only costs for the African continent will be given here.

Production Costs of Tilapia Fry and Juveniles

The costs given here (Table 3) were obtained from full-scale experiments in ponds in Niger (Sona nursery station; 34 ponds of 350 m^2 and a pumped water supply) and in small floating nursing cages immersed in the Niger river.

Table 3 indicates:

[a]Data extrapolated from results obtained in experimental stations.

Table 3. Economic data on the production of *Oreochromis niloticus* fry (0.5-1 g), juveniles (5 g) and fingerlings (30 g) in different production systems in Niger (Parrel et al. 1990).

	Infrastructure		
	Earthen ponds (Sona nursery) 3,120,000 fry·year^{-1}	Earthen ponds (0.4 ha) 3 cycles·year^{-1} 520,000 fingerlings·year^{-1}[a]	Floating cages (5 m^3) 3 cycles·year^{-1} 7,200 fingerlings·year^{-1}
Fixed expenses*	10,200,000	-	13,800
Variable expenses*	3,250,000	-	144,000
Total expenses*	13,450,000	4,950,000[b]	157,800
Unit price/ fish*	4.3	9.5	21.9

*In F CFA 1990 (US$1.00=250-300 F CFA).
[a]In the context of a private farm (no depreciation).
[b]Including profit margin.

- a cost price (production cost) for 0.5-1 g *O. niloticus* fry of 4.3 F CFA[b] (3.67 F CFA without depreciation);
- a cost price for 5 g *O. niloticus* fry of 6.5 F CFA (without depreciation of the ponds; 7.5-8 F CFA including depreciation); and
- a cost price for 20-25 g *O. niloticus* fingerlings produced in cages of 22 F CFA (48 F CFA per male fingerling; sex ratio = 45% ♂); in ponds, the production cost of the male fingerlings would be 45 F CFA without depreciation (53 F CFA with depreciation) according to Parrel et al. (1986).

Production Costs of Catfish Fry and Juveniles

Production costs for *C. gariepinus* fingerlings produced in CAR in 1984 were as follows (Table 4):

- for a 1-g fingerling produced in a hatchery: 9.7 F CFA with depreciation and 6.7 F CFA without depreciation; and
- for a 1 to 3-g fingerling produced in a hatchery, then nursed in ponds: 8.2 F CFA with depreciation and 5.15 F CFA without depreciation.

[b]US$1=250-300 F CFA until 1 January 1994; US$1=600 F CFA in 1996.

The production cost for *H. longifilis* fry (150 mg) in a hatchery (fed with Artemia and trout feed) is 4-6 F CFA per fingerling. In cages immersed in ponds, this cost is 3.6 F CFA (with a 20% survival rate) and 1.7 F CFA (with a 50% survival rate). The production cost of *H. longifilis* fingerlings (60% survival rate) is 41 F CFA per fingerling (45 g mean body weight). For the latter species, costs were calculated under experimental conditions without considering the investment depreciation (Barros 1990).

The comparative status of tilapia and catfish production costs is given in Table 5.

Fry Production of Aquaculture and Development

General Operation

Fry intended for fattening after grow-out by artisanal fish farmers may come from three sources (Lazard et al. 1991).

- Option 1: from the farm itself (backyard hatchery)[c] or from neighboring

[c]In this case, 10-20% of the total surface area used for aquaculture must be devoted to *O. niloticus* fry and fingerling production (20-30 g required size for manual sexing; Lazard 1984).

Table 4. Economic data on the production of six-day-old *Clarias gariepinus* fry in the Central African Republic (Janssen 1984).

Items	Infrastructures	
	Hatchery Spawning + fry raising 1,242,000 units·year^{-1}	Hatchery + earthen ponds Spawning + start (6 days) 1,242,000 units·year^{-1}
Fixed expenses*	5,330,000	5,770,000
Variable expenses*	6,740,000	4,490,000
Total expenses*	12,070,000	10,260,000
Unit price/ fish*	9.7	8.2

*In F CFA 1990 (US$1.00=250-300 F CFA).

Table 5. Comparative economic data on the production cost of individual tilapia and catfish fry and juveniles for: *Oreochromis niloticus* (Lazard 1984; Parrel et al. 1986; Parrel et al. 1990); *Clarias gariepinus* (Janssen 1984); and *Heterobranchus longifilis* (Barros 1990).

Progeny weight	*Oreochromis niloticus*[a]	Catfish
150 mg		*Heterobranchus longifilis*[b] Hatchery: 4 to 6 F* Hatchery + cage: 1.7 to 3.6 F*
0.5-1 g (*Oreochromis niloticus*) 1-3 g (*Clarias gariepinus*)	Pond 3.7 F* 4.3 F**	*Clarias gariepinus*[a] Hatchery + Pond: 5.15 F* Hatchery + Pond: 8.2 F** Hatchery: 6.7 F* Hatchery: 9.7 F**
5 g	Pond 6.5 F* 7.5 to 8 F**	
10 g	Pond 10 to 12 F*	
	Pond 20 F* 24 F** Cage 25 F**	
40-50 g		*Heterobranchus longifilis*[b] Pond: 41 F*

F: in F CFA.
*Without depreciation.
**With depreciation.
[a] Production station.
[b] Experimental station.

farms that have surplus fry or specialize in the production of juveniles.

- Option 2: from nurseries. Today, these are still government-run operations, managed by a project or by government employees.
- Option 3: from the wild. The fish farmers catch the juveniles for stocking their ponds straight from the wild, or buy it from a fisher.

These various options are chosen and/or vary in time and space according to three major criteria:

- Culture Species: Only tilapia lends itself to an artisanal and decentralized development of backyard hatchery (Option 1) because its production and nursing are simple and do not call for any sophisticated techniques. Aquaculture development of other species (catfish, carps or other species being domesticated) involves a more or less complex phase of reproduction and larval culture that can hardly be considered, at least for the moment, except in the context of a nursery or hatchery (Option 2). These species may also be caught in the wild subject to all the risks involved in Option 3: seasonality, heterogeneity of sizes, species mixing, need for storing and transportation after the capture.
- Development Policy (who can be fish farmers?): Because nursing is still largely an "assisted" activity, the option chosen depends in fact on the project's (or the government's) philosophy.

Some development projects favor Option 1 for tilapias, whereas others favor Option 2 and perhaps even Option 3. For tilapia, the use of nurseries poses many problems (high production costs that generally require a subsidized sale price, transportation costs, the transfer of only a part of the profit from fry sales to the nursery, etc.). These problems also apply to the other species, but those species can only be either captured in the natural environment or produced in a hatchery. However, when a mass production of tilapia juveniles is necessary, the use of a hatchery is unavoidable.

- Development over Time: The projects that have opted for nurseries have seen fish farmers move gradually but systematically toward backyard production of tilapia fry. This can be explained both by the professionalization of some fish farmers and the economic failure of the nurseries.

For Clarias, the rare existing hatcheries heavily subsidize the fry and generally hardly survive the projects that created them: supply thus remains much lower than demand and fish farmers must often resort to Option 3.

Options 1 and 3 tend to become common when projects relying on hatcheries and nurseries end.

Case Studies

TILAPIAS

The majority of tilapia culture projects in Africa have regarded fry production as the cornerstone of their strategy (Lazard et al. 1991). This is why many fry production facilities have been built or rebuilt in various countries (Côte d'Ivoire, CAR, Congo, Cameroon, Madagascar, Niger, etc.) in the last few years.

The experience of operating these stations reveals the following constraints:

- high operating costs for stations running according to the administrative model;
- low levels of technical expertise on the part of agents responsible for these stations, leading to a low yield of fry per unit area; and
- problems of logistics in delivering the fry or fingerlings to fish farmers.

Thus, operational problems of the La Landjia Station in CAR (poor water supply

and lack of resources) have forced the services responsible for aquaculture extension projects to buy from some fish farmers to resell to others.

In Côte d'Ivoire, the situation is more complex. The demand for tilapia fry from the FAO-Water and Forests Project is decreasing because fish farmers tend to produce their own fry and market them directly among themselves. In 1989, 60% of all tilapia fry used by the project's fish farmers were produced by the fish farmers themselves (directly or selling among themselves). The same year, the project facilities produced 800,000 fry of which only 300,000 were delivered to fish farmers—the difference being due mainly to the lack of resources for the transportation of the fry. The cost price of the *O. niloticus* fry (3-10 g) was 7 F CFA per fry and that of the male fingerlings (30 g) was 20 F CFA. The selling prices to fish farmers were 3 and 10 F CFA per unit, respectively, the difference therefore being subsidized by the project.

CATFISH

In addition to the facilities necessary for the different reproductive stages (hatchery with tanks, Zoug jars or incubation troughs and nursing ponds in good conditions), the captive breeding of these species requires high quality feed (for broodstock and juveniles), hormones to induce spawning and specific feeds for the larvae (generally, Artemia).

These requirements are generally met for as long as the project that initiated the hatchery continues.

The initiating project ensures the technical and scientific support necessary for its smooth operation, and the operating costs—which are generally high—usually result in a significant subsidy to fish farmers.

In Côte d'Ivoire, for example, an examination of the operation of the La Loka catfish hatchery reveals:

- difficulty in solving the technical problem of nursing fry from 25 mg to 5 g, resulting in a very high mortality. In 1988, there was a 2% survival rate between the larval stage ex-hatchery and the 5-g fingerlings delivered to the fish farmers (39,000 fingerlings delivered out of 1,500,000 larvae produced in the hatchery);
- the high subsidy level, with an estimated cost price for Clarias fry of 15-20 F CFA and a selling price to fish farmers of 10 F CFA;
- the problem of the technical training of station personnel, which became obvious when a hatchery supervisor left and was not replaced; and
- the difficulty of supplying expensive inputs (pituitary extracts and Artemia) after the project ends.

In the CAR, the ending of the various development projects resulted in the closure of the La Landjia Clarias hatchery, which, by 1990, was no longer producing Clarias fry for reasons detailed earlier.

In Cameroon, the Ku-Bomé nursery-hatchery (near Bamenda) produces tilapia fry, common carp fry and *C. gariepinus* fry. The hatchery, built in 1986, has never functioned (in 1991) due to a lack of funding to complete the incubation facilities. The reproduction of carp and Clarias therefore is done in ponds (*kakaban* method for the former; simulated flood for the latter). Lack of credit makes the acquisition of hormones to induce spawning difficult. In 1989, this station produced 20,000 common carp fry and 15,000 Clarias fry (marketed at 25 F CFA per fry with a mean body weight of 12-18 g). This volume of production is still much lower than the demand also because fish farmers, among whom

farming techniques for these two species have been promoted, are reluctant to return to tilapia culture. Furthermore, the delivery of fry to fish farmers poses unsolved problems of logistics (need for vehicle and fuel).

Conclusion

Has the spontaneous reproduction of tilapia in captivity been a true driving force for the development of tilapia culture in Africa?

In addition to the disadvantages of tilapia sexuality for the production of marketable fish (anarchic proliferation of fry), it also involves a number of constraints for the controlled and planned mass production of fry and fingerlings that relate to low individual fertility and spawning asynchrony:

- large pond surfaces;
- high number of broodstock;
- relatively complex management of broodstocks to maximize the quantity and quality (grading, survival rate, etc.) of fry production;
- good level of technical competence from fish farmers; and
- relatively complex planning of pond use (stocking of broodfish, spawning and nursing of fry) for a regular supply downstream of the fish farmers practicing grow-out in various rearing structures.

In short, the low level of technical competence apparently required for tilapia culture has long led to the belief that it could be open to all, including fry production. Nursing has long been done in the same ponds used for the production of marketable fish.

Analysis of production costs for *O. niloticus* fry indicates a relatively high cost price for a good quality production (homogeneous and planned) that should be taken into account in establishing selling prices to fish farmers. The failure of the nurseries was no doubt largely caused by this high cost price related to the lack of control of production costs and insufficient technical competence. Nevertheless, tilapias have the considerable advantage of being producible by fish farmers, in ponds, and as part of artisanal culture.

Catfish, whose production has long been limited by a scarcity of fry, could be the second group of fish to ensure the development of African aquaculture. Production costs of *C. gariepinus* and *H. longifilis* juveniles do not differ very much from those of tilapia. In addition, the level of technical competence required to reproduce these species of catfish is no greater than that needed for the efficient operation of a tilapia fry production station (planned, graded and monosex).

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Selective Broodfish Exchange of *Oreochromis niloticus* in Large Breeding Hapas Suspended in Earthen Ponds

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LITTLE, D.C., D.J. MACINTOSH and P. EDWARDS. 1996. Selective broodfish exchange of *Oreochromis niloticus* in large breeding hapas suspended in earthen ponds, p. 92-99. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Broodstock exchange and artificial incubation can be used to improve the productivity of intensive tilapia breeding systems. Eggs and yolksac larvae are removed from the mouth of incubating female fish within a five-day period. The exchange of females from spawning hapas, with fish conditioned for 10 (T1), 15 (T2) and 20 (T3) days in separate, single sex, high density groups was compared in a 100-day trial. The selective exchange of females from spawning hapas, i.e., spawned or unripe fish females exchanged for fish that were visually ripe and conditioned for a minimum of 10 days was also compared (T4). A further treatment compared the exchange of males as well as females (T5); both sexes were conditioned in single sex, high density groups for 10 days. Increasing the duration of female conditioning from 10 to 15 days did not improve seed output, but doubling the conditioning period (20 days) reduced it by 18%. Total seeds harvested from females selectively exchanged were almost 50% higher than if all females were exchanged at each harvest (90.1 and 64.6 seeds·m^{-2} of spawning hapa·day^{-1}, T4 and T1, respectively). Efficiency of brood female production was also improved by selectively exchanging females (314 and 233 seeds·kg^{-1}·day^{-1}, T4 and T1, respectively). Exchange of males also significantly increased seed output (80.5 seeds·m^{-2} spawning hapa·day^{-1}). Clutch size of seeds removed from selectively exchanged female and male treatments was significantly increased over the control treatment (T1).

Introduction

A major problem for the mass production of Nile tilapia (*Oreochromis niloticus*) fry is the asynchrony of tilapia breeding. In contrast to the synchronous shoaling and breeding of many other fish species, the complex reproductive biology and behavior of *Oreochromis* spp. constrain mass production of seeds. High and sustainable production of fry has been shown to be possible in tank and hapa systems if management practices are intensified. Broodfish condition can be maintained by reducing the period of natural spawning and incubation to only five days and then removing the seeds from the mouthbrooding female. Following seed harvest with a period of single sex, high density "conditioning" improves productivity further. A period of 10 days of such conditioning before release into a lower density spawning environment has been shown to improve synchrony of spawning and productivity of the system as a whole, but analysis revealed that the mean spawning intervals (MSI) varied between 15 and 20 days (Little 1989). A recovery period of more than 10 days may

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therefore further enhance seed production and synchrony of spawning. However, a longer period of conditioning would increase production costs since the required number of breeding fish would be increased substantially.

These earlier experiments showed that simple exchange of all female fish after each five-day period of spawning opportunity did not optimize the number of ripe fish in the hapa; despite 10 days conditioning, a proportion of females did not develop obvious ripeness and readiness to spawn. Also, after a five-day period of spawning opportunity, some females not only did not spawn but also remained in an unripe condition. Sorting of females to be stocked in the spawning hapa, in the same way that carp broodfish are sorted for induced breeding, could result in an increased proportion of breeding fish.

Readiness to spawn and fry production have been assumed to be only dependent on female condition (Uchida and King 1962; Peters 1983) but the demands made on males when spawned females are continually replaced with conditioned, ripe females are likely to be greater. Exchange of males was found to improve *O. niloticus* seed output more than female exchange alone in a hapa system with a 21-day interharvest period (Lovshin and Ibrahim 1989) but the consequences for male exchange in a more intensive system in which spawning activity is more concentrated are unknown. Fry producers have normally allowed several days for territory establishment and courtship by males (Rothbard et al. 1983) and the advantages of using conditioned males may therefore be outweighed by lack of time to court and spawn with receptive females.

Materials and Methods

Pure *O. niloticus* (Chitralada strain) were obtained from an experiment on broodfish in which fry of the same origin (individual size: 1.5-3 g) were raised over a period of five months to maturity in fertilized earthen ponds using supplementary feeding. Broodstock were then sexed and maintained in spawning and conditioning hapas suspended to a depth of 60 cm in the same earthen pond (area 1,740 m^2) over an experimental period of 106 days through the hot dry season to the beginning of the rains (March-June 1987). Ambient daily temperature ranged from 26 to 35°C. Broodfish from different treatments were released in single spawning net hapas (8.0x5.0x0.9 m) at a rate of 2 fish·m^{-2} and a sex ratio of 1:1. Each treatment had a minimum of three female replicate groups (Table 1) which were exchanged successively in the spawning hapa throughout the experimental period; a total of 20 harvests were made at five-day intervals. After each five-day period, the bottom strings of the hapas were untied, the broodfish were concentrated at one end of the hapa using a bamboo pole slid under the hapa and every broodfish removed one at a time using a double handnet. The mouth of each broodfish was checked and any eggs and yolk-sac fry were removed by washing the seeds from the females' mouth; seeds passed through the coarse mesh and concentrated in the fine mesh handnet below. Individual seed clutches were held in plastic bowls to allow staging before preservation in 4% formaldehyde (Peters 1983) in sealed glass or plastic bottles. Same stage seeds from females in the same treatment were combined. The numbers and average weight of seeds were estimated by counting and weighing three subsamples of 100 seeds and then bulk weighing the total batch on a top-pan balance (Mettler Pe 3600) to two decimal places. Surface moisture was removed before weighing by holding the eggs in a fine-mesh net on dry absorbent tissue. Seeds were staged at harvest using an arbitrary scale of development that could be determined at the pond side: (1) uneyed

Table 1. Experimental design showing treatments and numbers of broodfish (*Oreochromis niloticus*).

	Duration of conditioning prior to exchange (days)		Sets of broodfish (x 40 individuals)	
Treatment	Females	Males	Females	Males
T1	10	-	3	1
T2	15	-	4	1
T3	20	-	5	1
T4	A minimum of 10 days and then used according to ripeness	-	3	1
T5	10	10	3	3

eggs, (2) eyed eggs, (3) prehatched and (4) hatched, yolksac larvae.

Harvested broodfish were placed in floating net hapas before being lifted onto the pond side for estimation of weight change.

Female and male broodfish were conditioned separately, according to treatment, in small nylon nets (3.0x1.8x0.9 m) at a density of 7.5 fish·m^{-2} (Table 1). All female broodfish were removed and replaced with conditioned females after five days spawning opportunity in Treatments (T) 1, 2, 3 and 5. In Treatment 4 (T4), two categories of females, (a) spawned females and (b) unspawned but unripe females were removed at harvest and replaced with an equal number of females selected for ripeness from fish in the conditioning hapa. The main criterion for selection of ripe females was a soft, rounded abdomen. Selection of females was therefore made to maximize the number of such females stocked in the spawning hapa at the beginning of each spawning period.

In Treatment 5 (T5), males were also removed and replaced every five days with fish raised in a conditioning hapa for 10 days.

All hapas were replaced every 10 days and cleaned thoroughly with brush and hose before reuse.

Early morning dissolved oxygen (Winkler titration) and chlorophyll *a*, total ammonia and pH were measured in water samples removed at 0900 hours.

Fish in spawning and conditioning hapas were fed a floating catfish pellet (Charoen Pokphand, Bangkok; crude protein 30%) to satiation three times daily.

Observations of mean daily seed output, number of seeds·kg female·day^{-1} and number of seeds·kg fish·day^{-1} were statistically compared using a paired *t*-test.

Mean percentage of total seeds harvested as uneyed eggs were compared using the Kruskal-Wallis one-way analysis of variance (ANOVA) test.

ANOVA was used to determine the relative effects of stage and treatment on seed clutch characteristics. Spearman rank correlation was used to describe the relationship between stage and clutch size of harvested seeds.

Results

Mean daily seed output was significantly increased ($P<0.05$) from hapas in which females were selectively managed (T4) or in which all males were exchanged compared

to treatment (T1) where only females were exchanged after a five-day spawning opportunity (Fig. 1).

A conditioning period of 10-15 days gave similar production in terms of total seeds·m^{-2}·day^{-1} but an increase to 20 days reduced seed output by around 18% (53 seeds·m^{-2}·day^{-1} compared to 65 seeds·m^{-2}·day^{-1}) (Fig. 1). Duration of female conditioning more obviously affected the number of seeds harvested relative to the total weight of females used. Using fewer total broodfish (T1), the output of seeds almost doubled compared to that when more females were used to enable a period of 20 days conditioning (T3).

Productivity of females, and broodfish total, declined with duration of conditioning (Fig. 1). The use of selective female exchange and a single group of males (T4) gave the best efficiency of broodfish use overall (T5, 120 seeds·kg broodfish total·day^{-1}). The variability of seed output with time was high, particularly for selective exchange of females (T4, Fig. 1).

There were no significant differences in synchrony of spawning among treatments (Table 2). Late spawning occurred within a range of 23-35% of total spawns (Stage 1) and early spawning, as indicated by the harvest of well-developed seeds (Stages 3 and 4), between 40 and 60%.

Individual mean seed weight was related to stage but not treatment, hatched yolksac larvae were heavier than unhatched eggs.

Variation in clutch size was explained by both treatment and harvested seed stage and there was significant interaction between the two sources of variation. Mean clutch size of seeds harvested from selectively exchanged females (872; T4) and females spawned with exchanged males (844; T5) was larger than from females subjected to a simple exchange strategy (673; T1) and this effect was pronounced when yolksac fry were harvested (early spawned seeds T1, 249) (Table 3). Relative clutch size (number of seeds·g^{-1} female spawned) did not vary between treatments. Mean clutch weight and size were correlated with the stage at which seeds were harvested ($P<0.001$, $r^2 = 0.4$). Late spawned clutches of seeds tended to be both heavier and larger than early spawned eggs/hatched fry. Clutch size after hatching (Stage 4) was nearly 20% less than seed harvest as prehatched eggs.

A longer conditioning time inevitably reduced the total time spent by individual female fish in the spawning net from a total of 35 days (T1) to only 21 days (T3) during the experiment. Although mean spawning intervals (MSI) increased with female conditioning period (Table 4), there was no difference in spawning intensity or the mean number of spawns recorded per day individual females spent in the spawning hapa. Females spawned more frequently after male exchange (T5) or when females were selectively exchanged (T4) compared to simple exchange of all females (T1) ($P<0.05$).

Comparison with the productivity of other systems can be made after allowing for the losses during artificial incubation. Seed production expressed as first feeding or yolksac absorbed fry followed the same trends.

Discussion

The optimization of broodfish use is particularly important for species that produce relatively few seeds from each spawning. The use of strategies to optimize individual output needs to be balanced with the extra cost of handling larger numbers of fish in more complicated ways.

The type of broodfish exchange used to increase spawning synchrony and productivity in intensive systems (those in which broodfish can be caught and handled easily) has consequences for ease of management and cost of fry production. An optimum strategy would maximize breeding activity and enhance readiness to spawn, while reducing the number and hence the cost of broodstock.

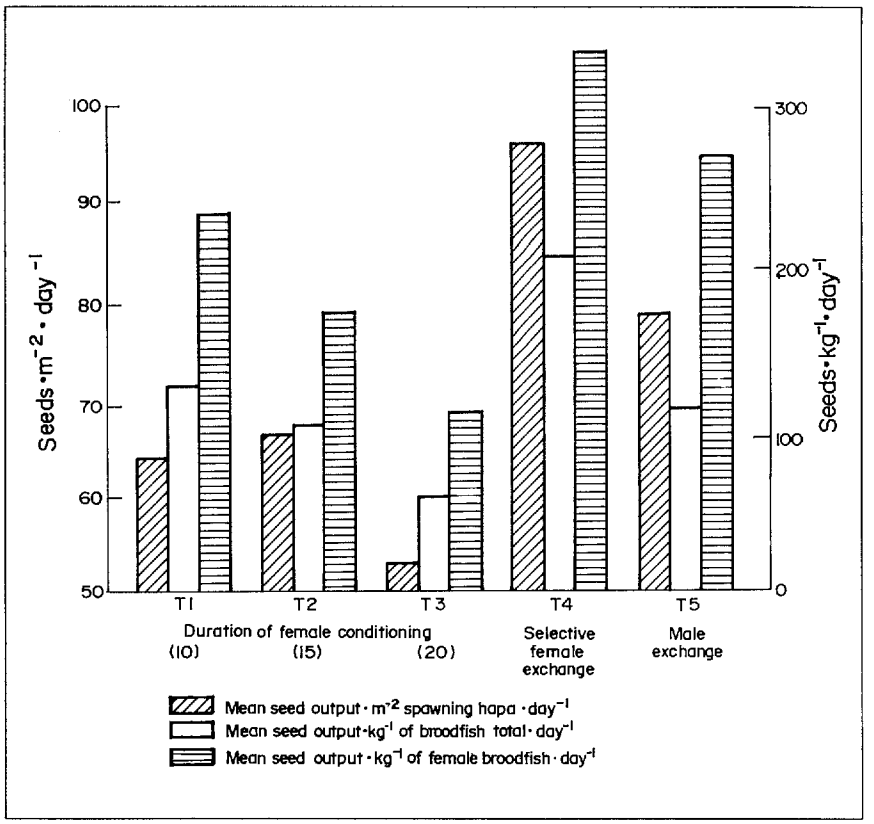

Fig. 1 Mean values for performance of Nile tilapia (*Oreochromis niloticus*) broodfish spawned in large nylon hapas after different periods of female conditioning (10 days, T1; 15 days, T2; and 20 days, T3), selective female conditioning (T4) and male conditioning (males and females conditioned for 10 days, T5).

Table 2. Mean percentages of total seeds harvested as uneyed eggs (Stage 1) from spawning hapas (area=40 m^2, stocked with 80 broodfish per unit [female:male ratio=1]; time=106 days) managed under different conditioning and exchange regimes.

Treatment	Mean percentages of total harvested stage 1 seeds (n)
T1	32.8 (20)
T2	23.2 (20)
T3	34.4 (20)
T4	31.2 (19)
T5	31.8 (19)
Chi-square	5.1[a]

[a]Chi-square value of the Kruskal-Wallis one-way ANOVA; P>0.05.

Table 3. Clutch size of seeds[a] harvested from mouthbrooding females subjected to different broodstock management strategies.

Treatment	Procedure	Mean ± SE[b]
T1	10 days conditioning	673 ± 58.7c
T2	15 days conditioning	704 ± 38.9c
T3	20 days conditioning	721 ± 34.4c
T4	Selective exchange of spawned females	872 ± 44.6d
T5	Male exchange only	844 ± 39.8d

[a]Mean weight of all seeds in a single clutch wet weight. Means with the same letters are not significantly different (P>0.05).
[b]Standard error.

Table 4. Mean spawning intervals (MSI) and percentages of females spawning during a five-day opportunity.

Treatment	Procedure	MSI (± SE)	Mean % spawning
T1	10 days conditioning	36.7 ± 1.7	41.0
T2	15 days conditioning	49.2 ± 4.6	43.0
T3	20 days conditioning	67.0 ± 5.1	38.5
T4	Selective exchange of spawned females	34.4 ± 5.6	48.0
T5	Male exchange only	30.4 ± 2.5	51.0

This study indicates that individual broodfish productivity is not improved if females are conditioned for longer than 10 days. Actual seed output declined with duration of female conditioning, and when compared in terms of seeds produced per weight of total broodfish, this trend was even clearer. Selective exchange of females optimized total seed output and individual clutch size. The improved performance of females managed in this way could have been a result of the selected fish spawning more completely and/or the eggs produced being of higher quality and thus surviving better.

Females conditioned for periods longer than 15 days in contrast may have produced a larger proportion of overripe, poorer quality eggs. Peters (1983) observed that reabsorption of eggs in the ovary began within as little as one week of reaching a critical gonadosomatic index (GSI) for *Oreochromis* spp. but that this did not prevent spawning. However, the eggs from "overripe" fish in which the maximum GSI had been attained for more than a critical period, gave rise to poor embryo survival, which would explain the smaller individual clutch size and total seed output at harvest.

The high variability in seed output in the selective exchange of females (T4) suggested that the maintenance of ripe fish was not always optimized. This was certainly

influenced by the experimental design since, if a high proportion of females spawned, a large number had to be removed from the spawning hapa and exchanged with fish from the finite number maintained in the conditioning net.

The ability of *Oreochromis* spp. males to spawn several times within the same day (Lowe-McConnell 1959; Peters 1971; Polder 1971) is countered by a declining capacity to fertilize successive clutches of eggs (Rana 1986). The improved seed production, larger clutch size and reduced MSI observed in the male exchange treatment compared to female exchange only may have resulted from a higher level of fertilization in spawned clutches of eggs and/or increased vigor of courtship and spawning. The importance of male "condition" has been recognized in common commercial practice and reflected in the sex ratios of broodfish stocked (Mires 1982; Hughes and Behrends 1983). However, sex ratio may be a poor indicator of actual availability of spawning males since hierarchical effects may prevent subordinate males from taking part in spawning activities in intensive systems (Turner 1986; Little 1989). Moreover, regular spawning may be constrained in intensive systems by a tendency for females to prefer unspawned males with ripe gonads (Silverman 1978a, 1978b). Nakatsuru and Kramer (1982) reported that lemon tetra (*Hyphessobrycon pulchripinnis*) females tended to choose males that had not recently spawned. The results of this study support those of Lovshin and Ibrahim (1989) who reported improved seed output after male exchange.

Their results suggest that male exchange is more important than female exchange but their harvest, and therefore female conditioning interval, was prolonged (21 days) which may have affected female productivity (see above). The fertilization capability of a single male Nile tilapia increased from 22 to 100% after a rest period of one week (Rana 1986) and it may be expected that the conditioning period of males will be reduced to five days, and that only two groups of broodfish will be used, with consequent savings.

The reduction in individual clutch size with harvested seed stage or duration of natural incubation can be interpreted with reference to Rana (1990). Removal of unfertilized eggs from the buccal cavity, contacts with pharyngeal teeth after hatching and dislodgement of the yolksac during the churning movements of natural incubation were the most common causes of loss from females held in tanks and aquaria. Marshall (1979) found no relationship between the number of eggs in the mouth and the egg stage at harvest of *O. macrochir* by seine netting in a natural lake, but he rejected females that appeared to already have lost eggs or fry. Other authors (Aronson 1949; Lowe-McConnell 1959; Riedel 1965) have associated the decline in fry numbers during mouthbrooding to the limited size of the buccal cavity but Rana (1986) demonstrated that this was not a limitation.

There were no significant differences in spawning synchrony between treatments, as determined from differences in seed stages, but more than 40% of all females spawned within five days if conditioned for 10-15 days prior to stocking. When females were selectively exchanged or males were also exchanged, frequency of spawning improved to around 50%.

Exchange and conditioning of both female and male broodfish should be carried out selectively and frequently to optimize seed output and the productivity of individual broodfish. Individual selection of female broodfish for ripeness however requires extra time and the maintenance of twice as many males would increase broodstock costs. Early and frequent removal of seeds also necessitates their artificial incubation before a strict comparison with methods harvesting first feeding or advanced fry can be made but the current study obtained seed yields almost twice the highest level previously reported (Little et al. 1993).

Acknowledgements

This study was conducted whilst the first author was engaged as an EEC Research Associate. Drs. P. Edwards and D.J. Macintosh were seconded to AIT by the Overseas Development Administration, UK. The research was funded by contract TSD-A-090-UK(H) awarded by the Commission of the European Communities under its "Science and Technology for Development" Programme. The research was carried out in its entirety on AIT campus and the assistance of the AIT staff is gratefully acknowledged.

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The Effect of Sex Ratio at Stocking on Growth and Recruitment in Nile Tilapia (*Oreochromis niloticus*) Ponds[a]

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Abstract

Nile tilapia (*Oreochromis niloticus*) juveniles were manually sexed and 50, 80, 95, 98 and 100% male populations (two replicates) were stocked at a density of 1 fish·m^{-2} in 200-m^2 earthen ponds. Growth of stocked fish and recruitment were assessed from biweekly sampling. Ponds were completely harvested after 24 weeks. Recruitment was observed in all ponds with recruits representing 4.7 to 31.4% of total biomass at harvest. Errors from manual sexing resulted in small numbers of females being stocked and subsequently some recruits occurring in the "all-male" ponds. Analysis of variance (ANOVA) showed a significant (P=0.01) effect of the proportion of males on biomass of recruits and suggested an effect on mean weight gain of the stocked fish. Multiple regression analysis showed a significant (P=0.001) positive effect of proportion of males on the growth of the stocked fish and a negative (P=0.01) effect on biomass and number of recruits. Growth and recruitment patterns were similar in the ponds stocked with 95, 98 and 100% males. These data suggest that, although increases in growth and decreases in recruitment were observed when ponds were stocked with high proportions of males, recruitment levels can still be unacceptably high in these ponds. Efforts should be made to eliminate *all* females from ponds so as to negate fully the deleterious effects of recruitment in tilapia growout ponds.

Introduction

The farming of monosex all-male tilapia has long been proposed as the solution to the problem of uncontrolled reproduction and overpopulation in tilapia culture ponds. Existing methodologies (manual sexing, hybridization and androgen sex reversal) generally fail to give 100% male progenies consistently. In research to develop a technology for generation of all-male *Oreochromis niloticus* by genetic manipulation of sex determination to produce novel YY males (Scott et al. 1989; Mair et al. 1991), we noted that several YY male sires produced small proportions of female offspring. If the aberrant occurrence of this small proportion of females is under genetic control, contrary to the hypotheses of

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monofactorial sex determination in this species, it may be possible to select against this over several generations of production of YY males. However, such a selection program would require considerable investment. Thus, the question "how desirable is it to produce reliably 100%-male populations?" needs to be addressed.

Several authors have suggested that if >95% male progeny can be achieved, problems related to reproduction and overpopulation will be negligible (Mires 1977; Macintosh et al. 1988). However, there is little data to support this. Studies on the effect of small proportions of females (as low as 2.5%) suggested that these can have a very significant effect on the growth of stocked fish and the yields of culture ponds with recruits forming up to 70% of the harvest. In the case of sex reversed fish, harvests including only a single female (0.35%) still resulted in yields containing up to 44% recruits (Anderson and Smitherman 1978).

In a study on the effect of the introduction of small proportions of female *O. niloticus* to ponds stocked with all-male hybrids (*O. niloticus* x *U. urolepis hornorum*), Lovshin et al. (1990) demonstrated that all-male fish grew 121% and 69% larger than those in populations containing 2.5% and 5.0% females, respectively. Total yield, including recruits, was highest in the 5.0% female populations.

This study was designed to determine whether there exists a minimum acceptable proportion of females in pond cultures of *O. niloticus* by determining the effects of known proportions of females on growth, yield and recruitment, concentrating on populations containing very high proportions of males.

Materials and Methods

First feeding fry were obtained from mass spawning of a number of locally available Philippine strains of *O. niloticus*, including a proportion of red tilapia (*Oreochromis* sp.). Fry were divided into four batches and stocked in 5-m^2 concrete tanks at equal densities. Three of the four batches of fry were given feed containing 17α-methyltestosterone (40 $mg \cdot kg^{-1}$ of feed) for 28 days to generate a surplus of males.

Following treatment, all fish were pooled and grown for 114 days in fine mesh hapas up to a mean weight of 20-24 g, at which size they could be sexed by examination of the genital papilla. Fish were sexed and divided into five treatment groups: 50, 80, 95, 98 and 100% males, with each treatment duplicated. Fish were then stocked at 1 $fish \cdot m^{-2}$ in 200-m^2 earthen ponds in a randomized complete block design.

Fish were grown for 126 days with biweekly sampling of approximately 25% of the population. At sampling, the mean weight of stocked fish (MWT), and total biomass (FBIOM) and number (FNUMB) of sampled recruits (fingerlings >5g, caught by seine net) were ascertained. Ponds were fertilized biweekly at a rate of 1,000 $kg \cdot ha^{-1}$ of chicken manure and 50 $kg \cdot ha^{-1}$ of inorganic fertilizer (N-P-K, 16-20-0). No supplementary feeding was applied. Water quality parameters, temperature, pH, and dissolved oxygen were monitored biweekly at 0700 and 1600 hours.

At harvest, individual mean weights were taken for stocked fish and recruits (which could be easily distinguished from the larger stocked fish by the significant size differential) were graded, counted and weighed. Mean weight (MWT) and

biomass of stocked fish (STBIOM), and recruits (RECBIOM) and total biomass (TOTBIOM) were analyzed by analysis of variance (ANOVA).

Multiple regression analysis was used to analyze the effects of a range of predictor variables on three dependent variables: growth rate of the stocked fish (GRINC, in $g \cdot day^{-1}$), FBIOM and FNUMB. The predictor variables were: initial weight of the fish (INWT, in g); percentage of males at harvest (PMALE, in %); replicate (BLK, dummy variable); age of stocked fish since first feeding (AGE, in days); proportion of red tilapia in the population (PRED, in %), survival during pond growout (SURV, in %); dissolved oxygen concentration (DO, in $mg \cdot ml^{-1}$); water temperature (TEMP, in °C); and pH (PH). All variables were measured for each 14-day sampling period, resulting in a dataset of 90 records (N=90: 5 treatments*2 replicates *9 sampling periods) according to a method first developed by Pauly and Hopkins (1983).

For each dependent variable and for the log-transformations of FBIOM and FNUMB, a regression model was constructed by combining predictor variables that together explained as much of the variation as possible (highest possible R^2). For each model, violations of the assumptions of multiple regression (multicolinearity, zero mean error, constant error variance and autocorrelation of the residuals) were checked for and avoided (Costa-Pierce et al. 1993). Variables that expressed proportions (PRED, PMALE and SURV) were transformed to the arcsine of their square root (TPRED, TPMALE and TSURV). The relative importance of predictor variables in a model was assessed using standardized regression coefficients (Costa-Pierce et al. 1993).

Results and Discussion

Errors in manual sexing at stocking resulted in small deviations from expected sex ratios at harvest. This is important in the case of the ponds stocked with "all-male" fingerlings. One female was found in one "all-male" duplicate pond and both contained recruits. Ponds were supplied with pumped well water and the drains screened, so contamination from feral fish can be discounted. It was assumed that "all-male" populations contained 0.5-1.0% females, making them almost indistinct from the 98% male treatment.

Highest mean weight gains were achieved in the three treatments that were 95% male or higher. Also, the biomass of recruits in these treatments was lower than in the 50 and 80% male treatments (Table 1). The treatment effect on mean weight gain, biomass of stocked fish and total biomass was not significant, but there was a significant block effect (ANOVA, see Table 2). For biomass of recruits, the block effect was not significant, but the treatment effect was (Table 2). However, linear contrasts showed significant differences in mean weight gain and biomass of stocked fish between the 50 and 80% treatments, on one hand, and the 95, 98 and 100% male treatments, on the other (P=0.05; see also Fig. 1). With only two replicates, the power of the ANOVA was low (Van Dam 1990) and the probability levels of the nonsignificant variables in the ANOVA for MWT and RECBIOM (both around 0.08) indicate that with more replicates, these effects might have been significant. The block effect was probably due to differences in pond maturity: ponds in Block 1 were in their second production cycle, whereas those in Block 2 were in their first cycle.

Table 1. Weight gain and recruitment at harvest in Nile tilapia (*Oreochromis niloticus*) ponds stocked at different sex ratios. Where no females were stocked, the observed recruitment was due to sexing errors (see text).

Sex ratio stocked (♂ : ♀)	Sex ratio harvested (♂ : ♀)	Initial mean weight (g)	Harvest mean weight (g)	Weight gain (g)	Survival %	Biomass of recruits (g)	Recruits as % of harvested biomass
100:100	97:87	21.03	36.49	15.46	92.0	2,847	29.55
100:100	94:91	19.61	44.59	24.98	92.5	3,825	31.45
160:40	164:25	21.47	42.60	21.13	94.5	1,517	15.72
160:40	147:26	20.10	51.95	31.85	86.5	2,157	19.18
190:10	181:9	21.81	46.13	24.32	95.0	973	9.85
190:10	170:9	21.51	57.93	36.42	89.5	654	5.87
196:4	181:4	21.68	40.69	19.01	92.0	538	6.52
196:4	174:6	20.71	59.96	39.25	90.0	1,097	9.14
200:0	189:0	21.38	50.40	29.02	94.5	472	4.68
200:0	174:1	21.25	58.91	37.66	87.0	1,063	9.25

Table 2. Mean squares of the analysis of variance (ANOVA) on mean weight gain (MWT, in g), biomass of stocked fish at harvest (STBIOM), biomass of recruits at harvest (RECBIOM, in g) and total fish biomass at harvest (TOTBIOM) for the whole 126-day experiment. Significance levels are indicated (*=5%, **=1% and ***=0.1%).

Effect	DF	MWT	RECBIOM	STBIOM	TOTBIOM
Replicate	1	374.789**	604668.100[b]	6571193.756*	11162500*
Treatment	4	49.089[a]	2482037.650**	1816682.803	218532.696
Residual	4	10.836	116443.850	514865.264	609053.481

[a]Probability level was 0.086.
[b]Probability level was 0.085.

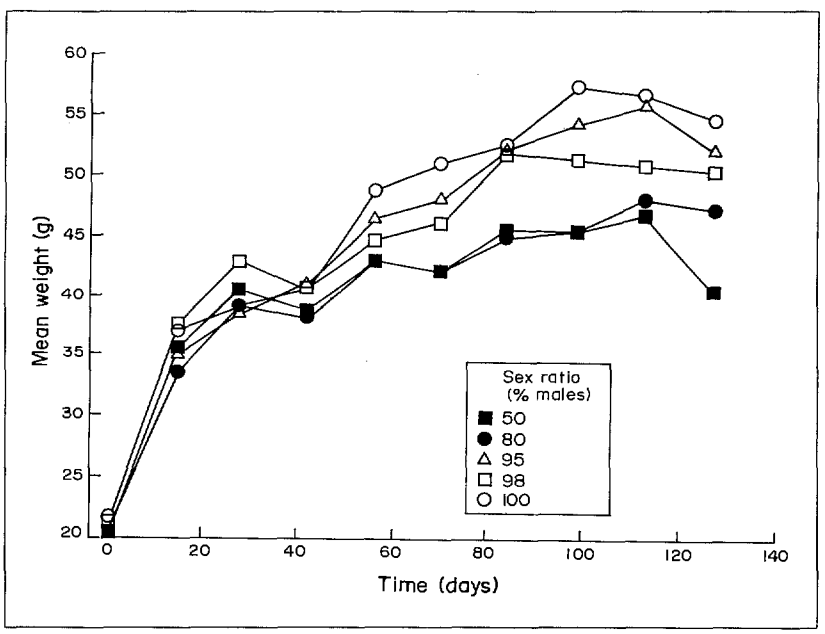

Fig. 1. Growth of *Oreochromis niloticus* populations stocked in ponds at different sex ratios (mean of duplicates).

There were no clear trends in recruitment during the whole period and the results were highly variable (Fig. 2), possibly due to the inconsistency of sampling procedure. However, recruitment commenced later and at a lower rate in the high percentage male treatments. Also, recruitment declined over time in all treatments, possibly due to a reduction in spawning frequency of the stocked fish as pond biomass increased, or to cannibalism of younger fry by older juvenile recruits.

Total biomass was not significantly different between any treatments suggesting that all the ponds may have reached their maximum carrying capacity (in the absence of supplementary feed) and that differences among treatments were manifested in the difference in relative proportions of recruits and stocked fish (Table 1, Fig. 3).

Multiple regression analysis resulted in significant models for GRINC (R^2=0.669), FBIOM (R^2=0.553) and FNUMB (R^2=0.432). The initial weights of the fish and replicate number were the most important explanatory variables for GRINC (Table 3 and Fig. 4). The proportion of males was positively correlated with GRINC.

Age of the stocked fish was most important in explaining the variability in FBIOM and FNUMB (Fig. 4). The decline in recruitment (Fig. 2) over time is not expressed in this analysis because the effect of age is described linearly. We did not attempt an analysis with nonlinear effects of age.

DO was positively correlated with both FNUMB and FBIOM, indicating the importance of oxygen in fingerling survival and growth. Temperature affected only the recruit biomass, suggesting that it was less important for survival. Proportion of males was significantly and negatively correlated with recruit numbers and biomass.

There was no significant positive or negative serial correlation of the residuals (Durbin-Watson test, P=0.001). Using log-transformed values of FBIOM and FNUMB resulted in a much better fit of the models (Table 4; LOGFBIO: R^2=0.773; and LOGFNUM: R^2=0.702). These models, however, did show positive serial correlation of the residuals in the Durbin-Watson test.

These results showed that increasing the male sex ratio at stocking did significantly affect marketable yield by increasing weight of stocked fish and decreasing recruitment. However, the effect was not as great as that observed in other similar studies (Macintosh et al. 1988; Lovshin et al. 1990). This may be due to the shorter growout period used in the present study although it is notable that all other studies incorporated supplementary feeding which may amplify the effects of direct competition among stocked fish and recruits.

There was no evidence to indicate that predominantly male (>95%) populations have more homogeneous individual weight distributions. Indeed, there were few significant differences in the population characteristics in ponds stocked with 95, 98 and "100%" male populations. Evidence from other studies (Lovshin et al. 1990; Mair, unpubl. data) suggests that significant additional increases in yield can be gained from the absence of recruitment in completely all-male populations and thus the elimination of all females should be an important objective in sex control programs.

Acknowledgements

The authors thank Lolita Santiago for her technical assistance. This paper is a contribution of Freshwater Aquaculture Center/Central Luzon State University-University College of Swansea Research

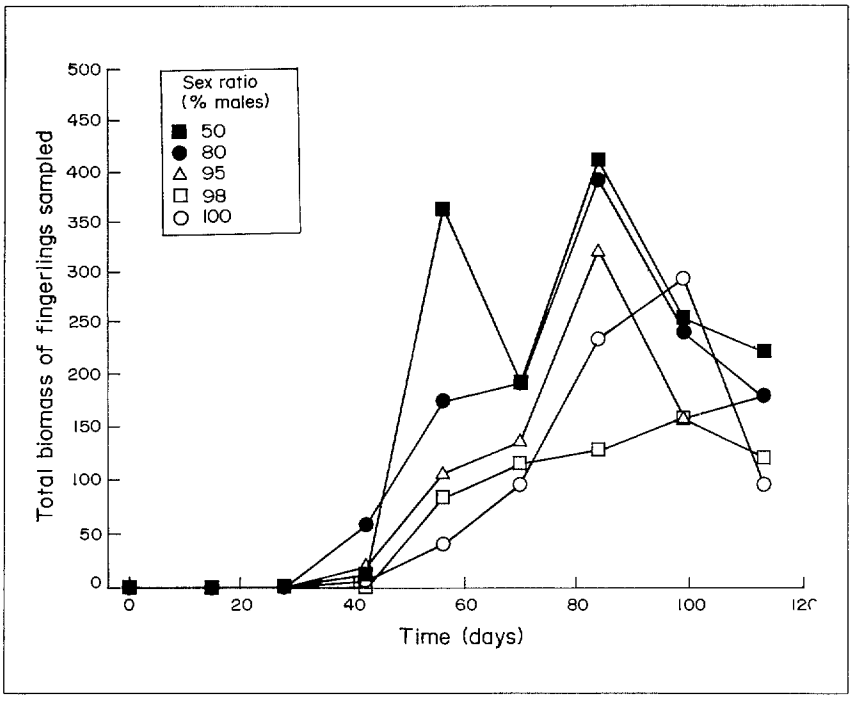

Fig. 2. Recruitment during culture of *Oreochromis niloticus* populations stocked at different sex ratios (means of duplicates).

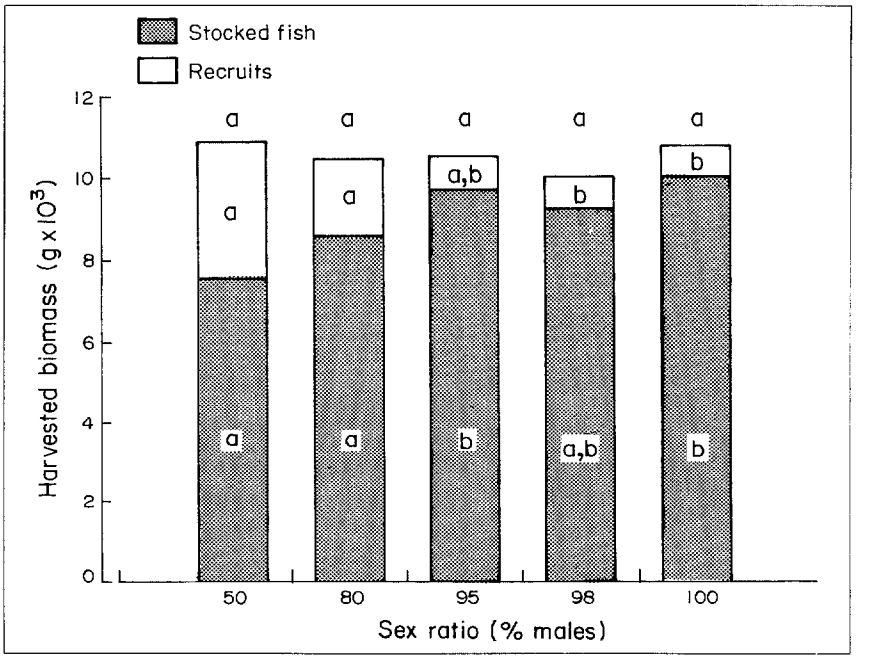

Fig. 3. Mean harvested yields of stocked fish and recruits in *Oreochromis niloticus* culture ponds stocked at different sex ratios. Columns with different letters have significantly different means (P=0.05).

Table 3. Multiple regression results for growth rate (GRINC, in g·day^{-1}), biomass of recruits (FBIOM, in g) and number of recruits (FNUMB) in every 14-day sampling period of the experiment with various predictor variables (N=90). Significance levels of variables are indicated (*=5%, **=1% and ***=0.1%). For explanation of predictor variable abbreviations, see text.

	GRINC		FBIOM		FNUMB	
	b	s.e.[a]	b	s.e.	b	s.e.
Predicted variable						
INWT	-0.031	0.002***				
PMALE	0.379	0.099***	-104.916	37.148**	-38.843	10.836**
BLK	0.488	0.057***				
TEMP	0.050	0.019*	13.691	7.194[b]		
DO			17.304	6.155**	4.794	1.779**
AGE			2.546	0.259***	0.541	0.076***
Constant	-0.653		-712.216		-48.111	
Adjusted R^2	0.669		0.553		0.432	
F-value	46.050		28.486		23.543	
Durbin-Watson	1.787		1.905		1.878	
Probability	<0.001		<0.001		<0.001	

[a]Standard error.
[b]Probability level was 0.060.

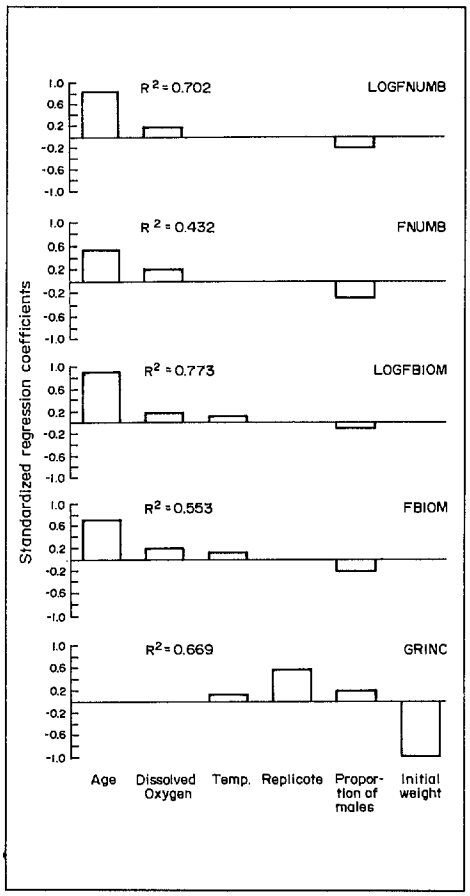

Fig. 4. Standardized regression coefficients of models for growth rate, recruit biomass and recruit numbers. For explanation, see text and Tables 3 and 4.

Table 4. Multiple regression results for log-transformed values of recruit biomass (LOGFBIO) and number of recruits (LOGFNUM) in every 14-day sampling period for the experiment with various predictor variables (N=90). Significance levels of variables are indicated (*=5%, **=1% and ***=0.1%). For explanation of predictor variable abbreviations, see text.

	LOGFBIO		LOGFNUM	
	b	SE[a]	b	SE
Predicted variable				
TPMALE	-1.078	0.515*	-1.319	0.420**
TEMP	0.226	0.100*		
DO	0.270	0.085**	0.228	0.069**
AGE	0.062	0.004***	0.041	0.003***
Constant	-14.688		-4.668	
Adjusted R^2	0.773		0.702	
F-value	76.623		70.919	
Durbin-Watson	1.280		1.300	
Probability	<0.001		<0.001	

[a]Standard error.

Project on Genetic Improvement of Tilapia supported by a grant from the Overseas Development Administration (R.4452) to J.A. Beardmore and D.O.F. Skibinski. A.A. van Dam is seconded to ICLARM by the Directorate General for International Cooperation, Ministry of Foreign Affairs, The Netherlands.

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Comparison of the Growth of *Oreochromis karongae* and *O. shiranus* in Fishponds in Malaŵi

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Abstract

The results are presented of two growth trials using an indigenous tilapia species *Oreochromis karongae* in ponds at Mzuzu, Malaŵi. The first compared the weight gain of *O. karongae* at two stocking densities in 150-m^2 ponds (1 and 2 fish·m^{-2}) with that of *O. shiranus*, the most commonly used species for aquaculture in Malaŵi. At both densities, *O. karongae* grew from 54 g to 104 g on average in 16 weeks, compared with *O. shiranus* which grew from 39 g to 55 g on average. *O. shiranus* produced much greater number of fry and fingerlings (over 8,000/pond compared with 200-300 for *O. karongae*). The second trial compared the growth of juvenile *O. karongae* in 300-m^2 ponds, using three different fertilizers (chicken manure, diammonium phosphate and grass compost) without feeding. Each treatment was triplicated. The fry grew from 1.3 g to 14-20 g in 12 weeks. There was no significant difference ($P>0.05$) in the final mean body weights of fish from the three fertilizers. The results of these trials are discussed relative to the appropriateness of *O. karongae* as candidate species for fish farming in Malaŵi.

Introduction

Malaŵi has a very rich fish fauna which is divided into two main ecological zones: the Lake Malaŵi catchment (including the upper Shire River) and the lower Shire, which shares the same fauna as the lower Zambezi. The Lake Malaŵi fauna is particularly rich, with perhaps 1,000 fish species, many of which are undescribed. The Central and Northern Regions Fish Farming Project operates wholly within the Lake Malaŵi catchment. One of its main aims is to investigate the suitability of indigenous fish species for fish farming. Two indigenous tilapias, *Oreochromis shiranus* and *Tilapia rendalli*, have been used for farming in Malaŵi since the 1950s, but are relatively slow growing and mature precociously in ponds (Vincke 1981).

Indigenous tasselled tilapias assigned to subgenus *Nyasalapia*: *Oreochromis lidole*, *O. saka*, *O. karongae* and *O. squamipinnis* (Trewavas 1983) are important species fished for food, locally referred to as *chambo*. Their aquaculture potential has not been studied in detail as they could not breed in shallow ponds (Msiska 1988). However, a few *chambo* stocked in a pond at Fisheries Station at Nkhata Bay bred in early 1990 at the same time work in southern Malaŵi was also showing that *chambo* could breed in ponds. These observations were encouraging enough for more research efforts to be directed at *chambo* as candidates for fish farming.

Stocks of each of the species were collected from Lake Malaŵi and allowed to spawn at Nkhata Bay and Mzuzu. Recent work by Turner at Monkey Bay

in southern Malaŵi has suggested that *O. saka* forms a subgroup of *O. karongae* and that there are only three species of subgenus *Nyasalapia*: *O. karongae*, *O. lidole* and *O. squamipinnis* (Turner and Robinson 1991). This fits with our observations and is the nomenclature used in this paper. We found that *O. karongae* was the easiest species to collect and breed in captivity.

The two trials described here were to determine how *O. karongae* grew in ponds. The first compared different stocking densities of *O. karongae* with *O. shiranus*. The second trial used smaller fish and different methods of pond fertilization, suitable for use by rural farmers.

Materials and Methods

Trial No. 1

Five 150-m^2 ponds were stocked with *O. karongae* fingerlings spawned from wild broodstock originating from Nkhata Bay and Monkey Bay, and with *O. shiranus* fingerlings spawned from broodstock at the station in Mzuzu.

The fingerlings were of the same age (16 weeks) with average body weights from 35 to 55 g. Each pond was fertilized every week with 200 g diammonium phosphate and fed daily with maize bran at 3% of total fish biomass. At the start of the trial, 30 fish from each pond were weighed and tagged. The tags were for fish identification during weighing, enabling the use of individual weights for statistical analysis rather than replication of treatments.

Two-thirds of the fish in each pond were bulk weighed in groups of 10 every 28 days to 1 g and the individual total and standard lengths of 20 fish were measured to 0.1 cm.

Water from each pond was also sampled at 0500 hours for measurement of dissolved oxygen, pH, total hardness, total alkalinity, chlorophyll *a*, ammonia, nitrite, nitrate and available phosphorus using methods described by Stirling (1985). The trial was terminated after 16 weeks when all the fish were counted and weighed.

Trial No. 2

Nine 300-m^2 ponds were each stocked with 900 *O. karongae* fingerlings of 1.3±0.5-1.3±0.8 g average weight. The following three treatments were each randomly assigned to triplicate ponds: dry chicken manure applied at 7 $kg \cdot pond^{-1} \cdot week^{-1}$; diammonium phosphate applied at 300 $g \cdot pond^{-1} \cdot week^{-1}$; and grass compost with an initial application of 1 m^3 of dry chopped grass (6.5% crude protein) into a compost crib in the corner of the pond, followed by weekly additions of 10 $kg \cdot pond^{-1}$.

No supplementary feed was given. Each pond was sampled every 28 days and the fish weighed and measured. Water samples were collected monthly and analyzed as in Trial 1. The trial was interrupted by a flood soon after sampling at the end of week 12. Data analysis was by Kruskal-Wallis nonparametric ANOVA using Statgraphics (Statistical Graphics Corporation, USA) software.

Results

Unfortunately, the fish lost their tags so statistical analysis among treatments was not possible. However, *O. karongae* apparently grew more than *O. shiranus* at both stocking densities ($P<0.05$ if all results for each species are grouped together) as shown in Table 1 for Trial 1. Overall production appeared to be highest with *O. karongae* stocked at 2 $fish \cdot m^{-2}$, and these fish appeared to grow just as well as the *O. karongae* stocked at 1 $fish \cdot m^{-2}$.

Table 1. Results of Trial 1. Comparison of the weight gains of *Oreochromis karongae* and *O. shiranus* stocked at 1 and 2 fish·m^{-2}. (*Ok=Oreochromis karongae*, *Os=Oreochromis shiranus*; NB=Nkhata Bay, MB=Monkey Bay, MZ=Mzuzu; MBW1±SD and 2=stocking and harvesting mean body weights, respectively; and SGR=specific growth rate. SD=standard deviation. Harvesting mean body weights having the same letter are not significantly different [P>0.05] according to the Kruskal-Wallis nonparametric ANOVA.)

Pond #	1	2	3	4	5
Species and source	*Ok* (NB)	*Ok* (NB)	*Ok* (MB)	*Os* (MZ)	*Os* (MZ)
Stocking density	1·m^{-2}	2·m^{-2}	1·m^{-2}	1·m^{-2}	2·m^{-2}
MBW1±SD (g)	55±6	52±7	53±9	42±10	35±12
MBW2±SD (g)	105±11b	106± 6b	102±9b	56±22a	54±31a
SGR (%·day^{-1})	0.57	0.64	0.58	0.26	0.39
Mortality (%)	7.3	8.7	14.7	18.0	43.3
Weight gain (kg)	6.35	13.44	5.11	0.59	-1.32
Weight of fingerlings (kg)	1.6	2.0	1.2	8.1	8.4
No. of fingerlings	228	274	309	8,100	8,367

Trial 2 was stopped by a flood and the data (Table 2) are from a sample. It was not possible to assess mortality or total production. The growth of *O. karongae* across the treatments was not significantly different (P>0.05).

In both trials, the pond water parameters remained within acceptable limits for tilapia growth. Water temperatures were within the range 21-26°C.

Discussion

These trials suggest differences in culture performance characteristics between *O. karongae* and *O. shiranus*. *O. shiranus* can breed at sizes as small as 8 g (Maluwa 1990), and once breeding commences, growth slows dramatically. *O. karongae* tend to grow for a longer period, and to a larger size, before breeding. At the start of Trial 1, *O. shiranus* were already of breeding size whereas *O. karongae*, despite being larger at stocking, did not start reproducing until attaining 90 g body weight. This difference would be important where large fish are needed at harvest; however, most markets in Malaŵi readily accept small fish. In such cases, it would not matter that *O. shiranus* is small at harvest. The yield, in terms of weight per unit area, would be the most important factor for farmers. Trial 1 also suggests that *O. karongae* can give higher overall production than *O. shiranus*.

Continuing research efforts are needed to establish the future of *O. karongae* in aquaculture. However, it does seem to have advantages over *O. shiranus* for culture in Malaŵi, and the greater one is that of being an indigenous species.

Acknowledgements

This work forms part of the research activities of the Central and Northern Regions Fish Farming Project 5-ACP-MAI-047, Mzuzu, funded by the European Economic Community through the Malaŵi Government, Ministry of Forestry and Natural Resources, Fisheries Department.

Table 2. Results of Trial 2. Growth of *Oreochromis karongae* fingerlings under three fertilization regimes, without any supplementary feeding. (MBW±SD; 1 and 2=stocking and harvesting mean body weights, respectively. SD=standard deviation. Final mean body weights having the same letter are not significantly different [P>0.05] according to the Kruskal-Wallis nonparametric ANOVA.)

Treatment	Chicken manure	Diammonium phosphate	Grass compost
MBW1±SD (g)	1.3±0.5	1.3±0.7	1.3±0.8
MBW2±SD (g)	20.0±4.3a	14.0±5.7a	14.6±3.8a
SGR (%·day^{-1})	3.3	2.8	2.9

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Growth, Survival and Sex Ratios of *Oreochromis urolepis hornorum*, *O. niloticus* and Their Hybrid Treated with 17α-Methyltestosterone

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Abstract

Two experiments were designed to differentiate and quantify the observed growth enhancement due to sex reversal and the growth enhancement due to anabolic effects among 17α-methyltestosterone (MT)-treated and control *Oreochromis urolepis hornorum*, *O. niloticus* and their hybrid (*O. niloticus* female x *O.u. hornorum* male) cultured in hapas. Mixed-sex, all-male and all-female populations of fish were utilized in the studies. All fish treated with 60 mg MT·kg^{-1} of diet showed a positive growth response (P=0.05) when compared to their respective controls at the conclusion of the sex reversal period (day 30). All-male hybrids showed a 10% increase in growth in response to MT treatment. Since no sex reversal occurred among the hybrids, the observed growth enhancement of these fish represents solely the anabolic effect of the MT at the conclusion of the sex reversal period.

At 180 days, control male *O. niloticus* average weight was similar to the MT-treated *O. niloticus*. Control and MT-treated hybrids also had similar average final weights. The *O.u. hornorum* treated fish had final weights significantly greater than the control *O.u. hornorum* males (P=0.05).

The growth of all-female tilapias was not significantly different from that of the all-female controls after 30 days culture, but treated females were significantly heavier (16.2%) than control females at the conclusion of the grow-out phase (180 days) (P=0.10). It was inferred from the data from MT-treated females that MT-treated genotypic males had attained mean weights 55.3% and 23.0% greater than nontreated male fish at 30 and 180 culture days, respectively.

Introduction

Control of reproduction is of fundamental importance for the successful culture of tilapia (Chimits 1957; Hickling 1967; Lovshin 1975). Various management strategies have been developed to avoid unwanted reproduction in grow-out ponds (Hickling 1960; Clemens and Inslee 1968; Guerrero 1982). The most effective and, presently, most widely used technique is the treatment of sexually undifferentiated fry with synthetic androgens to sex reverse genotypic females.

Many investigators have reported enhanced growth in fish treated with androgens compared with controls (Yamazaki 1976; Donaldson et al. 1979). Tilapia treated with androgens do not always show superior growth compared

to untreated fish (Anderson and Smitherman 1978; Hanson et al. 1983). Androgen-treated tilapia are phenotypic males, but the population consists of both male and female genotypes. The superior growth of tilapia males has a genetic basis (Fryer and Iles 1972; Anderson and Smitherman 1978) and it would be expected that a genotypic female, sex reversed to the male phenotype (genotypic female/phenotypic male), would not grow as fast as a normal male.

The objective of these experiments was to differentiate and quantify the growth enhancement due to sex reversal and due to anabolic effects among methyltestosterone (MT)-treated and control *Oreochromis urolepis hornorum*, *O. niloticus* and their hybrid (*O. niloticus* female x *O.u. hornorum* male).

Materials and Methods

Fish Stocks

The experiments presented were conducted at the aquaculture facilities of the Panamerican Agriculture School (PAS), Honduras. Broodfish were obtained from the National Association of Banana Producers, San Jose, Costa Rica, and from the Pond Dynamics/ Aquaculture CRSP, Comayagua, Honduras. Additional *O. niloticus* were selected from PAS stocks.

Fry Production

All fry utilized for these experiments were produced in outdoor concrete tanks. Tanks were periodically seined to remove embryos and sac-fry from the mouth of incubating females. Yolksac fry were transferred to plastic trays, containing continuously aerated water, and embryos were artificially incubated there.

Each experiment was begun using fry that had completed yolksac absorption, determined through microscopic examination (15x) to ensure that all fish were at the same development stage when stocked. Body length was not used as a criteria for stocking fry due to possible maternal effects among tilapia species (Siraj et al. 1983).

Feed Preparation

During the first 60 days of each experiment, fry and fingerlings were fed a diet containing 42% crude protein. The feed was finely ground to enable ingestion of all particles and given to the fish ad libitum four times a day. During the remaining months of each experiment, fish were fed a similar diet containing 28% crude protein and fed ad libitum three times daily.

Feed containing hormone (60 mg $MT \cdot kg^{-1}$ of feed) was prepared as described by Shelton et al. (1978). Use of hormone-treated feed was suspended on day 30 of each experiment.

Experimental Units

Fish were initially reared in small hapas (0.30x0.60x0.37 m) constructed of fiberglass window screening material (Tave and Tave 1984). Hapas were suspended in one concrete tank (3.0x6.0x0.9 m). During a 15-day period, fish were stocked in the hapas as they completed yolksac absorption.

In May 1988, 12 hapas were each stocked with 50 *O. niloticus*, *O.u. hornorum* or tilapia hybrid fry. The hormone and control treatments were assigned to hapas containing each type of fry, in a completely randomized design. There was a total of 36 hapas with

six replicates of each treatment. In the second treatment, begun in August 1989, 24 hapas were each stocked with 50 *O. niloticus*, 12 with mixed-sex fry and 12 with all-female fry.

At 30-day intervals, all fish from each hapa were anesthetized (200 ppm of MS-222), counted, weighed collectively and measured for total body length. On culture day 30, fish from all replicates of each treatment were pooled and reallocated at 20 fish·hapa^{-1} for another 30 days. The hapas were maintained in the same concrete tank. On culture day 60, fish were transferred to nylon hapas (1.2x2.4x1.2 m with a 0.6-cm mesh) suspended in a 0.06-ha earthen pond for the remainder of the experiment.

Statistical Analysis

The effects of the control and MT diets were compared among the different tilapia in a factorial arrangement. Analysis of variance (ANOVA) was conducted on the observed mean body weights, mean body lengths and survival percentage of fish. Differences among treatment means were determined by using Duncan's multiple range test. Sex ratios were tested for goodness of fit by chi-square analysis.

Results and Discussion

Experiment 1

Pooled MT-treated fish had greater (P=0.05) mean body weights than pooled controls at 30 days (Table 1). No difference was found when the mean final weights for pooled MT-treated and control fish were compared.

MT-treated fish weighed significantly more than their respective controls after 30 culture days (Table 2). There was no difference (P=0.16) between final mean weights of the *O. niloticus* treated and control fish. Mean final weights for the treated and control hybrids were also similar. Only the *O.u. hornorum* MT-treated fish maintained a significantly greater mean weight throughout the 180-day experiment in comparison to their controls. The differences in mean weights of fish paralleled those of the mean total lengths (Table 3).

Survival of *O.u. hornorum* was 69%, significantly lower than the 90% survival rate observed among *O. niloticus* and hybrids (P=0.10). Control groups of the two species were composed of 60% male fish, not significantly different from the 1:1 ratio expected for tilapia. Only a single female was found among the control hybrids. All MT-treated fish were phenotypic males.

These results indicate a growth enhancement effect of the MT, observable principally during the sex reversal period. During the remaining months of the experiment, differences between treated and control groups were gradually reduced except for the *O.u. hornorum* fish. Goudie (1984) found that tilapia rapidly eliminated ingested radio-labelled MT. Curtis et al. (n.d.) reported rapid biotransformation of MT to polar metabolites and their excretion from tilapia within a few days.

The prolonged effect of the MT treatment on genotypically mixed-sex fish, which results in growth enhancement, is derived from the combination of the sex reversal of genotypic females to the male phenotype and of any anabolic growth effect of the MT. It was not possible to observe differences in the growth rates between genotypic male and female fish of the two species utilized in the present experiment.

At the conclusion of the sex reversal period, the MT-treated hybrids had a mean weight 10% greater than the hybrid controls (P=0.05). As the hybrid

Table 1. Mean weights (g) for combined MT-treated and control *Oreochromis urolepis hornorum*, *O. niloticus* and their hybrid (*O. niloticus* x *O.u. hornorum*) grown in hapas. Mean weights for each culture period followed by the same letter are not significantly different (P=0.05)

Treatment	Culture days					
	30	60	90	120	150	180
Control fish	0.80b	5.54b	33.8b	66.1b	95.0a	119.4a
MT-treated fish	0.90a	5.83a	37.7a	72.5a	102.2a	122.6a

Table 2. Mean weights (g) of *Oreochromis urolepis hornorum*, *O. niloticus* and tilapia hybrids (*O. niloticus* x *O.u. hornorum*) treated with MT and control fish grown in hapas. Mean weights for each culture period followed by the same letter are not significantly different (P=0.05).

Fish type/ treatment	Culture days					
	30	60	90	120	150	180
O. niloticus						
control	0.95b	6.84a	36.0b	77.3a	108.2ab	138.8a
MT-treated	1.04a	6.93a	41.5a	77.1a	100.8b	144.4a
Hybrids						
control	0.80d	6.52a	40.7a	69.8ab	100.8b	118.3b
MT-treated	0.88c	6.28a	40.6a	73.8ab	102.6b	117.6b
O.u. hornorum						
control	0.65e	3.26c	24.9d	51.3c	66.4d	93.8d
MT-treated	0.79d	4.30b	30.9c	66.5b	82.6c	110.4c

Table 3. Mean weights and mean lengths at completion of the 30-day, sex reversal period for *Oreochromis urolepis hornorum*, *O. niloticus* and *O. niloticus* and tilapia hybrids (*O. niloticus* x *O.u. hornorum*) treated with MT and control fish grown in hapas. Means followed by the same letter are not significantly different (P=0.05).

Fish type/ treatment	Mean weight (g)	Mean length (TL; mm)	
O. niloticus	0.99a	39.0a	
controls			38.1ab
MT-treated			39.9a
Tilapia hybrids	0.84b	36.8b	
controls			36.1b
MT-treated			37.4b
O.u. hornorum	0.72c	35.1c	
controls			33.8c
MT-treated			36.4b

fish were all males, no sex reversal occurred among the treated fish and the growth enhancement observed can be attributed solely to the anabolic effect of the MT.

Experiment 2

Combined MT-treated fish had significantly greater average weights and body lengths compared to pooled controls

at 30 and 180 culture days (Table 4). Similar to the results of experiment 1, the MT-treated *O. niloticus* had greater average weights than their controls at 30 culture days (Table 4). The weights of the treated all-female fish were not significantly different from controls. On culture day 180, treated females were significantly heavier than controls by 16.2% (P=0.10).

MT-treated fish were all phenotypic males. No males were observed in the all-female control groups. Mixed-sex control groups comprised 52% males.

It is possible, using data from the all-female MT-treated groups, to infer the weights of genotypic female/phenotypic male fish in the mixed-sex treated fish (Table 5). Taking into account the sexual composition of the mixed-sex control group, the inferred weight for a genotypic male MT-treated fish would be 0.56 and 78.1 g at culture days 30 and 180, respectively. Using a similar procedure with the weights of control all-female fish, the weight for control males at 30 days can be inferred equal to 0.38 g.

MT treatment produced an anabolic effect in the genotypic male *O. niloticus* fish which resulted in their attaining average weights 55.3% and 23.0% greater than control males at 30 and 180 culture days. This represents the anabolic growth enhancement of the MT treatment, independent of any sex reversal effect in the *O. niloticus*.

Table 4. Mean weights and body lengths of *Oreochromis niloticus* combined control (mixed-sex and all-female) and combined MT-treated fish (mixed-sex and all-female). Means for each culture day followed by the same letter are not significantly different (P=0.10).

Fish size	30 days		180 days	
Combined control fish				
Mean weight (g)	0.37b	-	54.0b	-
Mean length (TL; mm)	-	26.7b	-	142.4b
Combined MT-treated fish				
Mean weight (g)	0.43a	-	62.4a	-
Mean length (TL; mm)	-	27.6a	-	147.4a

Table 5. Observed and inferred weights for mixed-sex and all-female *Oreochromis niloticus* at the conclusion of the sex reversal period (30 days) and at 180 days. Inferred weights are indicated by an asterisk.

Sex of fish		Mean weight (g)	
Genotype/phenotype	Treatment	30 days	180 days
Male/male	Control	0.38*	64.4
Female/female	Control	0.36*	52.5
Male/male	MT	0.56*	78.1*
Female/male	MT	0.39*	56.8*
Female/female	Control	0.36	48.9
Female/male	MT	0.39	56.8

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Designing New Fish Farming Models Adapted to Rural Côte d'Ivoire

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Abstract

The study of constraints affecting the development of fish culture in rural Côte d'Ivoire has shown that it is impossible to use farming methods that require expensive inputs such as supplementary feed. In contrast, farmers are willing to devote much of their time to fish culture if their work is adequately compensated.

When available, most inputs really accessible to farmers have a poor nutritional and/or fertilizing value. The efficient use of the limited trophic resources can be done through: (1) the qualitative and/or quantitative improvement of the flow of substances in the different levels of the pond trophic web (direct feeding, autotrophic productivity and heterotrophic microbial productivity) and (2) improvement of the accessibility to trophic resources by the fish. The present study is based on on-farm and on-station experiments focusing on: (1) the improvement of culture environment (treatments based on use of rice bran to which green manure may or may not be added, and on combined fish and rabbit culture) and (2) the use of a substratum made of bamboo or branches (acadja) to improve fish accessibility to primary production (attempts to substitute commercial feed by acadja system in lagoon pens have already been giving promising results).

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The results from this study confirm the importance of adding substrate for primary producers, of combining fish and rabbit culture and of using green manure for the improvement of pond production. New approaches for research and R&D in low input fish culture are suggested.

Introduction

During the last 15 years, considerable efforts have been made to develop tilapia culture in rural environments in vast areas of Côte d'Ivoire (see also Koffi et al., this vol.). The extension of aquaculture to these areas has been based on three types of pond inputs:

- commercial feed containing about 25% protein;
- combined chicken and fish culture; and
- the use, in the form of a compost generally located in a corner of the pond, of inputs available on-farm and often of low nutritional and/or fertilizing value.

The treatments giving the highest fish yields are—provided that fish farms are adequately managed—those consisting in commercial feed and combined livestock farming. However, the use of these techniques in rural areas is paradoxically declining whereas "poor" treatments essentially based on the use of refuse from artisanal processing of rice or other cereals continue to develop (Morissens et al. 1993). For Copin and Oswald (1988), Oswald and Copin (1992), and Koffi (1989 and 1992), this phenomenon is basically due to the fact that returns on cash investments are considered by farmers to be lower in fish culture than in other agricultural undertakings. In contrast, farmers are willing to devote a substantial part of their time to fish culture if their work is adequately remunerated. This trend of culture diversification is reinforced by the current crisis affecting cash crops (coffee and cocoa).

Today, the development of fish culture in rural areas is influenced to a large extent by the possibility of introducing farming systems that are essentially based on labor inputs. The establishment of these systems will be all the more important as the requirements for working capital will be low, even nil.

In addition, the absence of agricultural by-products for use in fish culture is a general characteristic in most Ivorian villages and, generally, on the African continent. Only large villages, with small artisanal hulling machines, can produce rice bran or maize bran of relatively poor nutritional and/or fertilizing value for local use. In this context, the challenge is to introduce farming systems that would eliminate constraints related to supply of inputs for most farmers. Preserving self-reliance, the only guarantee of a sustainable development in the current context, is a concern that must lead to studies on the efficient use of fresh plant biomass (grass) for fish culture, by perhaps establishing a small-scale animal breeding unit for its transformation.

In this context, the use of resources of low nutritional and/or fertilizing value is inevitable. One of the major characteristics of these "poor" treatments is their poor palatability for the fish. Consequently, their effect through direct consumption by the fish is limited, but their fertilizing effect is certain (Dembélé et al. 1991).

The more efficient use of trophic resources produced by this type of fertilization is made possible through:

1. The qualitative and/or quantitative improvement of the flow of substances in different trophic levels of the "pond" ecosystem. Solutions proposed in this area are:

- combined rabbit and fish culture without supplementary rice bran;

- combined rabbit and fish culture with supplementary rice bran; and
- association of a burrowing fish, to resuspend sediments, and thus stimulates the heterotrophic web.

2. Improved accessibility of the fish to resources of the trophic web. Here, the proposed systems are polyculture (systematically practiced in rural areas) and the establishment of a substratum of bamboo or branches, based on the acadja system used for capture fisheries in the lagoons of Benin. Experiments using a bamboo substrate in freshwater ponds have shown significant increases in biomass harvested compared to control ponds without artificial increase of substrate (Hem 1991). This technique mobilizes the mineral resources of the pond for growth of algae or related organisms (periphyton or *aufwuchs*) around the bamboo poles, a primary resource that is accessible to fish. In the ponds without substrate, most of the primary production derives from nanoplankton that cannot be used by filter-feeders such as tilapia (Spataru 1977).

Experiments with farmers combine rabbit and fish culture, implantation of acadjas and use of rice bran. Other related experiments using green manure and acadjas are conducted at the fish research station of the Institut des Savanes (IDESSA) in Bouake.

On-farm Experiments in Rural Midwestern Côte d'Ivoire

Methodology

The small group of test farmers with whom experiments were conducted is socially homogeneous (Koffi et al., this vol.). All know fish culture well. Once the phase of pond construction was completed, all farmers met problems in the supply of pond inputs (rice bran or nutrients). This showed the poor level of adaptation of the model to socioeconomic conditions. However, some fish farmers showed interest in testing new production models. In Midwestern Côte d'Ivoire, test farmers were extremely motivated as fish culture constitutes an essential activity of their work calendar and their ponds are well constructed (dikes, monk drain and drainage system).

The results presented here are those of culture cycles in farms that are fully productive. This production setup implied a minimum of five ponds (average surface = 0.045 ha) of which two were used for broodfish and fingerling production. The water used for these ponds was not suitable for fish culture, with pH values varying between 5.5 and 7, and very low conductivity (siliceous soils or water high in humic acids).

Innovations for which the farmers showed interest were:

1. The acadja system: In ponds that can be drained, the implantation of a substrate made of 10 bamboo$\cdot$m^{-2} represents an investment that will be amortized over several years.

2. Combined rabbit and fish culture: The association of livestock to other cultures is always described as a very efficient production system. Under small-scale farming conditions, the only intensive (battery) production system that requires hardly any of the costly commercial feeds—and therefore involves limited cash expenses—is rabbit culture. Farmers who attempted the intensive farming of pigs and chickens were soon confronted with diseases caused by inadequate feeding practices. Moreover, intensive farming systems require large quantities of inputs and a working capital that farmers cannot sustain. The use of rabbits in integrated farming systems has been described as promising (Little and Muir 1987).

Fish culture here is always based on polyculture, the dominant species being the male *Oreochromis niloticus* in association with a strictly carnivorous fish (*Parachanna obscura* or *Hemichromis fasciatus*), *Heterotis niloticus* and catfish (generally, *Heterobranchus isopterus* and rarely *H. longifilis*). *Labeo coubie* was used on two occasions.

The proposed techniques were derived from the results of a biotechnical and socioeconomic analysis of the farms. Discussions with fish farmers were essential to assess their constraints.

Support services proposed a range of techniques among which the farmer could choose. They also had the option to reject the proposed techniques. When a particular technique required funds, the support services contributed to the investment (purchase of rabbits, transportation of bamboos, etc.) but they never contributed to operating costs.

It is difficult to draw scientific conclusions from the comparison of technical results obtained from different fish farmers. Too many varying factors are involved. However, even under these conditions, the results from a single culture operation (and its development with time) are interesting and their analysis by the fish farmers themselves contribute to trying new approaches when necessary.

Technical Results

Research was conducted on the following farming systems (see also Tables 1 and 2):

1. Rice bran-based treatments (Table 1):
   - use of rice bran alone;
   - use of rice bran in the acadja system; and
   - use of rice bran with rabbit culture (rabbit pens on stilts).
2. Treatments without rice bran (Table 2):
   - rabbit culture alone;
   - acadja fertilization using the feces of rabbits reared outside fishponds; and
   - acadja and rabbit culture (rabbit pens on stilts).

Discussion and Conclusions

Technical Results

The acadja production system, and the use of rabbits, are excellent techniques to complement rice bran-based treatments. Yields of 2.3 $t \cdot ha^{-1} \cdot year^{-1}$ (acadja-rabbits) are produced without any input other than grass (fresh plant biomass), i.e., this technique does not require any cash expense. Fertilization is assured by using three rabbits, weighing a total of about 5 kg and producing 800 g of $feces \cdot day^{-1}$; which corresponds to about 200 g of dry matter (4.6 kg $DM \cdot ha^{-1} \cdot day^{-1}$). Inputs of dry matter remain very low compared to the usual standards applied to tropical integrated livestock farming, i.e., 75-50 kg $DM \cdot ha^{-1} \cdot day^{-1}$ (Hopkins and Cruz 1982; Morissens et al. 1993).

It appears, but this should be confirmed, that the association of tilapia-*Labeo* with acadja is detrimental to tilapias because of their identical, and therefore, competing diet. It seems also that *H. longifilis* is a predator of *P. obscura*, another predator of tilapias. This arrangement would reduce effective control of *O. niloticus* populations.

Several parameters are still to be defined: stocking densities for different species used, number of rabbits and bamboo density. In any case, the acadja-rabbit technique can be efficient only if fish farmers gain mastery of the stocking of ponds with carnivorous fish to control the proliferation, due to sexing errors, of tilapia fry and of the rigorous management of pond water (no overflow at the monk drain).

Table 1. Results of culture experiments using rice bran alone, rice bran and acadja, and rice bran with rabbits and 0.04 to 0.05-ha fishponds.

Treatment	Cycle	Species	Density (ind·m^{-2})	Duration (day)	IDG[a] (g·day^{-1})	Net yield (t·ha^{-1}·year^{-1})
Rice bran alone	1	*Oreochromis niloticus*	1.6	253	0.3	1.1
	2	*O. niloticus*	1.7	181	0.3	1.3
	3	*O. niloticus*	1.3	95	0.6	1.6
Rice bran with acadja[b]	1	*O. niloticus*	1.43	134	0.88	4.75
		Heterobranchus isopterus	0.1	134	0.86	0.3
Rice bran[c] and rabbit culture in pens on stilts	1	*O. niloticus*	1.57	119	1.2	6.1
		Heterotis niloticus	0.01	119	2.9	0.1
(283 rabbits·ha^{-1})		fry *H. niloticus*	-	-	-	0.3
13 rabbits /0.046 ha		*H. isopterus*	0.11	119	0.9	0.3
		Parachanna obscura	0.03	119	0.2	0

[a]IDG: individual daily growth.
[b]6 kg rice bran·day^{-1} and 10 bamboos·m^{-2}.
[c]6 kg rice bran·day^{-1}.

Table 2. Combined fish and rabbit culture (hutch on stilts), with or without acadja, in 0.04 to 0.05-ha earthen ponds + rice bran.

Treatment	Cycle	Species	Density (ind·m^{-2})	Duration (day)	IDG[a] (g·day^{-1})	Net yield (t·ha^{-1}·year^{-1})
Rabbits	1	*Oreochromis niloticus*	1.8	82	0.3	1.5
	17 rabbits/	*Heterotis niloticus*	0.01	82	0.3	0.2
(370 rabbits·ha^{-1})	0.046 ha	*Heterobranchus isopterus*	0.11	82	-0.3	0
		Parachanna obscura	0.03	82	0.3	0
	2					
(180 rabbits·ha^{-1})	9 rabbits/ 0.05 ha	*O. niloticus*	1.2	168	0.13	<0.6
Rabbits + acadja + fertilization[b]	1	*O. niloticus*	1.0	122	0.7	1.6
	feces	fry *O. niloticus*				0.9
	8 rabbits/	*H. isopterus*	0.13	122	-0.6	0.2
(178 rabbits·ha^{-1})	0.045 ha	*Heterobranchus longifilis*	0.004	122	12.9	0.2
		Labeo coubie	0.01	150	3.6	0.1
Rabbits + acadja[c]	1	*O. niloticus*	0.8	141	0.7	2.1
	3 rabbits/	*H. niloticus*	0.2	141	3.0	0.1
(71 rabbits·ha^{-1})	0.0425 ha	fry *H. niloticus*				0.3
		H. isopterus	0.08	141	-0.18	0
		P. obscura	0.03	141	0.1	0
		more fry (mean weight about 2 g)				
	2	*O. niloticus*	0.6	56	0.6	1.2
	7 rabbits/	*H. niloticus*	0.02	56	12.4	0.8
(156 rabbits·ha^{-1})	0.045 ha	*H. isopterus*	0.11	56	0.3	0.1
		L. coubie	0.01	56	1.6	0
		P. obscura	0.01	56	-0.2	0

[a]IDG: individual daily growth.
[b]Acadjas and fertilization using feces produced outside the farm. A bucket of the excreta of eight rabbits was poured each day in the pond (an average of 3.75 kg of manure per day corresponds to 500-600 g of dry matter. Pond = 0.045 ha).
[c]During this cycle, water management was poor; frequent overflow at the monk drain with possible negative effects on production (loss of nutrients).

Changes in Fish Farmers' Behavior

Fish farmers have been favorably impressed by increases in yields and in growth rates observed in the cycles acadja+rice bran and rabbits+rice bran. However, the supply of rice bran is still a constraint for them. Surprisingly, the model rabbit+bamboo has modified the behavior of the two fish farmers concerned, i.e., they have been devoting more time to fish culture since they obtained their first results. One of them suggested to plant himself a second pond with bamboos (only the transportation of bamboo is subsidized). The most characteristic comment on their part was: "these fish are gifts!"

Experiments without rice bran did not yield expected results. Yields in marketable fish remained low compared to the amount of work involved. A significant increase in yields is still possible with a few improvements in water management, stocking, and in the number of rabbits used.

Nevertheless, the motivation expressed and demonstrated by the farmers to continue the experiment, and their appreciation of the first results, are encouraging. This will contribute to the establishment of culture systems likely to resolve the constraints that today hinder the development of fish culture in rural Côte d'Ivoire and generally in subSaharan Africa.

On-station Experiments

Supplementary fertilization using rice bran with dry chicken manure or grass

OBJECTIVE

The experiment aimed at assessing the effects of supplementing coarse rice bran (first polishing) as standard feed in the fishponds with dry chicken manure or grass. Dry chicken manure is readily available in peri-urban areas.

METHODOLOGY

The experiment was conducted in 400-m^2 ponds at IDESSA fish culture station in Bouake using a mixed population of *O. niloticus* males (1.95 ind·m^{-2}) and *Clarias gariepinus* (0.25 ind·m^{-2}). Three treatments were applied and replicated at a different time: rice bran alone (control); rice bran with supplementary fertilization using dry chicken manure; and rice bran with supplementary fertilization using fresh grass (*Pennisetum purpureum*).

Daily rice bran application rates varied from 52 kg·day^{-1}·$pond^{-1}$ at the start of the culture period, to 11.6 kg·day^{-1}·$pond^{-1}$ at the end of the period (seventh month). For the entire production period, dry chicken manure was used at a rate of 2.4 kg·day^{-1}·$pond^{-1}$ and fresh grass at 32 kg·day^{-1}·$pond^{-1}$. These two fertilization regimes were almost equivalent in terms of nitrogen input. At the end of the culture period, the quantity of dry matter used in the ponds reached a maximum of 240 kg· ha^{-1}·day^{-1} for rice bran alone, 300 kg· ha^{-1}·day^{-1} for rice bran+chicken manure and 360 kg·ha^{-1}·day^{-1} for rice bran+grass.

RESULTS

Tables 3 and 4 present the results of the experiment. Comparisons of treatments relying on randomized block analysis of variance (ANOVA) did not show any significant difference among treatments in terms of yields for *O. niloticus* and for combined *O. niloticus* and *C. gariepinus,* nor in terms of feed conversion ratios. However, results on mean daily growth and mean yield were higher in the grass treatments. Additional replicates are needed to confirm this trend. The analysis of variance revealed a "block" effect and significantly higher values in terms of growth and yield for *C. gariepinus* in the treatment using rice bran+grass.

DISCUSSION

Yields ranging from 4 to 6.3 t·ha^{-1}·$year^{-1}$ are "normal" with regard to feed and fertilizers. We can, of course, question the need to cut and distribute 292 t·ha^{-1}·$year^{-1}$ of fresh grass for an increase in production of 1.4 t·ha^{-1}·$year^{-1}$ over the volume obtained using only rice bran. The remuneration for cutting the grass depends essentially on the quantity of grass available and its proximity, and on the time spent for a given harvest. We can estimate, therefore, that an annual distribution of 290 t·ha^{-1} will require a minimum of 4,500 hours of work! However, it is possible that in the context of small-scale farming

characterized by limited access to rice bran and low or irregular inputs of this resource, the "grass effect" could be more significant even if the yields are lower than those recorded here (see above for the yield results in treatments using rice bran in on-farm experiments).

The significant differences recorded between the grass treatment and the other treatments in terms of individual growth and yield for *Clarias* show a link between treatment and stimulation of a trophic resource specifically accessible to *Clarias*. This demonstrates that in these conditions, polyculture is more likely to optimize the use of this treatment than monospecific stocking using *O. niloticus* males.

It would be interesting to assess the effect of a grass treatment on a culture combining *O. niloticus*, catfish, and *H. niloticus*. The burrowing behavior of *H. niloticus*, which is largely established in rural fish farms, is likely to promote the suspension of great quantities of grass-derived sediments which, in turn, promote microbial production (Costa-Pierce and Craven 1987; Costa-Pierce and Pullin 1989) and may have positive effects on the entire heterotrophic and autotrophic webs (Schroeder 1983; Spataru et al. 1983).

In addition, following the first tests conducted in lagoons and in ponds (Hem 1991), an experiment was set up at the IDESSA station using four replicated treatments. These treatments consisted in: (1) using rice bran (without substrate); (2) providing a substrate made of 10 bamboos·m^{-2} without feed supplement; (3) providing a substrate constituted by 10 bamboos·m^{-2} with rice bran as feed; and (4) providing a substrate made of 32 branches of *Cassia*·m^{-2} with rice bran as feed. The objective of this experimental design was to assess the significance of feed-substrate interaction and the ability of a substrate made of ordinary branches to provide a vertical surface roughly equivalent to that of the bamboo substrate.

The few tests done so far on-farm as well as on-station have not yet led to the development of farming techniques that would totally free the farmers from their input constraints. Further testing is needed on systems combining substrate, green manure, polyculture and resuspension of sediment, as well as on the stocking densities best adapted to the productivity of these systems. The identification of the interactions that the association of these techniques involves requires much experimental support. Ideally, this type of research should be supported by a more fundamental study of the complex trophic mechanisms that link farming techniques and treatments, on the one hand, and farming techniques and production, on the other. Regarding

Table 3. Summary results from different tests (net yields in t·ha^{-1}·year^{-1}).[a]

	Rice bran-based treatment		Culture system without rice bran
	Without rabbits	With rabbits	With rabbits
Without acadja	< 2 (3)	6 (1)	< 2 (2)
With acadja	5 (1)	(0)	2.3 (2)

[a]Figures in parentheses represent the number of cycles studied.

Table 4. Supplementary fertilization using dry chicken manure or grass in a culture experiment with *Oreochromis niloticus* and *Clarias gariepinus* fed with rice bran in 400-m^2 ponds.

Species/treatment	Density ($ind \cdot m^{-2}$) Block 1	Density ($ind \cdot m^{-2}$) Block 2	Individual daily growth ($g \cdot day^{-1}$) Block 1	Individual daily growth ($g \cdot day^{-1}$) Block 2	Individual daily growth ($g \cdot day^{-1}$) Mean[a]	Feed conversion ratio Block 1	Feed conversion ratio Block 2	Yield ($kg \cdot ha^{-1} \cdot year^{-1}$) Block 1	Yield ($kg \cdot ha^{-1} \cdot year^{-1}$) Block 2	Yield ($kg \cdot ha^{-1} \cdot year^{-1}$) Mean[a]
O. niloticus										
Rice bran alone	1.95	1.95	1.12	0.82	0.97 (0.15)	11.91	15.23	5,488	4,023	4,755.5 (732.5)
Rice bran + manure	1.95	1.95	1.13	0.92	1.025 (0.115)	10.32	13.57	6,337	4,545	5,441 (896)
Rice bran + grass	1.95	1.95	1.13	1.16	1.145 (0.015)	10.97	10.06	5,643	5,864	5,753.5 (110.5)
C. gariepinus										
Rice bran alone	0.25	0.25	0.55	0.30	0.425 (0.125)	11.91	15.23	236	44	140 (96)
Rice bran + manure	0.25	0.25	1.56	0.30	0.59 (0.03)	10.32	13.57	379	23	201 (178)
Rice bran + grass	0.25	0.25	0.96	0.62	0.79 (0.17)	10.97	10.06	674	294	484 (190)

[a]Figure in parentheses are standard deviations.

this point, research work based on two complementary approaches is proposed:

1. Study of the flow of matter in the different levels of the food chain by plotting stable isotopes of carbon (C^{12} and C^{13}) (Schroeder 1978, 1983 and 1987), and investigation on what becomes of the major minerals contributed by the treatments (Krom et al. 1985 and 1989).

2. Companion study on the environment potential trophic resources and of the fish's stomach contents (Spataru et al. 1983; Milstein et al. 1985a, 1985b and 1988; Hepher et al. 1989).

These approaches should lead to a comprehensive assessment of the impact of different techniques or combination of techniques used at different levels of the ecosystem and to the identification of bottlenecks and trophic deadends.

The adaptability of the proposed techniques to rural conditions will be determined by the fish farmers themselves. To this effect, it is important to note that the feedback between fish farmers and support services has had a major impact on the direction of the ideas presented here on the use of "poor" treatments.

It is possible to imagine that a technical model tested in rural conditions (example: acadja+grass+polyculture including burrowing fish) will be massively adopted by farmers. In this context, there will be scope for its rapid optimization by the fish farmers themselves depending on their specific constraints.

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Growth Performance of *Oreochromis lidole*, *O. squamipinnis*, *O. shiranus* and *O. karongae*, New Candidate Species for Aquaculture in Open Waters and Fishponds in Malaŵi[a]

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MSISKA, O.V. and B.A. COSTA-PIERCE. 1996. Growth performance of *Oreochromis lidole*, *O. squamipinnis*, *O. shiranus* and *O. karongae*, new candidate species for aquaculture in open waters and fishponds in Malaŵi, p. 129-134. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

There are several tilapia species (Fam. Cichlidae) indigenous to Lake Malaŵi whose growth potential is little known outside their natural environment. Past aquaculture research efforts in Malaŵi have concentrated on *Oreochromis shiranus* and *Tilapia rendalli*. The performance of both species in ponds is limited by a high reproductive capacity and slow growth. Therefore, new candidate species for aquaculture, such as *Oreochromis lidole*, *O. squamipinnis* and *O. karongae*, are under investigation. *O. lidole* grows well in open waters ($\phi'=\log_{10}K+2\log_{10}L_\infty=2.79$) but does not spawn readily in ponds, while *O. squamipinnis* appears to have a low growth potential ($\phi=2.58$, also based on standard lengths). *O. karongae*, on the other hand, breeds in ponds. Selected growth comparisons were made between their populations in Lake Malaŵi and those kept in fishponds at the National Aquaculture Centre, Domasi, Zomba. Their growth potential is high, with $\phi'=2.76$ and 3.03 for the lake and fishpond populations, respectively. Thus, growth performance and spawning success in shallow pond make *O. karongae* an attractive candidate species for aquaculture.

Introduction

The need to increase fish production in Malaŵi to keep pace with increasing demand has been recognized (DEVPOL 1987; GOPA 1987). One approach is to expand and intensify fish farming activities. Most aquaculture research in Malaŵi has involved *Oreochromis shiranus* (a microphagous species) and *Tilapia rendalli* (predominantly macrophytophagous). *Tilapia rendalli* tastes good but is slow-growing and broodstocks produce relatively low numbers of fingerlings (Costa-Pierce and Chikafumbwa, this vol.). *O. shiranus* shows fast growth while young but matures early and can become stunted in fishponds (Msiska and Cantrell 1985; Pauly et al. 1988; Maluwa 1990).

The work of Lowe (1952) and Trewavas (1983) suggest that the search for indigenous tilapias that would perform

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better in aquaculture must consider *O. karongae* and *O. lidole*. Recently, the successful breeding of *O. karongae* in shallow ponds (O.V. Msiska, unpubl. obs.) has further spurred interest in this species (see Maluwa and Dixon, this vol.).

This paper compares the growth of *O. karongae*, *O. lidole* and *O. squamipinnis* from published data with preliminary growth studies in ponds, using ϕ' ($=\log_{10}K+2\log_{10}L_\infty$) (Pauly 1979; Pauly and Munro 1984) as an index of growth potential. The technique was chosen because of its demonstrated applicability to tilapias (Moreau et al. 1986; Pauly et al. 1988).

Methods

Capture Fisheries Data

Mean length-at-age estimates for *O. lidole*, *O. saka* (now regarded as a junior synonym of *O. karongae*), *O. shiranus shiranus* and *O. squamipinnis* as reported by Lowe (1952), based on samples collected in 1945-1947, were used to estimate the von Bertalanffy growth parameters (L_∞; K) from which the ϕ' values were calculated (Table 1).

Capture and Transportation of Live Fish to Ponds

Fingerlings were collected by beach seining, assisted by diving for specific schools of fish. For pond studies, fish were obtained from Cape Maclear and Kakoma Bay in Lake Malaŵi and from Lake Malombe (Fig. 1). The most successful fishing season was from January to March, after natural breeding, when most fry had become free-swimming.

Before transferring fingerlings to the National Aquaculture Centre (NAC), Domasi, Zomba, they were kept unfed in cages for at least 48 hours to allow them to void their guts. During this period, a prophylactic (terramycin) at 0.1 $mg \cdot l^{-1}$ and a vitamin premix were given by adding the powder forms of these medications into the cages. In the absence of terramycin, egocin (oxytetracycline hydrochloride and calcium pantothenate) was used. While the former drug is approved by the US Environmental Protection Agency (EPA) for use on food fish (Schnick 1988), the latter is commonly used for poultry.

Growth Trials in Ponds

All tilapia fingerlings were initially stocked into separate ponds, according to their origin. Various attempts at visually separating these immature forms into species proved futile, as were similar attempts by other workers (Lowe 1952; Tarbit 1969; Trewavas 1983; Turner et al. 1989). Thus, identification of fish was not confirmed until they had attained large sizes of over 100 g when breeding colors became conspicuous.

Sixty *O. karongae* fingerlings were stocked in each of the two 500-m^2 ponds. One of the most reliable characteristics used to help separate the three tilapias of subgenus *Nyasalapia* (*O.N. lidole*, *O.N. karongae* and *O.N. squamipinnis*) is the number of tooth rows on the lower jaw and their mode of arrangement (Turner and Robinson 1991). Because this parameter could be used without killing fish, it was extensively utilized in this study and fish which were classified as *O. karongae* but had over four rows were stocked separately from those whose rows were less than four. The two groups had, in an earlier study, been observed to differ in spawning requirements (Msiska, unpubl. obs.). The mean size at stocking was (+SD) 19.5+1.8 cm TL and 134.2+38.2 g body weight. Further, morphometric measurements

Table 1. Estimates of growth of *Oreochromis* spp. in Lake Malaŵi (adapted from Lowe 1952). The mean length-at-age estimates (total length) were obtained by length-frequency analysis and the counting of rings on the opercular bone. Standard lengths are in brackets.

Species		Age groups I		II		III		IV	
O. shiranus shiranus	Length (cm)	10.0	(7.8)	18.0	(15.8)	22.0	(19.8)	25.5	(23.3)
	Weight (g)	16.0		110.0		210.0		342.0	
O. karongae	Length (cm)	12.0	(9.3)	22.0	(19.3)	27.5	(24.8)	30.0	(27.3)
	Weight (g)	28.0		198.0		412.5		545.5	
O. squamipinnis	Length (cm)	9.0	(7.0)	17.0	(14.3)	24.0	(21.3)	26.5	(23.8)
	Weight (g)	12.0		86.0		264.0		366.0	
O. lidole	Length (cm)	13.0	(10.3)	23.0	(20.3)	28.5	(25.8)	31.0	(28.3)
	Weight (g)	40.0		220.0		463.5		609.0	

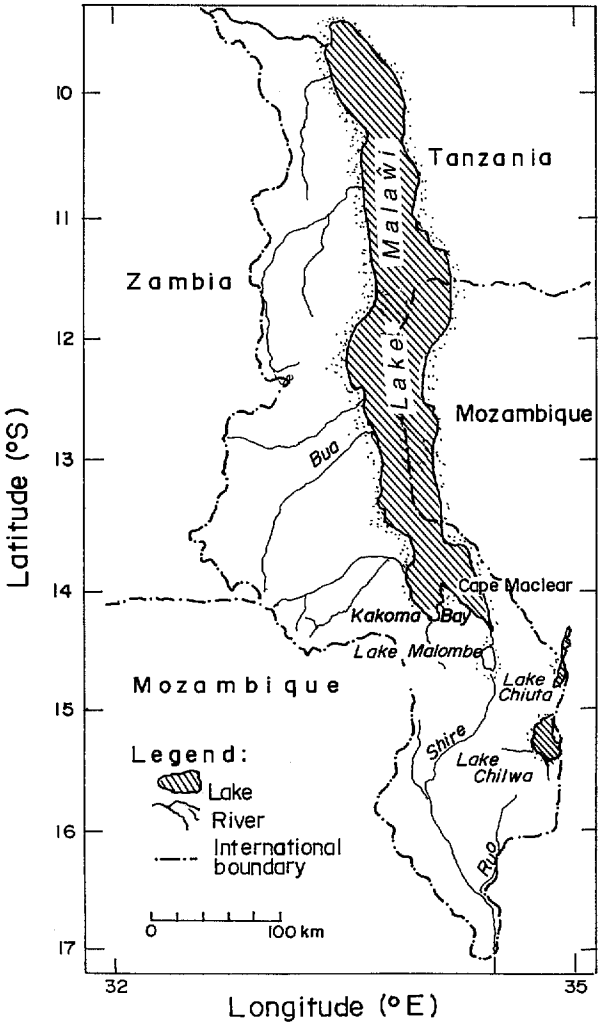

Fig. 1. Distribution of species of *Oreochromis* subgenus *Nyasalapia*: Lakes Malombe and Malaŵi.

were obtained for the two populations according to recommendations by Turner et al. (1989).

Five hundred *O. squamipinnis* fingerlings were stocked in a 500-m^2 pond. The mean size at stocking (±SD) was 16.4±1.7 cm TL and 91.3±28.0 g body weight. Confirmation regarding the identification of these fish was done in consultation with G.F. Turner who has extensively studied the taxonomy and ecology of the wild stocks of *Oreochromis* subgenus *Nyasalapia* in Lake Malaŵi.

Every month, a sample of 20 to 30 fish per pond was taken to record lengths and weights. During sampling, fish were anesthetized using benzocaine (Ross and Ross 1984). All the fish were fed maize bran at 4% body weight which was adjusted downwards to 2.5% body weight per day, six days per week, after the fish attained an average weight of over 200 g. Data were collected for a period of 275 days and ϕ' values were calculated from the von Bertalanffy parameters L_∞ (SL; in cm) and K ($year^{-1}$) following Vakily (1988). Morphometric measurements taken on the two groups of *O. karongae* were first converted to fractions of standard length and the Microstat program of Ecosoft Inc. was used to analyze the data on an IBM compatible personal computer.

Results

Table 2 summarizes the growth parameter estimated from wild and cultured tilapia populations. Pond data are restricted to *O. karongae* and *O. squamipinnis* following confirmation by G.F. Turner that these pond populations did not contain *O. lidole*.

Discussion

The values of ϕ' determined for *O. karongae*, *O. lidole* and *O. squamipinnis* are comparable to or higher than those published for tilapias regarded as having acceptable growth performance: *O. niloticus* (2.30-3.11), *O. aureus* (2.31-2.61), *O. andersonii* (2.46-2.63) and *O. mossambicus* (2.05-2.60) (Pauly et al. 1988). If growth performance using ϕ' were the only criterion for selecting species for aquaculture, then *O. lidole*

Table 2. Growth performance index (ϕ')[a] and related statistics of wild (Lake Malaŵi) and captive (National Aquaculture Centre, Zomba) stocks of Lake Malaŵi tilapia species.

Species	Stock	W_∞ (g)	W_{max} (g)	L_∞ (cm)	L_{max} (cm)	K($year^{-1}$)	ϕ'	Source
O. shiranus shiranus	Wild	-	-	27.8	39.0	0.481	2.57	This study
	Captive	53.5	-	11.0	-	9.87	3.08	Pauly et al. (1988)
O. karongae	Wild	781	857	30.3	34.0	0.631	2.76	Lowe (1952)
	Captive	656	-	27.7	-	1.391	3.03	This study
O. squamipinnis	Wild	760	758	31.9	33.0	0.375	2.58	Lowe (1952)
	Captive	537	-	23.6	-	1.739	2.99	This study
O. lidole	Wild	891	1,110	31.3	38.0	0.631	2.79	Lowe (1952)
	Captive	-	-	-	-	-	-	

[a] $\phi' = \log_{10}K + 2\log_{10}L_\infty$; K($year^{-1}$), L_∞(cm; SL).

would rank highest, with $\phi'=2.79$. However, its reluctance to breed in fishponds should first be resolved before using it in aquaculture (A.O.H. Maluwa and M. Dickson, pers. comm.).

Our morphometric data (not shown) suggest at least two variants of *O. karongae* in the pond populations. The presence of several strains in the wild has since been confirmed by differences in spawning success and nest characteristics, and tooth row arrangements and pharyngeal dentition (Turner and Robinson 1991). Consequently, it has been suggested that *O. karongae* is a nominal species comprising several variants of which *O. saka* is a junior synonym (Turner et al. 1989).

According to Lowe (1952) and Trewavas (1983), *O. lidole* is the fastest growing *Oreochromis* in Lake Malaŵi, followed by *O. saka* (now *O. karongae*), *O. squamipinnis* and *O. shiranus shiranus*. Such differences could not, however, be distinguished by calculating specific growth rates. Thus, the estimation of ϕ' appears to have more practical value during initial screening of candidate fish species than the conventional specific growth rate. More research needs to be done using this index of growth potential to examine other species of *Oreochromis* and/or *Nyasalapia*.

Acknowledgements

This study was made possible by funding from the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) through ICLARM, while fish collections were funded by the International Development Research Centre (Project No. 3-P-85-0265). We are grateful to Dr. R.S.V. Pullin for his support. Our thanks are due to Dr. D. Pauly for useful discussions on the growth performance index used in this study. Permission to publish the results has been given by the Secretary for Research and Environmental Affairs, Government of Malaŵi.

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Management of Tilapia (*Oreochromis shiranus* and *Tilapia rendalli*) in Ponds of Smallholder Farmers in Mwanza and Zomba West Districts of Malaŵi

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Abstract

A pond management system and intensified activities were introduced into Mwanza and Zomba West Districts, Malaŵi, through a development project, with the intention of achieving higher fish production and self-reliance amongst the smallholder farmers who had ventured into aquaculture. The extension efforts were simple and comprehensible. The major components of the model were: a semi-intensive polyculture system with *Oreochromis shiranus* and *Tilapia rendalli*, a stocking density of two fish (mixed sex) per m^2, a short culture period (four to six months) and complete pond draining at harvest using a locally made basket. Particular emphasis was placed on feeding with locally available resources.

Since January 1988, there has been a significant increase in the overall productivity from 303 $kg \cdot ha^{-1} \cdot year^{-1}$ to 804 $kg \cdot ha^{-1} \cdot year^{-1}$ with project farmer cooperators achieving 1,400 $kg \cdot ha^{-1} \cdot year^{-1}$ in Mwanza. In Zomba West, farmers exhibit an average productivity of 1,900 $kg \cdot ha^{-1} \cdot year^{-1}$. However, observations at Zomba West Extension Station showed low average weights at harvest (30-50 g) for both species. This was consistent with what the farmers obtained in their ponds.

The pond management system has two major constraints: farmers do not have enough of the required resources (feeds and fertilizers) and the two species of fish do not grow to large enough sizes to attract the more adventurous and commercially minded farmers.

Introduction

In 1988, a modified pond management system (MPMS) and intensified extension activities were introduced in Mwanza and Zomba West Districts in Malaŵi. The major objectives were to achieve increased fish production and self-reliance among the smallholder farmers. It was hoped that the MPMS would eventually spread to other parts of the country where potential for fish farming existed. Strong emphasis was placed on the use of locally available farm resources. The MPMS was devised from trials made at the Zomba West Extension Post, Chinseu (Otte 1990).

The MPMS was not drastically different from the system used previously and it was thought to offer a new option while decreasing the dependence on the scarce extension services of the Fisheries Department: a constraint to aquaculture development. The MPMS placed emphasis on the following:

- A suitable pond site with a permanent water supply, ideally a permanent stream or spring which could easily be diverted to feed the pond by gravity.

[a]Both authors are now deceased. Limited quantities of reprints are available from the senior editor.

- Construction, for new farmers, of drainable ponds of at least 200 m^2 in size, because smaller ponds can be uneconomical. However, farmers take up fish farming for various reasons and for extensionists to insist on the 200 m^2 minimum was unrealistic. Farmers were encouraged to minimize the cost for pond construction using family labor and locally available materials.
- Stocking with mixed-sex fingerlings of *Oreochromis shiranus* and *Tilapia rendalli* (1:1) at 2 fish·m^{-2}.
- Feeding with madeya (maize bran), the most commonly available feed (Msiska 1988) at rates determined from trials at Chinseu. Farmers were advised to feed at a rate of 4% of the total body weight per day. To make such measurements understandable to farmers, the weights of feed were converted to fit the receptacles (plates, buckets and other containers) that the farmer might have. The use of various other fish feed (on-farm by-products) were also encouraged.
- Inorganic fertilization with diammonium phosphate (DAP), chosen because many farmers grow maize and are encouraged by the agriculture extension workers to apply this fertilizer. They may have some fertilizer left over which could be applied to fishponds. DAP is more effective in stimulating algal growth in ponds than other locally available fertilizers. The recommended rate of fertilization was to be 1 g·m^{-2}·$week^{-1}$.
- Organic fertilization with livestock manure, although most farmers do not have enough livestock to accumulate manure to add to the ponds. They were encouraged to add as much manure as possible and where inorganic fertilizer and manure were scarce, composting of vegetation in the ponds was encouraged.
- Harvesting when the fish had produced enough fingerlings for the next culture period and had attained a marketable size: usually after four to six months.
- Harvesting by draining using a locally made basket attached to the outlet pipe and saving the fry and fingerlings and transferring them immediately to an adjacent holding pond (10-50 m^2).

The MPMS stressed the independent production of fingerlings by farmers and the farmers ability to harvest their own ponds without dependence on the use of a seine net provided by fisheries extensionists.

Results

Zomba West (Chinseu)

Up to the beginning of the program (June 1988), the area had been served by the Domasi Extension Service and there were only seven farmers with a total pond surface area of 1.44 ha. There were no records of production. Most of these farmers had been assisted by an OXFAM project in the late 1960s and early 1970s. By December 1988, there were 19 farmers with a total of 1.78 ha of ponds which yielded 917 kg of fish. In 1989, there were 60 farmers with 3.5 ha of ponds, yielding 2,467 kg. In 1990, 89 farmers produced 4,314 kg from 4.8 ha. The number of farmers has since risen to about 100, having a total pond area of 5.2 ha and an estimated annual productivity of about 1.9 t·ha^{-1}.

Between January and December 1990, 67 farmers were closely monitored with monthly production figures (Table 1).

Mwanza (Kunenekude)

Until 1987, the Fisheries Department had been providing very limited extension services to farmers in Mwanza. There were two extensionists, with only one motorcycle, to cover 13 villages

Table 1. Monthly production from fish farmers in Zomba West, Malawi: January to December 1990.

Month	No. of farmers per month	No. of ponds	Total pond area (m^2)	Fish production (kg)
January	3	3	615	33.80
February	3	4	2,526	151.30
March	4	4	3,891	166.30
April	6	6	1,978	232.00
May	6	7	3,747	196.20
June	3	3	1,028	58.50
July	8	9	3,370	171.60
August	10	10	2,887	276.00
September	11	12	3,791	260.00
October	4	4	802	94.00
November	4	4	4,213	302.20
December	5	5	1,279	100.50
Total	67	71	3.01 ha	2,042.40[a]

[a]Probably an underestimate because of difficulties collecting data during the farmers' second culture period. The annual total may be as much as twice this figure.

and 513 farmers having 649 ponds covering 6.72 ha. The budget set aside for extension was at MK1,000 (US$348) per year. At the end of 1987, a survey revealed a total production of 2,038 kg of fish: about 300 $kg \cdot ha^{-1} \cdot year^{-1}$.

In January 1988, 11 farmers, with a total pond surface area of 2,589 m^2, adopted the program's MPMS concept. Six months later, the net production was measured at 123 kg with an average net productivity of 950 $kg \cdot ha^{-1} \cdot year^{-1}$. By November, the number of farmers adopting the MPMS had risen to 22 with an average productivity reaching 1,400 $kg \cdot ha^{-1} \cdot year^{-1}$ (Table 2).

Overall Production

The total numbers of ponds and farmers (1983 to August 1991), for Mwanza and Zomba West, are given in Table 3. The figures for 1991 were extrapolated since the data for 1991 were available only up to August for Zomba West and no meaningful data were available from Mwanza.

Discussion

Increased extension efforts have here certainly improved fish production. There were a few technical problems with launching the MPMS. The harvest method by drainage and subsequent handling of fingerlings proved very difficult for farmers. In many cases, there was massive fingerling and fry mortality, resulting in increased demand for fingerlings at the extension posts, rather defeating the self-reliance objective.

In Zomba West (Chinseu), extension was done in an area where fish farming had only been minimally practiced: only seven farmers were known at the beginning. The farmer's choice of pond size, whether or not the pond was drainable, etc., depended on the extensionist. The average pond size here

Table 2. Fish production by the 22 farmers in Mwanza who adopted the MPMS from December to November 1990 (average culture period = 210 days).

Farmer	Pond area (m^2)	Production (kg)	Extrapolated net productivity ($kg \cdot ha^{-1} \cdot year^{-1}$)
1	270	10.5	661
2	756	58.0	1,304
3	176	16.0	1,546
4	302	5.5	310
5	119	5.0	714
6	216	8.2	645
7	188	18.0	1,623
8	NA	NA	NA
9	168	11.9	1,204
10	231	24.0	1,766
11	NA	NA	NA
12	144	6.0	708
13	270	27.8	1,700
14	120	12.0	1,700
15	150	10.0	1,133
16	171	5.0	497
17	253	15.0	1,008
18	145	14.3	1,677
19	452	69.2	2,595
20	264	19.5	1,256
21	576	61.7	1,821
22	330	38.0	1,958

Table 3. Fish farming development among smallholder farmers in Mwanza and Zomba West, Malaŵi.

Production factors	1983	1984	1985	1986	1987	1988	1989	1990	1991[b]
No. of farmers	87	225	337	425	513	523	628	559	669
No. of ponds	105	300	405	512	690	714	806	818	967
Total pond area (ha)	1.6	4.5	6.1	7.7	9.7	11.2	14.6	15.85	21.67
Total production (kg)	800	2,700	3,660	4,620	5,820	7,330	16,100	16,600	25,330
Value (MK)[a]	1,200	4,050	5,490	6,930	11,640	13,905	32,550	34,360	48,764

[a]Malaŵi Kwacha -2.00 in 1983; 2.71 in 1991 = US$1.
[b]Extrapolated data.

was about 335 m^2 in Zomba West. The average productivity in Zomba West was recorded at 1,700 $kg \cdot ha^{-1} \cdot year^{-1}$, twice that obtained in Mwanza.

In Mwanza, a lot of ponds had been constructed on sites that would not allow a pond to be drained by gravity. To the farmers, a pond is a small lake which is not drained when fish are to be caught. Moreover, the use of a basket for harvesting is a strange and unconventional method, not easily accepted.

The emphasis on a minimum pond size of 200 m^2 was viewed as unacceptable by farmers in Mwanza: 649 ponds, measuring little more than 100 m^2, had been constructed during an earlier program supported by UNICEF. Thus, the 200 m^2 minimum caused confusion among farmers. Under the UNICEF program, any waterbody was a "pond," regardless of size. Farmers could expand its size if they benefitted and as they saw fit, but it was difficult to increase the size of small ponds because of land constraints. After all these extension efforts, the average pond size was still only about 110 m^2 and as the project emphasized farmers with ponds of at least 200 m^2, many farmers were left out.

The quality and quantity of inputs required by the pond management system was almost impossible for the farmers to comprehend. Maize bran (madeya) is seasonal and becomes human food at some critical times of the year. Farmers cannot afford adequate fertilizer even for their maize. The assumption that farmers would have surplus fertilizer from the maize or other crops was wrong.

Although there are a lot of on-farm resources which could be fed to fish, the extension workers and farmers do not know of any suitable rates of feeding. No systematic work had been conducted on conversion factors of such resources. Most of these resources are also seasonal and are often available in small quantities. Attempts are being made to quantify these resources throughout the year (see Noble, this vol.).

The MPMS has been considered to select genetically for small fish. Msiska (1988) reported average weights of farmed *O. shiranus* of 125 g, but current average weights are about 70 g among farmers and 40-60 g at government stations.

It is very unlikely that the MPMS will significantly increase production beyond 2 $t \cdot ha^{-1} \cdot year^{-1}$ among smallholder farmers. Farmers need more technological options, including hatchery and broodstock management to produce better quality fingerlings. Also, different methods for harvesting, presently being developed at the National Aquaculture Centre at Domasi, should be tried with farmers.

The experience gained during these three years of extension has given food for thought about how to continue the extension program. Virtually all projects enjoy a very positive response from the target groups when a new idea/concept is being introduced—in this case a better pond management system. But it is obvious that, when only one option is given, it will only benefit a certain percentage of the target group and upper limits in outputs are easily reached. If we compare the government's yearly budget of MK1,000 in extension for the Mwanza District with the massive effort spent by this externally-funded project in the same area (years later), it is clear that a positive benefit:cost ratio only comes out during the first year. The relative gain in production in subsequent years, for both Mwanza and Zomba West, does not seem to justify the effort.

It seems that any project which emphasizes only one model/ technique will only achieve part of their goals. It is also clear that when a new project comes into an area where another project has

already been working, only marginal changes may result.

The most important lessons learned from this program are that: (1) any developing country must, at the beginning of every development project, have a clear concept/strategy which should be strictly followed by the various development agencies; and (2) such a concept/strategy must be *dynamic*. Development means change and when addressing non-uniform target groups, it should be the responsibility of those funding and executing the project to develop and offer a "basket of options" to the target groups, and leave it up to them to choose which option(s) best suit(s) their needs.

The present policy where new projects only offer "their solution" will eventually lead to more confusion and a detrimental outcome. Such efforts, time and money cannot ensure self-reliance and sustainability among farmers. Dynamics and coordination seem to be two key words which should be taken into account in development projects.

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Utilization of On-farm Resources for Aquaculture in Rural Africa[a]

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NOBLE, R.P. 1996. Utilization of on-farm resources for aquaculture in rural Africa, p. 141-151. *In* R.S.V. Pullin, J. Lazard, M. Legendre and J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

In Africa, aquaculture has not been successfully adopted although it is the home of tilapias. A major reason is that little account is taken of the bioeconomic resource base of rural African smallholder farmers. Most agriculture is at subsistence level with only a small excess crop production providing a meagre cash income for the rural household. Malaŵi provides an example; 85% of its eight million people are involved in agriculture with smallholders contributing 84% of the agricultural GDP. However, three-quarters of this contribution does not enter the cash economy. Under these circumstances, aquaculture will only succeed if it is integrated into the farming system and is based on recycling farm resources such as crop and livestock residues. Utilizing such materials as pond inputs would not only result in production of a valuable crop of fish, but may also improve the recycling efficiency of farm resources, thus enhancing overall farm productivity. Before taking such an approach, the bioresource base of farms must be estimated. This was done for a variety of crop and animal residues on 20 farms in Zomba District, Malaŵi. All the farms had ponds and were growing *Oreochromis shiranus* and *Tilapia rendalli*. Residues from maize (i.e., bran, stovers, and cobs) and rice (i.e., bran and straw), weeds from crop and fallow land, and animal manure were estimated. All crop residues and weeds were estimated from 50-m^2 quadrats placed in several fields and fallow areas on each farm. Resource-flow diagrams were produced showing the direction of movement of major crop and animal residues in the farm system. From this flow model, it was possible to pick out residues which could be redirected to the pond system to improve pond fertility and provide direct feed for fish. Some smallholders had grasses and weeds on fallow land (approximately end-of-season biomass, 7 $t \cdot ha^{-1}$ dry weight), and ash from cooking fires (over 500 $kg \cdot year^{-1}$ per farm, dry weight) which went unutilized in the farm system. Approximately 2,500 $kg \cdot ha^{-1} \cdot year^{-1}$ (dry weight) of maize stovers are produced, a part of which could be used directly in the pond, or composted to produce a high quality pond input. Most farms in this study did have unutilized bioresources which could be used more efficiently by processing them through fishponds. These provide the potential for increasing overall farm efficiency and production, by converting low value agricultural wastes into a valuable fish crop at low cost to the farmer.

Introduction

Under current economic and environmental conditions in Africa, small-scale aquaculture for rural families will only succeed if it is ecologically based and integrated into the diverse enterprises which are common to smallholder farms. The usual commodity-oriented approach to aquaculture has limited applicability in communities where farming is operating at or close to subsistence, and where cash incomes are too low to purchase the required feed and fertilizer for fish culture. The World Resources Institute and the International Institute for Environment and Development (1988) stated that average per caput GNP in Africa was only US$300 in the late 1980s

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and the position has worsened since then. In Malaŵi, which is the subject of this paper, the average income of rural families is just US$130 per year and such smallholders comprise 75% of the population (Experiments in International Living 1991).

Low incomes are coupled to problems of land availability. In Malaŵi, the average population density per square kilometer of arable land is 169 persons and this will rise to 242 by 2012 if population growth continues at its present rate (3.3% per annum; Experiments in International Living 1991). Currently, a third of smallholders cultivate less than 0.5 ha. Such pressure on land will require new ways to regenerate environments and marginal areas so that household income and nutrition can be improved (Lightfoot 1990).

Dommen (1988) pointed out that African agricultural production under growing population pressure is a constant struggle to maintain soil fertility and crop yields as fallow periods shorten and resources are used more intensively. Intensification of recycling of farm resources and integration of agricultural enterprises will be essential in order to maintain farm productivity when available arable land is shrinking. Aquaculture may provide a partial solution by aiding in efficiency of resource recycling and farm integration.

However, small-scale aquaculture has not been successful in alleviating nutritional problems in rural African communities or indeed improving the cash income of farming families (Noble and Costa-Pierce 1992). This is because aquaculture has often been introduced as a stand-alone enterprise in the farming system requiring its own suite of off-farm inputs in order to produce a reasonable crop of fish (GOPA 1987). With the land and income constraints experienced by many smallholder farmers, stand-alone enterprises are too risky and capital intensive. Therefore, aquaculture must operate within the resource base of the smallholder and act as a preliminary step to intensification of efficient utilization of on-farm bioresidues and integration of farm enterprises.

On-farm Bioresidues as Feed or Fertilizer for Aquaculture

On Malaŵian farms, there is a range of bioresidues which could act as suitable feed and/or fertilizer inputs for ponds. These are crop wastes, livestock manure and vegetation from fallow land. Ash from cooking fires also has potential as a source of nutrients for improving pond fertility.

Materials and Methods

Assessment of Bioresidues

MAIZE RESIDUES

In 1989, a small survey was conducted to establish availability of on-farm resources for aquaculture on 20 smallholder farms in Zomba District, Southern Malaŵi. These smallholdings all had crop-based farming systems with maize as the main food staple. Small-scale fish farming was also a minor enterprise on these farms. In total, 52x50-m^2 quadrats were placed in maize fields on these 20 smallholdings. The biomass of maize seeds and residues were measured from each quadrat. The residues were maize cobs, sheaths, stalks and maize bran (left after seeds have been pounded).

WEED RESIDUES

End-of-season biomass of weeds in 1989 was also measured in 42x50-m^2 quadrats on 15 farms. Six farms also

had fallow land on which weed biomass was estimated in 6x50-m^2 quadrats in the same way as for maize fields.

In 1990, five 50-m^2 quadrats were placed on "dambo" areas (low-lying land with high water table) on four farms. Six 50-m^2 quadrats were placed on fallow land with low water table on four farms. Productivity of weeds could be estimated because the length of the growth period was known in each case.

Production figures for weed growth were initially calculated as $kg \cdot ha^{-1} \cdot month^{-1}$ dry matter (DM) then converted to $kg \cdot ha^{-1} \cdot year^{-1}$ DM. Measurements were made during the wet season (1990) and then extrapolated to yearly production figures by assuming that high dry areas have a shorter growing season than low-lying damper areas.

For those weeds growing in higher parts of a farm where surface soils dry out in May and remain dry till November, the production period was taken approximately as six months. For those weeds growing in low-lying soils where surface soil moisture persists till August, production period was taken as nine months. For example, monthly grass production for low-lying areas was estimated as 745 $kg \cdot ha^{-1}$ DM which converts to 6,705 $kg \cdot ha^{-1} \cdot year^{-1}$, not 8,940 $kg \cdot ha^{-1} \cdot year^{-1}$ if growing season is only nine months.

DRY MATTER CONTENT AND NITROGEN VALUES

All biomass and production estimates for maize products and weeds were converted to dry weights by measuring moisture content of samples of plant material and drying them at 80°C until constant weight was achieved. Protein nitrogen content of maize residues and grasses was estimated using data from Miller (1975) and Gohl (1981).

ASH PRODUCTION IN HOUSEHOLDS

Ash production from cooking fires was estimated for six households. Total ash was collected and weighed after a period of three to seven days. Samples were taken to obtain dry weight measurements. Phosphorus content was estimated using values from Jamu (1990). The number of people involved in producing ash was noted so that ash production per head per day could be estimated.

LIVESTOCK MANURE PRODUCTION

This was extremely difficult to measure and the figures need to be treated with caution. Chicken manure production was estimated from four smallholdings by measuring accumulated manure over a period of days and converting to $kg \cdot day^{-1}$ DM. This could only be done for chicken house manure accumulated overnight.

RESOURCE PATHWAYS

Farmers were asked to indicate what happened to the maize, weed, ash and manure residues on their farms and the pathways for these resource flows were diagrammed.

Results

Tables 1 to 4 show the amount of bioresidues present on the smallholder farms in this survey. The most common bioresidue was maize stover (both hybrid and local varieties combined) with a mean production of approximately 2.5 $t \cdot ha^{-1} \cdot year^{-1}$ (range: 470-4,930). This presents about 186 $kg \cdot ha^{-1} \cdot year^{-1}$ DM nitrogen. This material was utilized as mulch and turned back into the soils or burnt at the end of the dry season when maize gardens were being

Table 1. Average production of maize residues on farms with fishponds in Malaŵi, April-May 1989.

Residues	Local maize[a]		Hybrid maize[b]	
	$kg \cdot ha^{-1} \cdot year^{-1}$	kg/farm	$kg \cdot ha^{-1} \cdot year^{-1}$	kg/farm
Stovers	2,479 (185)*	2,552 (190)*	2,789 (208)*	3,068 (229)*
Sheaths	181 (13)*	172 (13)*	122 (9)*	26 (2)*
Cobs	281 (21)*	152 (11)*	485 (36)*	321 (24)*
Bran	291 (6)+	297 (6)+	660 (13)+	466 (9)+

[a]Sample of 17 farms, 44x50-m^2 quadrats.
[b]Sample of three farms, 8x50-m^2 quadrats.
( )=Crude protein (CP) nitrogen estimated to nearest 1 kg.
*CP content taken as 46.6% per 1 kg DM (Miller 1975).
+CP content taken as 12.2% per 1 kg DM (Miller 1975). Conversions to kg DM nitrogen = CP content/6.25. All figures are dry matter (DM) estimates.

Table 2. Average terrestrial weed biomass on farms with fishponds in Malaŵi, June-July 1989.

Environment	Herbaceous plants		Grasses	
	$kg \cdot ha^{-1}$	kg/farm	$kg \cdot ha^{-1}$	kg/farm
Maize fields[a]	1,128	1,236	120 (2)	191 (2)
Fallow land[b]	322	41	4,252 (54)	2,516 (32)

[a]Sample of 15 farms, 42x50-m^2 quadrats.
[b]Sample of 6 farms, 6x50-m^2 quadrats.
( ) = Crude protein (CP) nitrogen estimated to nearest 1 kg. CP content taken as approximately 8.0% per 1 kg DM (Gohl 1981). Conversions to kg DM nitrogen = CP content/6.25. All figures are dry matter (DM) estimates.

Table 3. Production of grasses and herbaceous weeds on smallholder farms in Malaŵi, 1990.

Plants	Low land/high water table[a]	High land/low water table[b]
	$kg \cdot ha^{-1} \cdot year^{-1}$	$kg \cdot ha^{-1} \cdot year^{-1}$
Grasses	6,705±2,763 (86)	4,026±1,740 (52)
Herbaceous weeds	1,602±1,233	426± 222

[a]Sample of four farms, 5x50-m^2 quadrats (assuming nine months growth).
[b]Sample of four farms, 6x50-m^2 quadrats (assuming six months growth).
( ) = Crude protein nitrogen estimated to nearest 1 kg. CP content taken as approximately 8.0% per 1 kg DM (Gohl 1981). Conversions to kg DM nitrogen = CP content/6.25. All figures are dry matter (DM) estimates.
± = Standard deviations.

Table 4. Ash production from cooking fires on six smallholder farms in Malaŵi in dry season, 1990.

Farms	Number of people	Number of days	Total ash collected (kg DM)		Ash/head ($kg \cdot day^{-1}$)	
1	2	3	4.2		0.7	
2	2	2	1.1		0.3	
3	8	6	9.5		0.2	
4	10	4	11.5		0.3	
5	6	3	1.7		0.1	
6	16	7	26.9		0.2	
Mean	7.3	4.2	9.2	(0.5)	0.3	(0.02)
SD	4.9		8.8		0.2	

( ) = kg of phosphorus. Phosphorus content estimated at 5.6% per kg DM ash (Jamu 1990).

prepared for planting. Fig. 1 shows the major pathways for maize residues on farms in this study. The other residues (cobs and sheaths) were used as fuel for cooking.

Bran, which is the main food and fertilizer input for ponds, varied in production depending on whether farmers grew local or hybrid maize. Local varieties of maize produce less bran (mean: 290; range: 15-689 $kg \cdot ha^{-1} \cdot year^{-1}$ DM) than hybrid varieties (mean: 660; range: 230-910 $kg \cdot ha^{-1} \cdot year^{-1}$ DM). Bran as a nitrogen source is also relatively poor varying from 6 $kg \cdot ha^{-1} \cdot year^{-1}$ (local) to 13 (hybrid).

Weed biomass and production were fairly high on all farms. In maize fields, end-of-season biomass for grasses and herbaceous plants reached almost 1.5 $t \cdot ha^{-1}$ and on fallow land almost 4.4 $t \cdot ha^{-1}$ (fallow land only occurring on five farms). These figures are probably underestimates of production because they were measured after the maize harvests in May-June and do not take into account the weed production removed by farmers early in the maize growing seasons.

Table 3 shows estimates for grass and herbaceous weed production for different areas of farms in 1990. Dry fallow land productivity (4.5 $t \cdot ha^{-1} \cdot year^{-1}$ DM) is very similar to the end-of-season biomass estimates in 1989 (4.4 $t \cdot ha^{-1}$ DM) for fallow land (see Table 2). "Dambo" areas have the highest production of weeds (8.4 $t \cdot ha^{-1} \cdot year^{-1}$ DM) reflecting the greater persistence of high soil moisture content throughout the year. Nitrogen availability was estimated for grasses and varies from 2 $kg \cdot ha^{-1} \cdot year^{-1}$ (maize fields) to 86 $kg \cdot ha^{-1} \cdot year^{-1}$ (low-lying fallow land).

Mean ash production per household of five persons per year was 548 kg, i.e, 31 kg of phophorus usable to fertilize ponds (Table 4).

A mean production of 0.7 $t \cdot year^{-1}$ per farm (SD: 0.4) of chicken manure were produced by the four farms monitored in the survey which is equivalent to 25 kg of nitrogen.

Discussion

From the survey of farm resources, it is clear there are materials which might prove useful as sources of nitrogen and phosphorus for input to fishponds. The next problem is to determine if the farms have sufficient quantities of bioresidues to meet the nutrient demands of small-scale aquaculture, without unduly affecting use of bioresidues for other farm enterprises.

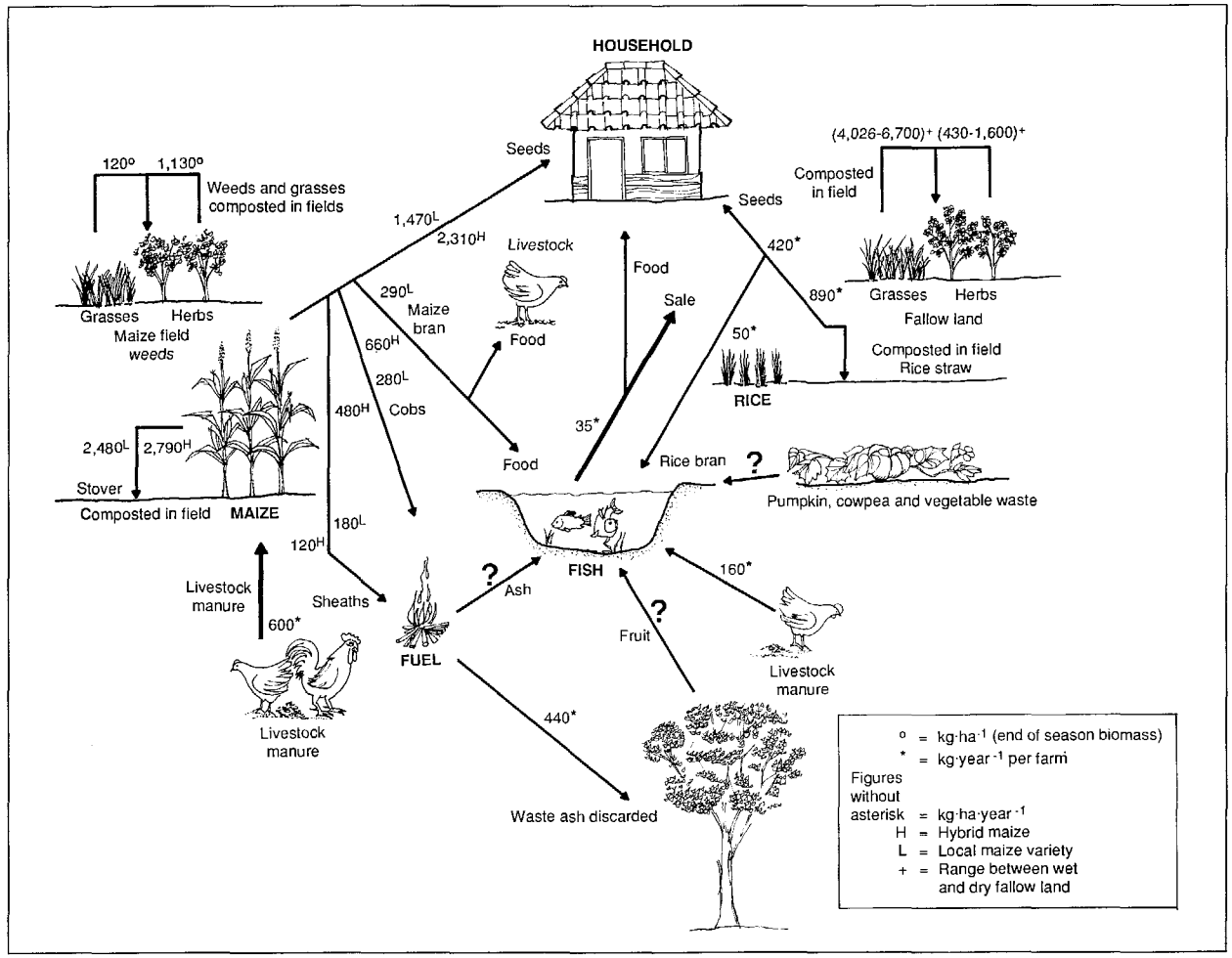

Fig. 1. Composite bioresource flow diagram for a Malaŵian smallholder farm practicing aquaculture.

Maize and Grass as Sources of Nitrogen for Ponds

Chikafumbwa (1990 and this vol.) demonstrated that napier grass (*Pennisetum purpureum*) input of 100 $kg \cdot ha^{-1} \cdot day^{-1}$ DM gave a relatively high on-station fish yield (1.4 $t \cdot ha^{-1} \cdot year^{-1}$) for *Tilapia rendalli* and *Oreochromis shiranus* in polyculture, slightly higher than on-farm (mean: 1 $t \cdot ha^{-1} \cdot year^{-1}$, Noble and Costa-Pierce 1992). Chikafumbwa stated that this input rate of napier grass is equivalent to approximately 1.6 $kg \cdot ha^{-1} \cdot day^{-1}$ N (nitrogen). Edwards (1987) recommended that the minimum input rate for nitrogen is about 8 $kg \cdot ha^{-1} \cdot day^{-1}$ N to support good algal growth in fishponds. However, Edwards (1987) stated that if low quality plant material is used as a nitrogen source, then maximum input rate should be adjusted to the equivalent of 4 $kg \cdot ha^{-1} \cdot day^{-1}$ N to prevent organic overloading of the pond.

The average size of ponds in Zomba District where the bioresidue survey was carried out was 338 m^2 (median: 196 m^2, SE: 34 m^2 for 209 ponds; Noble and Costa-Pierce 1992). Table 5 indicates the minimum land area which would be required to produce sufficient grass or maize residues to meet Chikafumbwa and Edwards' nitrogen input needs for aquaculture.

Excessive land areas are not necessary to produce sufficient maize stover residue to meet nitrogen input requirements for small fishponds. Between 0.1 and 0.3 ha of maize crops will supply enough crude protein nitrogen from stover residues to meet the annual needs of one 300- to 400-m^2 pond. If farmers rely solely on grass from fallow land to supply annual nitrogen input to ponds, then land requirements are higher, 0.3 to 1.1 ha for 300- to 400-m^2 ponds. Fallow areas are scarce because of pressure to use all available land for growing crops. Therefore, fallow land is unlikely to provide significant contributions of nutrients for pond systems. Obviously, if a farmer does have fallow areas, grasses and herbaceous weeds can provide a nutrient supplement in combination with crop residue inputs. Clearly, for many farms, sufficient nitrogen could be generated by maize stovers to supply the input needs for small-scale aquaculture.

Maize Bran Inputs for Fishponds

Chikafumbwa (this vol.) studied the effect of combined maize bran and napier

Table 5. Land area requirements needed to produce grass and maize residues sufficient to meet recommended annual nitrogen (N) pond input rates for tilapia polyculture.

	Maize area (ha)		Fallow land grass area (ha)	
Pond size (m^2)	A	B	A	B
100	0.1	0.03	0.3	0.1
200	0.2	0.1	0.5	0.2
300	0.2	0.1	0.6	0.3
400	0.3	0.1	1.1	0.4
500	0.4	0.2	1.4	0.5
1,000	0.8	0.3	2.7	1.1

A = land area needed based on input rate of 4 $kg \cdot ha^{-1} \cdot day^{-1}$ N (Edwards 1987).
B = land area needed based on input of 1.6 $kg \cdot ha^{-1} \cdot day^{-1}$ N (Chikafumbwa, this vol.).
Nitrogen data from Tables 1, 2 and 3 were used to compute area requirements.

grass inputs to ponds. With a grass input of 100 $kg \cdot ha^{-1} \cdot day^{-1}$ DM and maize bran input of approximately 24 $kg \cdot ha^{-1} \cdot day^{-1}$ DM, ponds yielded the equivalent of 3 $t \cdot ha^{-1} \cdot year^{-1}$ of *O. shiranus* and *T. rendalli* in polyculture. This is between two and three times current harvest yields from smallholder ponds.

Farms growing local maize varieties produce about 290 $kg \cdot year^{-1}$ of bran and those growing hybrid, 660 $kg \cdot year^{-1}$ (see Table 1). Table 6 shows the maize crop areas needed to support Chikafumbwa's input rates. If farmers are only growing local maize, then relatively large land areas are needed to supply sufficient bran (e.g., 0.9 ha for a 300-m^2 pond). In contrast, hybrid maize areas can be small (0.4 ha for a 300-m^2 pond) because hybrid varieties produce large quantities of bran on pounding.

Cooking Fire Ash as a Phosphorus and Buffering Source for Ponds

Jamu (1990) demonstrated that cooking fire ash was an effective liming agent for ponds and could act as a phosphorus source. Application of ash at a rate of 0.75 $t \cdot ha^{-1}$ over a two-week period neutralized the decrease in pH created by input of maize stover at 300 $kg \cdot ha^{-1} \cdot day^{-1}$ DM. As mentioned before, Chikafumbwa (1990 and this vol.) found that 100 $kg \cdot ha^{-1} \cdot day^{-1}$ DM, in combination with maize bran, gave the highest fish yields (3 $t \cdot ha^{-1} \cdot year^{-1}$). Assuming a pond has no natural buffering capacity to counteract lowering of pH by decomposition of grass, then to neutralize Chikafumbwa's grass input rates, 6 $t \cdot ha^{-1} \cdot year^{-1}$ of ash would be required. For a 500-m^2 pond, a household would only have to produce 300 $kg \cdot year^{-1}$ of ash. Average household sizes in Malaŵi are approximately five persons (ICLARM and GTZ 1991). Such a household would produce about 550 $kg \cdot year^{-1}$ of ash based on Table 4 and Jamu (1990). This ash production is well within the requirements laid down by Jamu (1990) for ponds with no natural buffering capacity. However, most ponds will have some buffering capacity, so ash production needs will probably be much lower than indicated above.

Ash is also a source of phosphorus. Edwards (1987) stated that minimum input rates for phosphorus to sustain algal growth is 0.8 $kg \cdot ha^{-1} \cdot day^{-1}$ (i.e., 292 $kg \cdot ha^{-1} \cdot year^{-1}$). Again, ash production by an average household can produce sufficient phosphorus (P) (31 $kg \cdot year^{-1}$ P) to meet the requirements of a pond up to 1,000 m^2 (29 $kg \cdot year^{-1}$ P).

Integrating Agriculture and Aquaculture

Consideration has only been given to necessary inputs which could act as feed or fertilizer for ponds. However, aquaculture cannot be operated just as an isolated enterprise receiving farm bioresources and not recycling materials back to other farm enterprises. Such an arrangement is inefficient and will ultimately lead to resource depletion in other parts of the farming system.

For example, Chimatiro (1991) showed clearly that ponds act as nutrient sinks. He looked at several farms and monitored all inputs to fishponds comparing them with output of fish. For four ponds, mean ecological efficiency of conversion of plant material into fish was 0.8% in terms of energy. Much of the plant inputs were probably being incorporated into the detritivore subsystem, thus leading to accumulation of nutrients in the pond mud.

Chimatiro (1991) verified this by using pond mud as fertilizer for cabbages. Mud came from ponds that had received napier grass as the sole input. Cabbage

Table 6. Land area needed to produce maize bran sufficient to meet annual pond input requirements for tilapia polyculture.

Pond size (m^2)	Land area of maize required to produce bran	
	Area of local maize[a]	Area of hybrid maize[b]
100	0.3	0.1
200	0.6	0.3
300	0.9	0.4
400	1.2	0.5
500	1.5	0.7
1,000	3.0	1.3

[a]Local maize variety, bran production: 291 $kg \cdot ha^{-1} \cdot year^{-1}$.
[b]Hybrid maize variety, bran production: 660 $kg \cdot ha^{-1} \cdot year^{-1}$.
Input rate from Chikafumbwa (1990): 8.8 $t \cdot ha^{-1} \cdot year^{-1}$.

yields on pond mud were equivalent to 120 $t \cdot ha^{-1} \cdot year^{-1}$ compared with 76 $t \cdot ha^{-1} \cdot year^{-1}$ for cabbages grown on unfertilized top soil. Likewise, Chikafumbwa (unpubl. data) demonstrated that very high yields can be achieved for vegetables receiving pond mud (i.e., approximately 200 $t \cdot ha^{-1} \cdot year^{-1}$ of economic yield for Chinese cabbage).

Vegetable waste can also be recycled back into ponds to act as feed and fertilizer. In the Chinese cabbage experiment, 74 $t \cdot ha^{-1} \cdot year^{-1}$ in waste leaf was produced. If a farmer had at least 100 m^2 of cabbage beds, approximately 740 kg of leaf waste would be generated each year. Assuming crude protein content to be approximately 2.5%, this would only provide 3 $kg \cdot year^{-1}$ N. Although this appears to be insignificant in terms of potential nitrogen input for a pond, the leaves may contribute other nutrients to the system.

Chimatiro (1991) and Chimatiro and Costa-Pierce (this vol.) demonstrated that waste cabbage or pumpkin leaves input at a rate of 50 $kg \cdot ha^{-1} \cdot day^{-1}$ DM into tanks with a polyculture of *O. shiranus* and *T. rendalli* gave mean yields of just over 1 $t \cdot ha^{-1}$ for a 134-day growth period. In Malaŵi, temperatures are favorable for fish growth for eight months of the year so the fish yield figures represent approximately 2 $t \cdot ha^{-1} \cdot year^{-1}$ when adjusted for eight months growth. These harvest yields are almost twice those from smallholder ponds.

Linking vegetables and ponds in this way can also improve income to farmers. Chikafumbwa found that sales of Chinese cabbages generated an income of US$25/month when sold locally in villages. Current smallholder incomes in Malaŵi average US$11/month (Experiments in International Living 1991). Thus vegetable-fish integration has the potential to improve incomes significantly for farmers with water and land resources to incorporate fishponds into their farm system.

Other types of aquaculture-agriculture integration are developing in Malaŵi. Farmers are just beginning to grow rice and fish together. Yields from rice-fish ponds vary from 2-4 $t \cdot ha^{-1} \cdot year^{-1}$ for rice and from 1.5-2.4 $t \cdot ha^{-1} \cdot year^{-1}$ for fish (Noble and Costa-Pierce 1992). Rice yields from fishponds are within the yield ranges (i.e., mean: 3.5 $t \cdot ha^{-1} \cdot year^{-1}$) expected from ricefields

receiving fertilizer and irrigation (Zomba Rural Development Project 1990). However, rice yields from fishponds are underestimates because figures represent only one cycle of rice. Farmers are now attempting to grow second crops of rice during the dry season. This will probably raise annual yields of rice from integrated rice-fish enterprises above 3 $t \cdot ha^{-1} \cdot year^{-1}$.

A 90-kg sack of unpounded rice costs US$18 (50 Malaŵian kwacha) at government agricultural centers. With the current yields from rice-fish ponds, farmers can expect to earn US$400 to $800 $\cdot ha^{-1} \cdot year^{-1}$ for the rice irrespective of any money earned from fish sales.

Concluding Remarks

This paper has tried to demonstrate that within maize-based farming systems, there are materials which have the potential to be used as feed and fertilizer for small-scale aquaculture. Fig. 1 shows that at present many materials such as cooking-fire ash, rotten fruit, vegetable waste and possibly some animal manure could be recycled through fishponds.

Given the limited economic resources of most Malaŵian farmers, more efficient utilization of on-farm bioresidues presents the main hope for developing sustainable aquaculture. However, a major problem for farmers is maintaining soil fertility and agricultural production as arable land availability declines. Therefore, aquaculture cannot be developed in isolation from other enterprises in the farming system. If food security is to be maintained for rural farmers, where they adopt aquaculture, it must contribute to the rehabilitation of the farming system by integration with other farm enterprises. Such interlinkages will aid in more efficient utilization and recycling of nutrients within the farm system.

Acknowledgements

We would like to thank Mr. Sloans Chimatiro for his assistance in the field and in computer entry of data; Mr. Fredson Chikafumbwa and Mr. Daniel Jamu for their very helpful comments on the manuscript; Mr. Fredson Chikafumbwa also for the use of his data on vegetable-fish integration; the ICLARM-GTZ staff at the National Aquaculture Center for their backup support; and the Malaŵi Fisheries Department extension staff for their assistance with field work.

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Effects of Pond Depth and Mechanical Mixing on Production of *Oreochromis niloticus* in Manured Earthen Ponds

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SZYPER, J.P. and K.D. HOPKINS. 1996. Effects of pond depth and mechanical mixing on production of *Oreochromis niloticus* in manured earthen ponds, p. 152-159. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

An experiment to assess the effects of water mixing on production of Nile tilapia *(Oreochromis niloticus)* in fertilized earthen ponds was performed at the Asian Institute of Technology in Thailand. Male fingerlings stocked at 3 fish·m^{-3} grew to final weights of 106-233 g in 173 days in nine ponds of approximately 370-m^2 individual surface area. Yields were statistically indistinguishable among treatments, averaging 5.4 t·ha^{-1}·$year^{-1}$ in three ponds of 1.5 m depth whose water was mixed for two hours each day; 6.7 t·ha^{-1}·$year^{-1}$ in three similar but unmixed ponds; and 6.9 t·ha^{-1}·$year^{-1}$ in three unmixed ponds of 0.9 m depth. Survival was significantly lower in the deep mixed ponds (72% compared with 91-93%). Fish grew more rapidly and had larger final weights in the shallow unmixed ponds, which had larger standing stocks of phytoplankton, as measured by chlorophyll *a*, and greater rates of gross dissolved oxygen (DO) production per unit volume during most of the growth period. Treatments did not differ in gross DO production per unit area. Daily mixing produced higher nighttime bottom oxygen concentrations up until 2300 hours, but did not change overnight DO minima. No treatment-related differences in ammonia concentrations or other water quality parameters were found. The oxygen-conserving effect of mixing was not effective in enhancing production of this species, which grows and survives well in unmixed ponds, tolerating or avoiding waters of low oxygen content. Mixing and aeration strategies must be examined carefully for both detrimental and beneficial effects.

Introduction

Tilapias are grown successfully in waste-fed ponds in warm climates, despite characteristic pond conditions that would be unsuitable for other species. Warmwater ponds tend to exhibit diel cycles of daytime density stratification and nighttime convective mixing. These create the potential for severe depletion of dissolved oxygen (DO), particularly near the pond bottom (Boyd 1990), but tilapias generally suffer little or no mortality in such ponds because they are tolerant of low DO and able to relocate from oxygen-depleted zones. Growth may nonetheless be inhibited under suboptimal oxygen regimes.

Stratification may, in addition, affect photosynthetic production of organic matter, which is an important source of the cultured animals' food. Under certain conditions, stratification may be beneficial in facilitating growth of the phytoplankton community; on the other hand, depletion of nutrient elements in isolated surface waters may limit upper-layer production of both organic matter and oxygen. Costa-Pierce and Pullin (1989) discussed observed and potential effects of stratification and mixing (including bottom turbation) on fish production. They recognized the species-specific nature of the relationships among fish production, production of microbial biomass and mixing, which will be discussed below.

Szyper et al. (1991) observed that: (1) in earthen ponds of depths ranging from 0.6 to 1.5 m, yields of *Oreochromis niloticus* were proportional to total stocking and fertilization inputs; and that (2) daytime pond mixing by internal water circulation can conserve DO during the day and improve nighttime concentrations near pond bottoms (Szyper and Lin 1990). Because ponds produced yields proportional to inputs, deeper ponds produced more fish per unit area, despite being more frequently and severely stratified. It was therefore reasonable to ask whether mixing might enhance production in deeper ponds by ameliorating conditions of low DO and potential nutrient-limitation of primary production. This paper reports the results of an experiment aimed at discerning the effects of such mixing on production of Nile tilapia.

Materials and Methods

The experiment was conducted in nine earthen ponds of about 370-m^2 surface area, maintained at depths of 0.9 and 1.5 m, at the Asian Institute of Technology near Bangkok, Thailand. Triplicate ponds for each of three treatments were stocked with juvenile male *O. niloticus* of 10-13 g individual weight (Chitralada strain: Tangtrongpiros 1988), hormonally sex determined following Guerrero (1979) and fertilized weekly with chicken manure, industrial grade urea and triple superphosphate (TSP), as in earlier experiments reported by Szyper et al. (1991). Stocking and fertilization rates (3 fish$\cdot m^{-3}$ and 3.5 gN$\cdot m^{-3} \cdot week^{-1}$, respectively) were proportional to pond volume.

Three ponds were maintained at 0.9 m depth and never mixed mechanically; three deeper ponds (1.5 m) were also left unmixed. Three 1.5-m deep ponds were mixed from 1500 to 1700 hours each day. An 0.5-hp (373 W) submersible pump took water in from 80-cm depth (below the thermocline) and discharged it horizontally at 10 cm, from a pipe of 6.4-cm diameter.

Standard methods were used to estimate physical, chemical and biological properties of the pond ecosystems (APHA 1985) with minor modifications (Pond Dynamics/Aquaculture [PD/A] CRSP 1989). Three pooled 90-cm water column samples were taken every two weeks from each pond and analyzed for total ammonia, oxidized nitrogen (nitrate plus nitrite), total Kjeldahl nitrogen, orthophosphate (soluble reactive phosphorus), total P, chlorophyll *a*, total suspended solids (TSS) and total volatile solids (TVS). Biweekly diel sampling programs determined DO and temperature at three depths (25 cm below surface, midwater, and 25 cm above bottom), six times during the diel cycle (at 0600 hours, 0900, 1200, 1600, 1800 and the following 0600). Gross photosynthetic oxygen production was estimated from each biweekly diel observation series (n=14 dates), according

to a modification of the free-water method of Hall and Moll (1975).

Fish were sampled monthly for length and weight (≥30 individuals taken by seining); total crop weight was taken at harvest. A short-term study of diel cycles of temperature and DO was conducted during the latter half of the trial using an automated monitoring system (Szyper and Lin 1990). Regression analysis and single-factor ANOVA were performed as described by Sokal and Rohlf (1981). Significance is referred to P=0.05 unless otherwise stated. Further analyses of effects of various factors on fish growth were performed using expanded Gulland and Holt plots (1957; see also Hopkins et al. 1988).

Results

Treatment mean values (n=3 ponds) of survival, final individual fish weight, extrapolated net yield and yield/input efficiency are presented in Table 1.

Survival ranged from 66.7 to 100% through the 173-day experiment; the mixed deep ponds showed significantly lower survival than the others (single-factor ANOVA). Final individual fish weights (pond means) ranged from 106 to 233 $g \cdot fish^{-1}$ and were significantly greater in the shallower unmixed ponds. Net yields, extrapolated to a hectare and yearly basis, ranged from 4.4 to 9.0 $t \cdot ha^{-1} \cdot year^{-1}$, and did not differ significantly among treatments. Yield/input efficiencies ranged from 1.64 to 5.41 kg $fish \cdot kgN^{-1}$, with the mean for shallower unmixed ponds significantly greater than for the other treatments.

The patterns of fish growth (Fig. 1) showed that growth was more rapid in the shallow unmixed ponds during most of the trial and that growth in both sets of deep ponds essentially ceased between three and four months while continuing in the shallow ponds.

Temperature regimes differed among treatments only in the degree of daytime stratification between surface and bottom waters. There were no treatment-related differences in mean (for three depths) water temperatures taken at 0930 hours on the days of the diel samplings. The mean temperature for the experimental period was 27.6°C (SD=2.1°C; range=22.9-31.6).

There were few treatment-related differences among pond DO regimes. The samples taken at 0930 hours discussed above showed (1) statistically indistinguishable mean DO concentrations among ponds of all treatments, at all three depths; and (2) similar ranges during the trial. Mean surface DO ranged from 4.5 to 6.2 $mg \cdot l^{-1}$; mean bottom concentrations ranged from 1.9 to 2.4 $mg \cdot l^{-1}$. The deep circulated ponds produced the lowest values in these ranges, the shallower uncirculated ponds the highest.

Diel patterns of DO were similar among unmixed ponds of both depths, with characteristic bottom DO levels <1 $mg \cdot l^{-1}$ during most hours of the day and night, and diurnal increases in surface waters ranging to maxima >20 $mg \cdot l^{-1}$ (Szyper and Lin 1990). In the mixed ponds, initiation of mixing at 1500 hours promptly raised bottom DO concentrations from <1 $mg \cdot l^{-1}$ to 2-4 $mg \cdot l^{-1}$ with levels >1 $mg \cdot l^{-1}$ maintained up until 2300 hours.

Mean area specific gross photosynthetic production of DO was similar among ponds of all treatments, averaging approximately 16 g $O_2 \cdot m^{-2}$ (Fig. 2A). Chlorophyll *a* (Chl) concentrations were greatest in the shallower ponds throughout the trial (Fig. 2B), as was gross DO production per unit volume (Fig. 2C). This pattern was also observed for total suspended solids and Secchi disk depth. Nutrient analyses showed concentrations of all dissolved forms of N and P to be continuously greater than

Table 1. Survival, growth, yield and yield efficiency during growth experiments with Nile tilapia (*Oreochromis niloticus*) in small ponds of different depths and with or without mixing. For details of treatments, see text. The data presented are the means and standard deviations (SD) from triplicate ponds, stocked at 3 fish·m^{-3}. Treatment codes are: DM = deep (1.5 m), mixed; D = deep (1.5 m), not mixed; and S = shallow (0.9 m), not mixed.

Treatment	Survival (%)		Final individual weight (g)		Yield (kg·ha^{-1}·year^{-1})		Yield/input efficiency (kg fish·kgN^{-1})	
DM	76.0		124.0		5,769		2.15	
	66.7		113.0		4,431		1.64	
	73.9		132.0		6,079		2.21	
mean (SD)	72.2	(4.9)	123.0	(9.5)	5,426	(876)	2.00	(0.31)
D	100.0		106.0		6,870		2.55	
	88.1		117.0		6,766		2.47	
	91.3		115.0		6,497		2.40	
mean (SD)	93.1	(6.2)	112.7	(5.9)	6,711	(192)	2.48	(0.08)
S	85.7		187.0		6,223		3.74	
	98.8		233.0		9,004		5.41	
	88.0		144.0		5,485		3.30	
mean (SD)	90.8	(7.0)	188.0	(44.5)	6,904	(1,856)	4.15	(1.11)

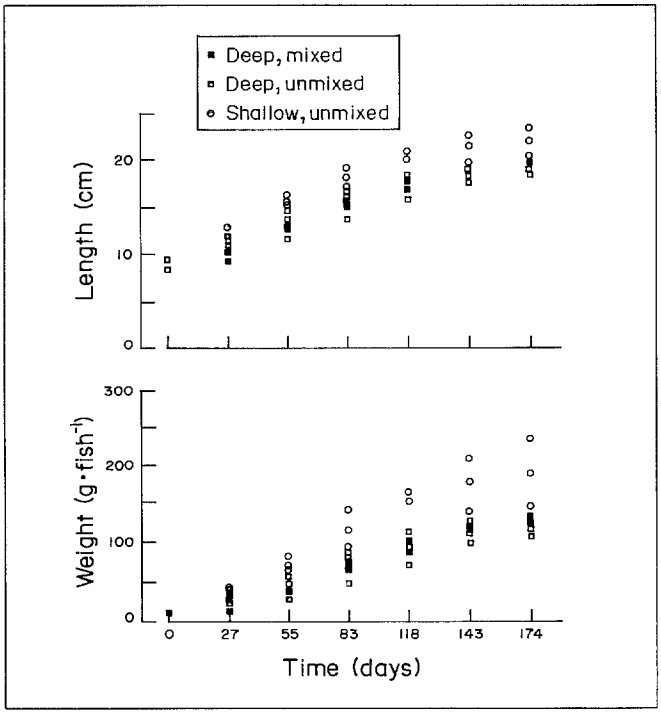

Fig. 1. Mean growth patterns of Nile tilapia (*Oreochromis niloticus*) in terms of length and weight during experiments in small ponds of deep and shallow depths, and with and without mechanical mixing. See text for details of treatments. Points are means for samples of ≥30 fish from a single pond; data for each pond is plotted, with some points overlapping.

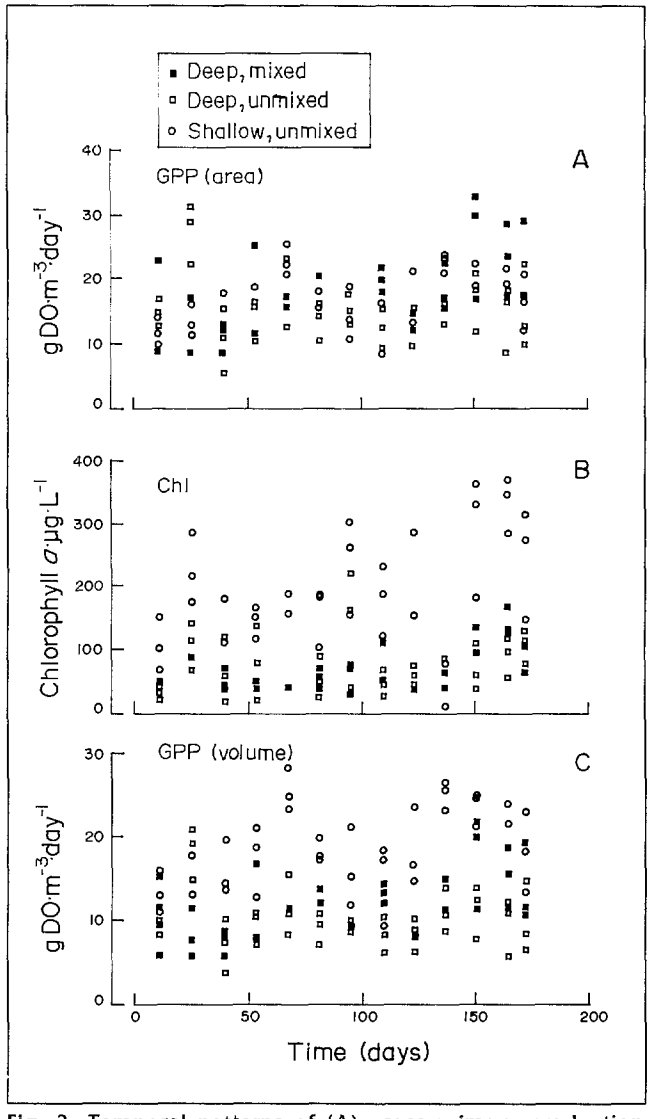

Fig. 2. Temporal patterns of (A) gross primary production of DO per unit area; (B) chlorophyll *a* (Chl) concentration; and (C) gross primary production of DO per unit volume during experiments in small ponds of deep and shallow depths, and with and without mechanical mixing. See text for details of treatments. Each point represents the DO production or chlorophyll concentration in a single pond on the date of biweekly sampling; all points are plotted, with some overlapping.

those known to limit phytoplankton growth; means were generally lower in the shallower ponds, probably reflecting the demand of the greater phytoplankton stocks.

Discussion

There was no evidence of a positive effect of pond mixing on tilapia production during this study. The

shallower unmixed ponds produced and maintained larger phytoplankton stocks than either mixed or unmixed deep ponds. These larger stocks presumably constituted a direct and/or indirect source of food for the fish (through the food web: Orachunwong et al. 1988). Phytoplankton stock and the primary production per unit volume are the only factors differing sufficiently to account for the more rapid fish growth in the shallower ponds.

Inclusion of volume-based gross primary production (GPP) substantially improved the fit of the Gulland and Holt model. Without consideration of GPP, the fit showed an R^2=0.32 (df=52). When GPP is added to the expression:

$$\Delta L/\Delta t = a + b_1 L + b_2(GPP)$$

where $\Delta L/\Delta t$ is the change in average length divided by the length of sampling period Δt; L is average length during the period; and GPP is the average gross DO production per unit volume during the period, the fit of this relationship is R^2=0.52 (df=51).

Mixing appeared to have little effect on most other ecosystem properties. The two sets of deep ponds exhibited overlapping ranges of measured parameters on most sampling dates. The enhancement of bottom DO levels by mixing appears to have been of little consequence to fish growth and production. If a mixing effect can be discerned here, it is a negative one on survival. The visible clay turbidity, seen in all ponds, does not account for this difference as levels of total suspended solids did not differ among treatments. It is possible that mixing in deep ponds inhibited early development of phytoplankton stocks during the first two months by destroying stratification during the time of highest DO concentration in unmixed ponds. The sampling regime used here does not permit detection of such an effect.

The results of this study are consistent with those reported by Szyper et al. (1991) from an earlier comparison among ponds of different depths, in that similar area-specific gross DO production was observed in ponds of all depths. Actual DO production rates were much greater (150-200%) in this study, however, as were the fish yield/input efficiencies, reflecting the increasingly effective fertilization protocols under development by the PD/A CRSP (Knud-Hansen et al. 1991). Fish yields during this study were somewhat greater than in the earlier work, but stocking densities were greater here in about the same proportion.

The two studies differed in relationships between pond depths and yields. Based on the earlier results, we expected yields from deep ponds to exceed those from shallow ones in proportion to the volume-indexed inputs (deeper ponds received more fish and fertilizer). Instead, we observed statistically indistinguishable area-specific yields from ponds of the two depths. During this study, ponds within treatments exhibited substantial variation in yield, rather greater than that seen in the earlier study, and there were fewer replicate ponds at each depth.

There has been relatively little study of mixing effects on pond production, particularly compared with the common demonstration of positive aeration effects. Lorio (1990) reported enhanced production of channel catfish in mixed ponds; Fast et al. (1988) found no significant effect of mixing on shrimp production. Costa-Pierce and Pullin (1989) discussed unpublished data indicating enhanced growth of *O. mossambicus* in ponds subjected to stirring of both water and sediments. All of these studies differed from the

present one in that prepared feeds were used.

The ambiguity attending effects of mixing on pond production can likely be attributed to the diversity of relationships among cultured species, pond microbial communities and diel oxygen regimes in ponds. The hypotheses presented by Costa-Pierce and Pullin (1989) are direct reminders of this point in many cases, and are indirectly related to it in others. The review presents considerable support for potential general enhancement of total microbial production by various mixing strategies. Different forms of mixing, however, affect diel oxygen regimes very differently, and these then interact with cultured species and their different sources of food, as the authors recognized. These latter factors might well confound the influence of a positive effect of mixing on total microbial production, even when the total microbial community is the major food source. A general connection between total microbial biomass production and enhancement of crop production should not be expected. Failure of the present controlled experimental application of water-column mixing to enhance either fish or autotrophic microbial production weighs against such generality, though total microbial production was not assessed here.

Fertilization experiments with Nile tilapia show increasingly that yields are connected with either inputs aimed at enhancement of autotrophic production, or with actual measured levels of such production (Knud-Hansen et al. 1991, 1993). The results presented here are consistent with this developing trend. One pertinent question to be addressed in our work is whether the nature of this connection is mediated more importantly through food itself or through diel oxygen regimes.

Acknowledgements

This research is a component of the Pond Dynamics/Aquaculture (PD/A) Collaborative Research Support Program (CRSP) supported by the US Agency for International Development, Grant No. DAN-4023-G-SS-7066-00, and by contributions from the University of Hawaii, the University of Michigan, Michigan State University, the Royal Thai Department of Fisheries and the Asian Institute of Technology. This is HIMB Contribution No. 907 and PD/A CRSP Accession No. 1091.

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Production of Florida Red Tilapia (*Oreochromis* sp.) Fry in Brackishwater Tanks under Different Feeding Regimes and Stocking Densities

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Abstract

Post-yolksac Florida red tilapia (*Oreochromis* sp.) fry (avg wt 0.010 g) were stocked in 530-l cylindroconical tanks at 5.7, 9.4 and 13.2·l^{-1} and reared for 30 days on an androgen-treated diet to induce sex reversal. Tanks were supplied with recirculated brackishwater (11-12 ppt). Final weights (avg 0.478 g), coefficients of variation (CV) of weights (avg 64%) and survival (avg 58%) did not differ significantly ($P>0.05$) among treatments, suggesting that higher densities are feasible. In another study, post-yolksac fry (avg wt 0.014 g) were stocked at 5.7 fish·l^{-1} and reared for 40 days under intermittent broadcast feeding (BF) or continuous automatic feeding (AF). Final weights, coefficients of variation of weights and survival were significantly ($P\leq0.01$, 0.05 and 0.05, respectively) higher in the AF group (4.04 g, 79% and 56%) than in the BF group (1.43 g, 70% and 38%). Thus, continuous AF produced higher fry survival and growth, but with greater variation in growth.

Introduction

Intensive saltwater tank culture may be preferable to large, open ponds for production of tilapia seed in tropical or arid coastal areas where freshwater resources are often limited (Hida et al. 1962; Uchida and King 1962; Al-Ahmad et al. 1988; Ridha and Cruz 1989; Ernst et al. 1991). The technical feasibility for commercial-scale production of Florida red tilapia (*Oreochromis* sp.) seed (eggs, yolksac fry and post-yolksac fry) in brackishwater (12-18 ppt) tanks was demonstrated in earlier studies (Watanabe et al. 1989; Ernst et al. 1991; Smith et al. 1991; Watanabe et al. 1992). Information concerning survival and growth of seed is also required for economic assessments.

Stocking densities and feeding regimens in fry culture tanks are often not quantified in tilapia hatcheries and many culturists "overstock" to compensate for cannibalism (Macintosh and De Silva 1984). High infrastructural and labor requirements of intensive tank culture necessitate that stocking densities and feeding regimes be optimized. The purpose of this study was twofold: (1)

to assess production of sex reversed Florida red tilapia fry under intensive, brackishwater tank culture at different stocking densities; and (2) to compare production under intermittent broadcast feeding and continuous automatic feeding, under commercial hatchery conditions.

Materials and Methods

The study was conducted at the Caribbean Marine Research Center tilapia hatchery (Ernst 1989) located on Lee Stocking Island (Exuma Cays, Bahamas).

The red tilapia broodstock used is a commercial strain known as Florida red tilapia, descendants of an original cross of *Oreochromis urolepis hornorum* and *O. mossambicus* (Behrends et al. 1982). Yearclass-one breeders were maintained in recirculating brackishwater (12 ppt) tanks (34.2-m^2) at a ratio of 180 females to 60 males. Seed, consisting of eggs, yolksac fry and post-yolksac (i.e., free-swimming) fry were collected by removing clutches from mouthbrooding females (Ernst et al. 1991) over a period of three days. Free-swimming fry were maintained in indoor 530-l tanks at 12 ppt, whereas eggs were incubated in 6.5-l upwelling jars supplied with recirculated brackish-water (12 ppt). Swim-up fry (i.e., swimming yolksac fry) were transferred daily to 530-l tanks until sufficient numbers were available for the experiments.

Production of Fry under Different Stocking Densities

Post-yolksac fry (avg wt 0.010 g; total length 9.6 mm) were stocked gravimetrically into 10, 530-l cylindroconical tanks at 5.7 fish·l^{-1} (3,000/tank) (three replicates), 9.4 fish·l^{-1} (5,000/tank) (four replicates) and 13.2 fish·l^{-1} (7,000/tank) (three replicates). Coefficients of variation (CV) of initial weights averaged 35% (Table 1). No significant ($P>0.05$) differences among treatment groups were observed for initial weight, length and CV of weights (Table 1). Average density ranged from 0.054 g·l^{-1} (5.7 fish·l^{-1}) to 0.128 g·l^{-1} (13.2 fish·l^{-1}) (Table 1).

Fry were fed Purina Trout Chow (48% protein) containing 17α-ethynyltestosterone (60 mg·kg^{-1} feed) for 30 days (Guerrero 1975) five times daily (at 0800, 1000, 1200, 1400 and 1600 hours) to 30-minute satiation and rations were adjusted daily depending upon the amount of food remaining on the tank bottom the next morning.

To monitor growth, a sample of 100 fish from each tank was weighed and measured (total length) on days zero and 30, whereas 50 fish were sampled on days 10 and 20. Sampled fish were not replaced. To assess survival, the number of fish remaining in each tank on day 30 was determined gravimetrically.

Rearing tanks were supplied with diffused aeration and water was recirculated through a biofilter (Ernst 1989). Maximum (30.4±0.1°C=mean± s.e.) and minimum (29.7±0.2°C) water temperatures, salinity (11.6±0.2 ppt) and dissolved oxygen (6.0±0.3 mg·l^{-1}) were measured daily. NH_4-N (0.38±0.19 mg·l^{-1}), NO_2-N (0.08±0.04 mg·l^{-1}), alkalinity (232±11 mg·l^{-1}) and pH (8.37±0.14) were measured weekly.

Production of Fry under Intermittent Broadcast Feeding and Continuous Automatic Feeding

In another study, post-yolksac stage (avg wt 0.014 g; total length 9.6 mm) were stocked into six, 530-l cylindroconical tanks at a density of 5.7 fish·l^{-1} (3,000/tank) and reared for 40 days under intermittent broadcast feeding (BF) or

Table 1. Growth performance of post-yolksac Florida red tilapia (*Oreochromis* sp.) reared for 30 days in brackishwater (12 ppt) tanks (530-l) at three stocking densities.

	Stocking density (fish·l^{-1})			
	5.7	9.4	13.2	P*
Initial				
Weight (g)	0.010 ± 0.001[1]	0.011 ± 0.001	0.010 ± 0.0003	-
Length (mm)	9.7 ± 0.2	9.7 ± 0.1	9.4 ± 0.1	-
CV of weights[2]	34.2 ± 1.6	33.4 ± 3.8	37.1 ± 8.2	-
Biomass (g·l^{-1})	0.054 ± 0.003[a]	0.099 ± 0.005[b]	0.128 ± 0.004[c]	< 0.001
Final				
Weight (g)	0.441 ± 0.033	0.485 ± 0.026	0.507 ± 0.003	-
Length (mm)	25.7 ± 0.3	26.3 ± 0.3	26.9 ± 0.2	-
CV of weights(%)[2]	68.3 ± 4.5	66.0 ± 1.8	57.7 ± 2.3	-
SGR (% bw·day^{-1})[3]	12.5 ± 0.4	12.8 ± 0.2	13.2 ± 0.1	-
Survival (%)	50.7 ± 4.7	65.9 ± 2.5	57.3 ± 6.1	-
FCR[4]	2.32 ± 0.26[a]	1.22 ± 0.07[b]	1.31 ± 0.11[b]	< 0.005
Biomass (g·l^{-1})	1.25 ± 0.07[a]	3.01 ± 0.17[b]	3.84 ± 0.39[c]	< 0.001
Yield (fish·l^{-1})	2.87 ± 0.26[a]	6.22 ± 0.23[b]	7.56 ± 0.08[c]	< 0.001

* Probability level when ANOVA was significant.
[1] Data are presented as means ± s.e. (n=3 for 5.7 and 13.2·l^{-1}; n=4 for 9.4·l^{-1}). Means within a row followed by different letters are significantly different (P<0.05, SNK).
[2] CV = coefficient of variation = s.d.·100/mean.
[3] SGR = specific growth rate = [(ln final wt - ln initial wt) ·100]/Δt.
[4] FCR = feed conversion ratio = dry wt fed/wet wt gain.

continuous automatic feeding (AF). Three replicate tanks were set up for each treatment. CV of the initial weights averaged 44.7% and biomass density averaged 0.08 g·l^{-1} (Table 2). No significant (P>0.05) differences among treatment groups were observed for initial weight, length, CV of the initial weights or biomass (Table 2).

Fish were fed the hormone-treated diet for 30 days, then a nontreated diet for the remainder of the study. Under the BF treatment, fish were fed five times per day (0800, 1000, 1200, 1400, and 1600 hours) by broadcasting feed manually over the top of the tank. At each feeding, fish were fed to satiation up to 30 minutes and rations were adjusted daily depending upon the amount of food remaining on the tank bottom the next morning.

Under the AF treatment, fish were fed continuously over a period of eight hours (0830-1630 hours) using automatic belt feeders (Zeigler Bros. Inc., Gardners, Pennsylvania, USA). Fish were fed to satiation, with rations adjusted as described above.

One hundred fish were sampled from each replicate tank on days 1, 10, 20, 30 and 40, weighed and measured, but were not replaced. To assess survival, the number of fish remaining in each tank on day 40 was determined gravimetrically.

Maximum (30.5±0.1°C) and minimum (30.1±0.1°C) water temperatures, salinity (11.4±0.3 ppt) and dissolved oxygen (6.0±0.1 mg·l^{-1}) were measured daily. NH_4-N (0.39±0.06 mg·l^{-1}), NO_2-N (0.08± 0.03 mg·l^{-1}), alkalinity (169±7 mg·l^{-1}) and pH (8.11±0.06) were measured weekly.

Treatment means for fish weights, lengths, CV of the initial weights, growth rates, survival, feed conversion ratios,

Table 2. Growth performance of post-yolksac Florida red tilapia (*Oreochromis* sp.) reared for 40 days in brackishwater (12 ppt) tanks (530 l) under intermittent broadcast feeding and continuous automatic feeding.

	Feeding method		
	Broadcast	Automatic	P*
Initial			
Weight (g)	0.012 ± 0.001[a]	0.015 ± 0.002	-
Length (mm)	9.43 ± 0.18	9.77 ± 0.35	-
CV of weight[b]	44.6 ± 9.4	44.7 ± 1.4	-
Biomass ($g \cdot l^{-1}$)	0.08 ± 0.002	0.08 ± 0.001	-
Final			
Wt (g)	1.43 ± 0.12	4.04 ± 0.35	< 0.01
Lt (mm)	39.9 ± 0.9	55.4 ± 1.2	-
CV of weight (%)[b]	69.6 ± 2.3	79.0 ± 0.01	< 0.001
SGR (% $bw \cdot day^{-1}$)[c]	11.9 ± 0.4	14.0 ± 0.2	< 0.05
Survival (%)	37.9 ± 1.9	56.0 ± 6.2	< 0.01
FCR[d]	2.87 ± 0.46	0.74 ± 0.03	< 0.05
Biomass ($g \cdot l^{-1}$)	3.25 ± 0.47	13.0 ± 0.56	< 0.001
Yield ($fish \cdot l^{-1}$)	2.24 ± 1.60	3.27 ± 0.34	< 0.05

*Probability level when a significant difference between treatments was observed.
[a]Data are presented as means ± s.e. (n=3).
[b]CV = coefficient of variation = s.d.·100/mean.
[c]SGR = specific growth rate = [(ln final wt - ln initial wt)·100]Δt.
[d]FCR = feed conversion ratio = dry wt fed/wet wt gain.

biomass and yield were compared by ANOVA. Multiple comparisons among means were performed by the Student-Newman-Keuls test. The significance level for all tests was $P \leq 0.05$.

Results

Production of Fry under Different Stocking Densities

Mean weights of fry reared under the different stocking densities were similar throughout the 30-day study. Final mean weights and lengths (avg 0.478 g, 9.6 mm), CV of weights (avg 64%) specific growth rates (avg 12.8% $bw \cdot day^{-1}$) and survival (avg 58%) did not differ significantly among treatments (Table 1).

Feed conversion ratio was significantly ($P<0.01$) higher among fish stocked at a density of $5.7 \cdot l^{-1}$ (2.32) than among those stocked at $9.4 \cdot l^{-1}$ (1.22) and $13.2 \cdot l^{-1}$ (1.31) (Table 1).

Final biomass increased significantly ($P<0.001$) with increasing stocking density and ranged from 1.25 $g \cdot l^{-1}$ (2.87 $fish \cdot l^{-1}$) to 3.84 $g \cdot l^{-1}$ (7.56 $fish \cdot l^{-1}$) (Table 1).

Production of Florida Red Tilapia under Intermittent Broadcast Feeding (BF) and Continuous Automatic Feeding (AF)

Mean weights of fish in the BF and AF groups remained similar through 20 days, but diverged thereafter (Fig. 1). After 40 days, mean weights and specific growth rates were markedly higher in the AF group (4.04 g, 14% $bw \cdot day^{-1}$) than in the BF group (1.43 g, 11.9% $bw \cdot day^{-1}$), with highly significant ($P<0.01$) differences observed among treatments (Table 2). CV of the final weights was significantly higher in the AF (79%) group

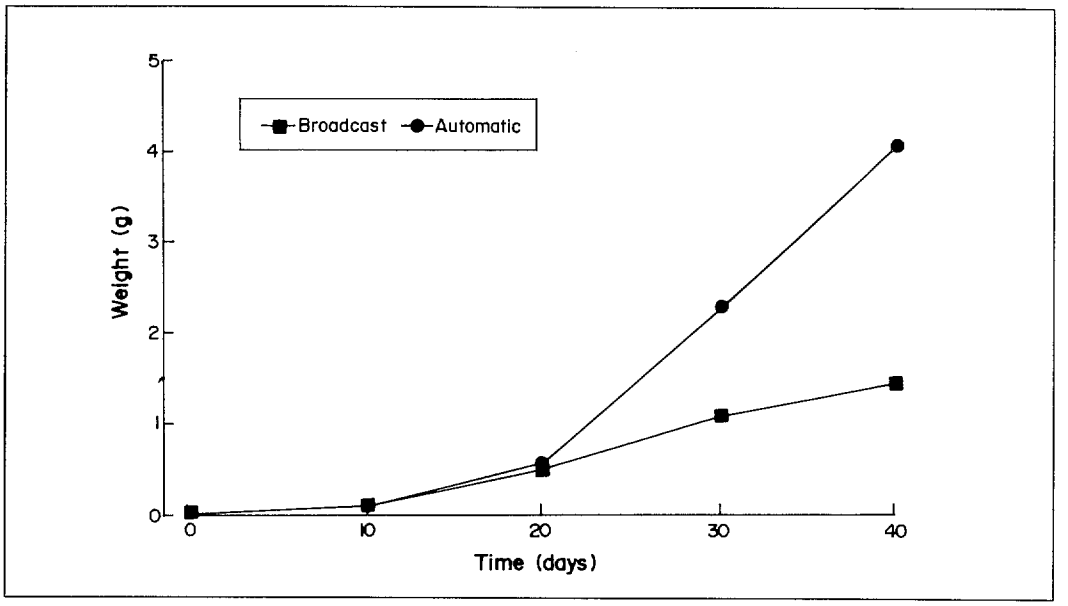

Fig. 1. Growth of post-yolksac Florida red tilapia (*Oreochromis* sp.) fry over 40 days in 530-l brackishwater (12 ppt) tanks under intermittent broadcast and continuous automatic feedings.

than in the BF group (70%) (Table 2, Fig. 2).

Survival was higher and feed conversion ratio lower in the AF (56% and 0.74) than in the BF group (38% and 2.87), with significant differences observed among treatments (Table 2). Final biomass and yield were higher in the AF (13 $g \cdot l^{-1}$ and 3.27 $fish \cdot l^{-1}$) than in the BF group (3.25 $g \cdot l^{-1}$, 2.24 $fish \cdot l^{-1}$), with significant ($P<0.001$ and $P \leq 0.05$, respectively) differences observed among treatments (Table 2).

Fish in both treatment groups were initially fed at 17% $bw \cdot day^{-1}$. Total feed dispensed to each tank over the 40-day study was significantly ($P<0.01$) higher in AF (avg 5,082±47 g) than in BF tanks (avg 4,596±84 g). However, on day 40, feeding rate in the AF group (4.52±0.17% $bw \cdot day^{-1}$) was significantly ($P<0.01$) lower than in the BF group (16.5±2.6% $bw \cdot day^{-1}$).

Discussion

Under commercial hatchery conditions, growth and survival of post-yolksac Florida red tilapia fry in brackishwater tanks were not impaired under stocking densities ranging from 5.7 to 13.2 $\cdot l^{-1}$, suggesting that higher stocking densities are feasible to increase production. This is consistent with the results of laboratory studies showing that survival and growth of post-yolksac *O. mossambicus* and *O. niloticus* x *O. aureus* hybrids in freshwater aquaria were not correlated with stocking density over the range of 2-12 $\cdot l^{-1}$ (Macintosh and De Silva 1984).

Average survival under all stocking densities in the present study (58%) was comparable to that reported for *O. spilurus* (46-68%) reared at densities of 2-8 $\cdot l^{-1}$ in 0.5-m^3 low-salinity (3-4 ppt) tanks (Al-Ahmad et al. 1988). Although higher survival rates have been reported

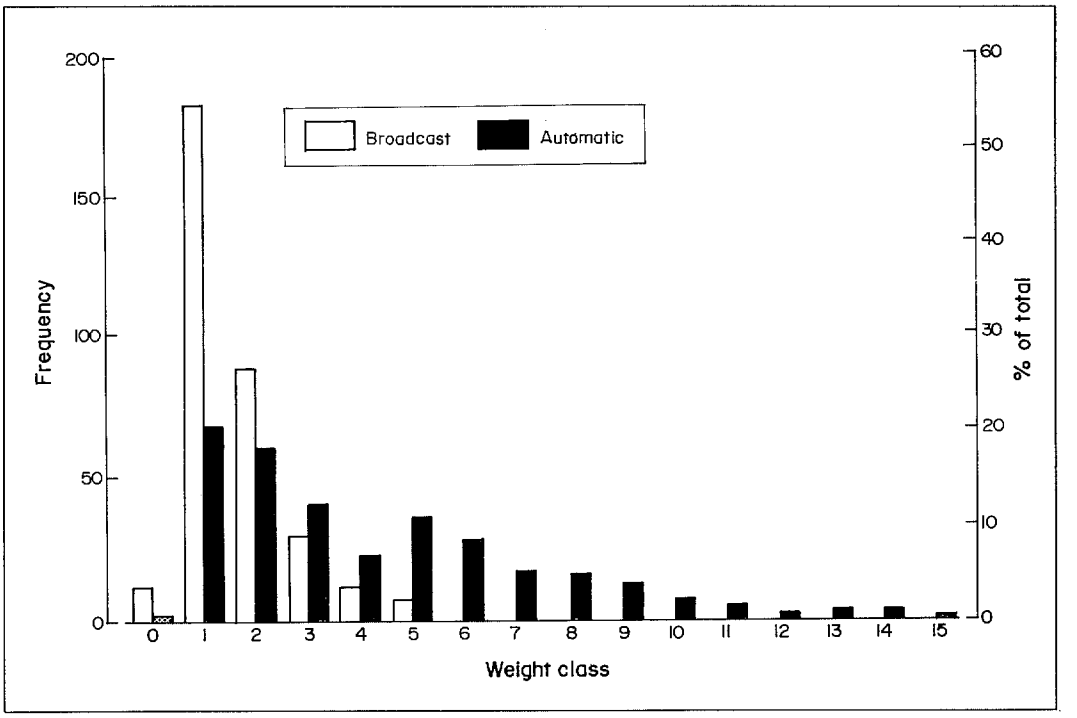

Fig. 2. Weight frequency distribution of Florida red tilapia (*Oreochromis* sp.) fry reared for 40 days in 530-l brackishwater (12 ppt) tanks under intermittent broadcast and continuous automatic feeding. Weight class 1 refers to a weight range of 0.30 to 1.28 g with each subsequent class increasing by 0.98 g to a maximum of 15 g. Weight class 0 refers to a weight range of <0.30 g.

for *O. niloticus* (78.1%) (Guerrero and Guerrero 1988) and *O. aureus* (82.3-96.8%) (Snow et al. 1983) in freshwater tanks (7.3-10 m^3), much lower stocking densities of 0.062-0.25·l^{-1} were used.

Under all stocking densities, few dead or moribund fish were observed during the study, suggesting that most mortalities were due to cannibalism (Uchida and King 1962; Macintosh and De Silva 1984; Pantastico et al. 1988; Watanabe et al. 1992). However, similar growth variation (i.e., CV of weights) and survival under the different densities suggest that behavioral interactions (i.e., aggression among fish) were not influenced by stocking density (Brett 1979), within the ranges used in this study.

Continuous AF produced better feed conversion ratios and higher growth and survival of post-yolksac fry than intermittent BF, perhaps because the digestive system of tilapias is adapted to process a continuous supply of small quantities of food (Jauncey and Ross 1982).

As observed for fish reared under different stocking densities, most mortalities of fish reared under AF or BF regimes were due to cannibalism. However, AF, with continuous availability of food, may have lowered the tendency for cannibalism among fry and thus improved survival. In *O. niloticus*, cannibalism of fry fed manually three times per day was inversely related to rate of feeding (6, 12 and 24% bw·day^{-1}) (Macintosh and De Silva 1984). In the

present study, more food was delivered to the AF tanks, but the feeding rate at the end of the study was much higher in BF than in AF tanks (16.5 vs. 4.52% bw·day^{-1}) due to the relatively high biomass in the AF tanks. Thus, feeding regime may be more important than feeding rate in reducing cannibalism.

Growth variation was higher under AF than BF. Under AF, the introduction of small quantities of food to the water surface over a confined area may have enabled dominant individuals to monopolize feed more effectively than under BF, where relatively large quantities were delivered over short periods. This may have intensified a size-hierarchy effect (Koebele 1985) in automatic-fed tanks.

Acknowledgements

This study was supported by a grant from the National Undersea Research Program, National Oceanic and Atmospheric Administration, US Department of Commerce and by the Perry Foundation. We thank Simon C. Ellis for technical assistance.

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Production of Florida Red Tilapia (*Oreochromis* sp.) in Flowthrough Seawater Pools at Three Stocking Densities

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Abstract

Sex reversed male Florida red tilapia (*Oreochromis* sp.) fingerlings (5.36 g avg wt) were stocked in nine seawater (37 ppt) pools (10 m^3) at densities of 15, 25, and 35 fish·m^{-3} and reared for 150 days on diets containing 32% and 20% protein. Each pool was supplied with aeration and flowthrough seawater (800%·day^{-1}). After 150 days, no significant ($P>0.05$) differences among treatments were observed in fish weight (avg 462 g), specific growth rate (avg 2.98%·day^{-1}), weight gain (avg 3.04 g·day^{-1}), survival (avg 94.4%), feed consumption (avg 5.17% bw·day^{-1}) and conversion ratio (avg 1.80). Coefficients of variation of initial and final weights averaged 34.4% and 22.8%, respectively, and did not differ significantly ($P>0.05$) among treatments. Final biomass (kg·m^{-3}) increased with increasing stocking density from 6.69, for tanks stocked at 15 fish·m^{-3}, to 15.4, for tanks stocked at 35 fish·m^{-3}. The results demonstrated that growth and survival in seawater were not impaired and that growth variation was not increased under stocking densities up to 35 fish·m^{-3}, suggesting that even higher densities are feasible to increase production.

Parasitosis by a marine monogenean (*Neobenedenia melleni*) was occasionally observed in fish under all densities. Treatment with brackishwater (18 ppt for 72 hours) alleviated symptoms of parasitosis for periods of 26-91 days.

Introduction

Intensive culture of salt-tolerant tilapias in flowthrough seawater tanks may be commercially feasible on Caribbean islands and other tropical coastal regions where seawater is abundant and favorable temperatures exist year-round, but where land and freshwater resources are limited (Watanabe 1991). The suitability of the Florida red tilapia strain (*Oreochromis* sp.) for culture at high salinities has been demonstrated by high growth rates and efficient feed conversion from fingerling to large, market-size fish in seawater (36 ppt) pools (Ernst et al. 1989; Clark et al. 1990).

Although there have been a number of studies on the effects of stocking

density on production of tilapias reared in cages (e.g., Wannigama et al. 1985; Carro-Anzalotta and McGinty 1986; McGeachin et al. 1987; Watanabe et al. 1990), little information is available on these effects in tank culture. In this study, the production of Florida red tilapia in flowthrough seawater pools was assessed at three stocking densities.

Materials and Methods

This study was conducted at the Caribbean Marine Research Center (CMRC) on Lee Stocking Island (Exuma Cays, Bahamas) from May to October 1989. The Florida red tilapia broodstock used was a hybrid: descendants of an original cross of *Oreochromis urolepis hornorum* (female) with *O. mossambicus* (male) (Behrends et al. 1982). Broodfish spawned naturally in outdoor pools containing brackishwater (12 ppt). Post-yolksac fry (<1 cm) were reared in 0.56-m^3 indoor tanks supplied with aeration and recirculated brackishwater (12 ppt). Fry were fed Purina Trout Chow (50% protein) with 17α-ethynyltestosterone (60 $mg \cdot kg^{-1}$ feed) for 28 days to sex reversed females (Guerrero 1975). Fry were then transferred to four outdoor pools (3.9 m^3), acclimated to seawater (37 ppt) at approximately 5 $ppt \cdot day^{-1}$, and reared for an additional 26 days. Fry were fed ad libitum three times daily with Purina Tilapia Chow (32% protein).

On 16 May 1989, fingerlings were graded and stocked in nine plastic-lined pools (5.4-m diameter, 10 m^3) at three densities: 15 $fish \cdot m^{-3}$, 25 $fish \cdot m^{-3}$ and 35 $fish \cdot m^{-3}$. There were three replicates for each treatment. Each pool was supplied with aeration and flowthrough seawater at an exchange rate of $800\% \cdot day^{-1}$.

On the day of stocking (experiment day 0), 35 fish from each pool were anaesthetized (0.3 ppt 2-phenoxy-ethanol), measured, weighed and replaced. Initial mean body weights (avg 5.36 g), total lengths (avg 66.2 mm) and coefficients of variation of initial weights (CV_w) (avg 34.4%) and lengths (CV_L) (avg 11.7%) did not differ significantly ($P>0.05$) among treatments (Table 1). Initial biomass ranged from 0.08-0.19 $kg \cdot m^{-3}$ (Table 1). Fish were sampled on days 15, 30, 45, 60, 91, 120 and 150.

Fish were fed three times daily (0800, 1300 and 1600 hours) the 32% protein diet from day 1 to day 55 and a 20% protein feed (Zeigler Tilapia Grower) from day 56 to 149. At each feeding, enough feed was provided such that an excess remained after 20 minutes, but was consumed before 40 minutes. For the 20% diet, 10- and 20-minute observation periods were used due to the feed's lower stability.

Maximum and minimum water temperatures, salinity and dissolved oxygen (DO) were measured daily, while NH_4-N, NO_2-N and pH were measured weekly.

Infestation by a marine monogenean parasite, *Neobenedenia melleni*, was observed during the study and was treated by reducing salinity to 18 ppt. Treated pools remained static for 72 hours before seawater flow was resumed.

Data on growth, growth variation, survival, feed consumption and conversion were compared among treatments by one-way analysis of variance (ANOVA). If the overall ANOVA was significant, differences between treatment means were analyzed using the Student-Newman-Keuls test. The level of significance in all tests was $P<0.05$.

Table 1. Growth, survival and feed conversion of all-male Florida red tilapia (*Oreochromis* sp.) fingerlings, produced by hormone treatment and reared for 150 days in flowthrough seawater pools (34 m^3) at three stocking densities.

	Stocking density (fish·m^{-3})		
	15	25	35
Initial			
Weight	5.63 ± 0.11[a]	5.10 ± 0.20	5.36 ± 0.12
CV of weight (%)[b]	31.9 ± 1.2	35.5 ± 1.5	35.7 ± 0.5
Length (mm)	67.3 ± 0.3	64.9 ± 0.7	66.4 ± 0.8
CV of length (%)[b]	11.6 ± 0.09	11.7 ± 0.4	11.9 ± 0.2
Biomass (kg·m^{-3})	0.08 ± 0.004a	0.13 ± 0.005b	0.19 ± 0.007c
Final			
Weight	470 ± 10	452 ± 9	463 ± 11
CV of weight (%)[b]	22.5 ± 2.1	24.3 ± 0.6	21.5 ± 2.0
Length (mm)	273 ± 3	266 ± 3	270 ± 3
CV of length (%)[b]	7.6 ± 1.2	12.0 ± 2.6	7.6 ± 1.4
Survival (%)	94.9 ± 0.2	93.9 ± 0.7	94.4 ± 0.2
DWG (g·day^{-1})[c]	3.10 ± 0.07	2.98 ± 0.06	3.05 ± 0.08
SGR[d] (% bw·day^{-1})	3.00 ± 0.05	2.99 ± 0.01	2.96 ± 0.03
Feed consumption[e] (% bw·day^{-1})	5.15 ± 0.17	5.24 ± 0.12	5.11 ± 0.13
Feed conversion[e] (dry wt·wet wt)	1.81 ± 0.16	1.83 ± 0.06	1.75 ± 0.08
Biomass (kg·m^{-3})	6.69 ± 0.15a	10.6 ± 0.16b	15.4 ± 0.27c

[a]Data are presented as means ± s.e. (n=3). Means within a row followed by different letters are significantly different ($P<0.05$, SNK).
[b]CV=coefficient of variation=SD·100/mean.
[c]DWG=daily weight gain=(final wt-initial wt)/Δt.
[d]SGR=specific growth rate=[(ln final wt-ln initial wt)·100]/Δt.
[e]Replicate data based on seven interval determinations during the 150-day study period.

Results

Growth of fish under the different stocking densities was similar throughout the 150-day study (Fig. 1). Final mean weights (avg 462 g), total lengths (avg 270 mm) and coefficients of variation of final weights (avg 22.8%) and lengths (avg 9.1%) did not differ significantly among treatments (Table 1). Thus, terminal weight data for all treatments were combined and the frequency distribution of body weights was determined (Fig. 2). Individual weights ranged from 98.6 to 688 g, with 75.2% and 66.9% of the individuals exceeding 393 g and 430 g, respectively.

Survival (avg 94.4%), daily weight gain (avg 3.04 g·day^{-1}), specific growth rate (avg 2.98% bw·day^{-1}), feed consumption (5.17% bw·day^{-1}) and conversion ratio (avg 1.80) did not differ significantly among treatments (Table 1). Final biomass increased with increasing stocking density from 6.69 kg·m^{-3} (15 fish·m^{-3}) to 15.4 kg·m^{-3} (35 fish·m^{-3}) (Table 1).

Average salinity (36.7 ppt), temperature (27.9°C), NH_4-N (0.11 ppm) and NO_2-N (0.005 ppm) did not differ

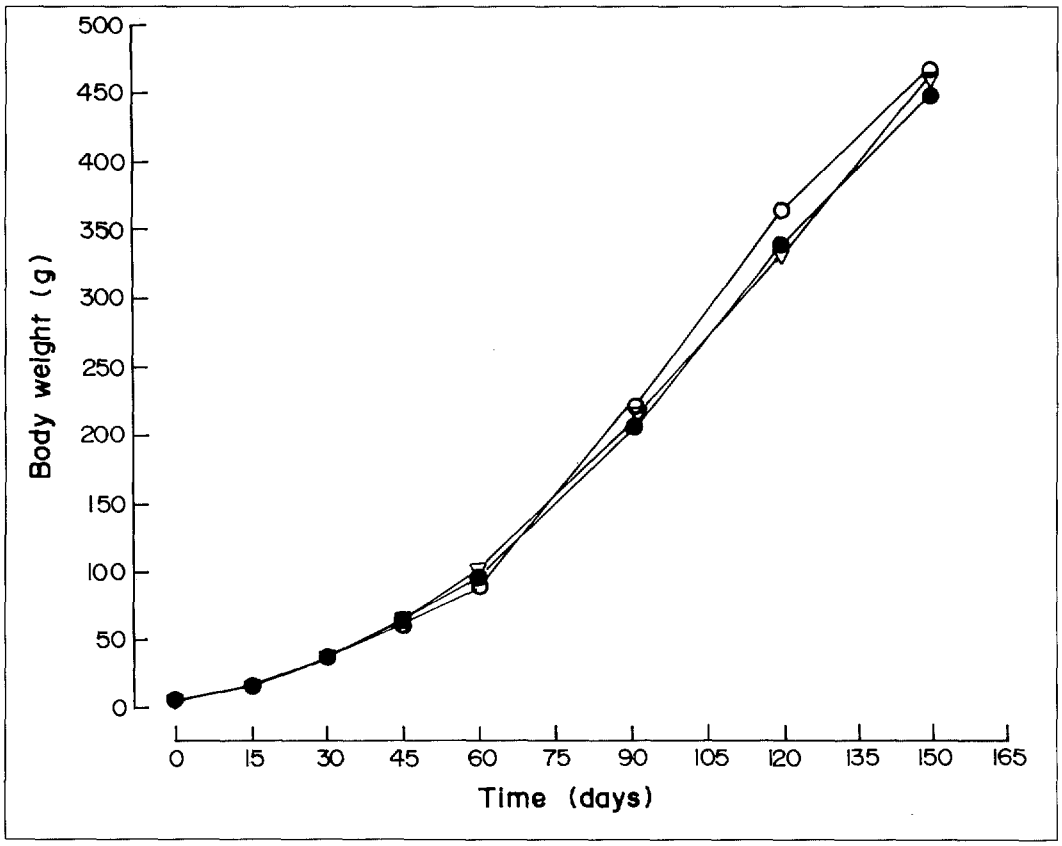

Fig. 1. Body weight of sex reversed male Florida red tilapia (*Oreochromis* sp.) fingerlings reared for 150 days in flowthrough seawater pools (10 m^3) at three stocking densities (○ 15 fish·m^{-3}, ● 25 fish·m^{-3} and ∇ 35 fish·m^{-3}).

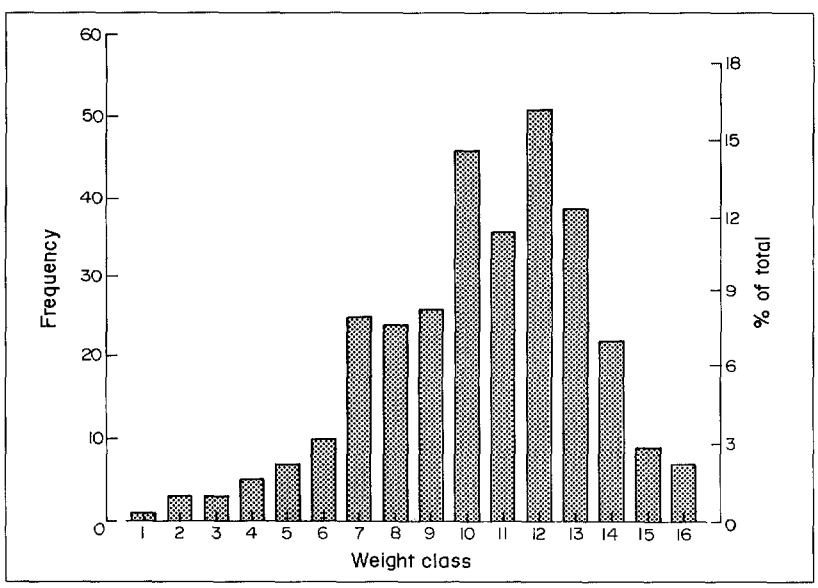

Fig. 2. Frequency distribution of body weights for all-male Florida red tilapia (*Oreochromis* sp.) fingerlings, produced by hormone treatment and reared for 150 days in flowthrough seawater pools (10 m^3) stocked at densities of 15, 25, or 35 fish·m^{-3} (combined data). Weight class 1 refers to a weight range of 98.6- 135.4 g, with each subsequent class increasing by 36.8 g to a maximum of 687.6 g.

significantly among treatments during the 150-day study (Table 2). Average DO showed a significant decline with increasing stocking density from 5.79 ppm (15 fish·m^{-3}) to 5.26 ppm (35 fish·m^{-3}) (Table 2).

On day 41, infestation by *N. melleni*, characterized by reduced feeding, fish resting on the pool bottom, excessive mucus production and corneal opacity, was detected among fish in one pool stocked at a density of 15 fish·m^{-3}. Infestation was subsequently observed in other pools at all stocking densities. Treatment with brackishwater (18 ppt for 72 hours) alleviated symptoms for periods ranging from 26 to 91 days.

Discussion

Growth, survival and feed conversion of Florida red tilapia reared in flowthrough seawater pools were not impaired under stocking densities of up to 35 fish·m^{-3}, and no effect of stocking density on size variation (Brett 1979) was evident, suggesting that even higher densities are feasible to increase production. Siddiqui et al. (1989) also observed no difference in growth (1.71-1.82 g·day^{-1}) survival (96-100%) or feed conversion (1.85-1.95) of *O. niloticus* reared in brackishwater (4 ppt) tanks (3.75 m^3) over 164 days at densities of 16, 32 and 42.6 fish·m^{-3}.

In this study, water exchange was kept constant (800%·day^{-1}) and flow rate was high (30-67 l·kg^{-1} fish·min^{-1}) at stocking, declining to a minimum (0.36-0.83 l·kg^{-1} fish·min^{-1}) when the study was terminated. Standing crops of up to 15.4 kg·m^{-3} were maintained with minimal effects on water quality. While initial flow rates could be reduced to minimize pumping costs, a trend toward lower average DO with increasing density suggests that flow rates during the latter stages of the study were suboptimal for tanks stocked at higher densities. Water flow rates of 0.3-1.0 l·kg^{-1} fish·min^{-1} have been reported to support tilapia yields ranging from 7.1 to 50 kg·m^{-3} in flowthrough tanks (Balarin and Haller 1982, 1983; Al-Ahmad et al. 1988; Siddiqui et al. 1989; Cruz et al. 1990). Yields of up to 52.2 kg·m^{-3} have been reached for Florida red tilapia grown in marine cages (Watanabe et al. 1990).

A potential constraint to culture of tilapias in full-strength seawater is susceptibility to infestation by *N. melleni* (Kaneko et al. 1988; Gallet de St. Aurin et al. 1990). Short-term immersion of infected fish in freshwater is known to be an effective treatment (Kaneko et al. 1988), but may be impractical on a commercial scale. In this study, a reduction of rearing tank salinity to 18 ppt for 72 hours was an effective, albeit temporary, therapeutic procedure. Reinfection of brackishwater-treated fish is probably due to the survivability of the eggs of *N. melleni*, which are considerably more resistant to hyposaline conditions than are the adults (Mueller et al. 1992). Recent studies have shown that exposure to 15 ppt for five days eliminates hatching of eggs in vitro as well as juvenile and adult stages of *N. melleni* in situ (Ellis and Watanabe 1993).

These results demonstrate that Florida red tilapia can be reared in flowthrough seawater pools from small fingerlings to market size (i.e., 5.36-462 g) in 150 days at densities of up to 35 fish·m^{-3}. Thus, each pool can produce 2.4 crops per year with a yield of approximately 37 kg·m^{-3}·year^{-1}. It is likely that productivity can be increased by stocking at higher densities and at larger initial sizes. Further study is required to determine maximum standing crop and associated water flow requirements for

Table 2. Water quality during culture of all-male Florida red tilapia (*Oreochromis* sp.) fingerlings, produced by hormone treatment, then grown for 150 days in flowthrough seawater pools at three stocking densities.[a]

	Stocking density (fish·m^{-3})		
	15	25	35
Salinity (ppt)[b]	36.8 ± 0.1	36.6 ± 0.1	36.8 ± 0.1
Dissolved oxygen (ppm)	5.79 ± 0.06a	5.51 + 0.01b	5.26 ± 0.02c
Temperature (°C)	27.9 ± 0.01	27.9 ± 0.01	27.9 ± 0.06
pH	8.23 ± 0.03a	8.14 ± 0.02b	8.00 ± 0.02c
NH_4-N (ppm)	0.10 ± 0.01	0.11 ± 0.01	0.13 ± 0.02
NO_2-N (ppm)	0.005 ± 0.000	0.005 ± 0.000	0.005 ± 0.000

[a]Data are presented as means ± SE (n=3 pools) and represent averages for the 150-day study period. Means within a row followed by different letters are significantly different (SNK).
[b]Salinities were lowered to 18 ppt for three days in certain pools in each treatment to treat trematode infection. Treatment periods were not included in the calculation of average salinities.

culture of the Florida red tilapia in seawater tanks.

Acknowledgements

This study was supported by a grant from the Office of the Undersea Research, National Oceanic and Atmospheric Administration, U.S. Department of Commerce and by the Perry Foundation, Inc.

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SESSION II. NUTRITION

Use of Terrestrial Plants in Aquaculture in Malaŵi[a]

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CHIKAFUMBWA, F.J.K. 1996. Use of terrestrial plants in aquaculture in Malaŵi, p. 175-182. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Terrestrial vegetation and agricultural crop residues have potential as fishpond inputs for farming herbivorous and planktivorous tilapias in rural Africa. Five experiments were performed to assess this for the culture of *Tilapia rendalli* and *Oreochromis shiranus* in Malaŵi. Screening of 29 terrestrial plants was conducted in ponds: 80% were eaten by *T. rendalli*; consumption ranged from 9 to 90% of plant dry matter (DM) per day. *T. rendalli* and *O. shiranus* fed with either maize bran or napier grass did not show significant differences (P>0.05) in growth or yield. Higher yields were obtained in polyculture with a combination of maize bran and napier grass. Presentation of whole, chopped and ground napier grass (*Pennisetum purpureum*) to a 50:50 polyculture of *T. rendalli* and *O. shiranus* gave no significant differences (P>0.05) in water quality parameters or fish growth. Application rates of napier grass to fishponds should not exceed approximately 50 $kg \cdot ha^{-1} \cdot day^{-1}$ DM. Higher loading decreased water quality, reduced fish growth and increased fish mortalities. A 50:50 stocking ratio of *T. rendalli* and *O. shiranus* gave significantly higher yields than other ratios.

Introduction

Development of aquaculture in rural Africa is limited because of cost and shortage of fish feeds and pond fertilizers, poor financial resources and knowledge base of small-scale farmers, plus the fact that it is a relatively new farm enterprise (Weatherley and Cogger 1977; Costa-Pierce et al. 1991; ICLARM/GTZ 1991). Terrestrial vegetation and agricultural crop residues are available on most African farms and could have potential as inputs for pond culture of herbivorous and planktivorous tilapias (Pullin 1986; Edwards 1987a). A survey conducted in Zomba, Malaŵi, indicated that pond polyculture of indigenous tilapias (*Tilapia rendalli* and *Oreochromis shiranus*) is the most common method adopted by smallholder fish farmers (Noble and Costa-Pierce 1992). However, little research has been done on the stocking ratios of these species for optimum utilization of the food niches in the pond ecosystem.

To assess further the use of terrestrial plants in aquaculture on small-scale farms, research was conducted at the National Aquaculture Center, Domasi, Malaŵi. This paper reports these results and discusses future prospects.

Materials and Methods

Experiments were conducted in 200-m^2 ponds, 5-m^3 (2.25x2.25x1 m) and 500-l (1x1x0.5 m) concrete tanks from January 1989 to March 1991. *T. rendalli* and *O. shiranus* were stocked in mono- and polyculture at different stocking

[a]ICLARM Contribution No. 990.

densities and mean body weights in tanks and ponds. A completely randomized design was used in all experiments.

Maize bran (MB) was given at 3% fish body weight, adjusted every two weeks at sampling; fresh napier grass (NG) was given at 100 $kg \cdot ha^{-1} \cdot day^{-1}$ DM and a combination (NG+MB) to indigenous tilapias (*T. rendalli* and *O. shiranus*) in mono- and polyculture in 200-m^2 ponds (Table 1). The times for cutting, carrying and chopping napier grass were recorded as person-hours$\cdot ha^{-1} \cdot day^{-1}$ (Table 2). Fresh napier grass was given whole, chopped (2-5 mm pieces) and ground at 100 $kg \cdot ha^{-1} \cdot day^{-1}$ DM to 50:50 polyculture of *T. rendalli* and *O. shiranus* in 500-l tanks (Table 3). Different application rates of fresh napier grass was given to 50:50 polyculture of *T. rendalli* and *O. shiranus* in both tanks and ponds (Table 4). *T. rendalli* and *O. shiranus* were stocked at different ratios (100:0, 75:25, 50:50 and 25:75) and were given whole fresh napier grass at 50 $kg \cdot ha^{-1} \cdot day^{-1}$ DM in 200-m^2 ponds (Table 5).

Fish were sampled with a seine net, sedated in benzocaine (Ross and Ross 1984), and individually weighed and measured. Over 70% of the total number of fish stocked in tanks and 15% in ponds were sampled at each sampling.

Water quality parameters were monitored as follows: maximum and minimum air and water temperatures, daily; pH, weekly (at 0800 hours); dissolved oxygen concentration (DO), weekly (at 0500 hours); ammonia, every two weeks (at 0800 hours); conductivity, weekly; and alkalinity, every four weeks. The methods used are outlined in APHA (1985).

At harvest, tanks and ponds were seined and drained to collect all fish. Fish were counted to determine percentage survival, weighed (g), and the total and standard length (cm) determined. Specific growth rates (SGRs) and mean final weights were compared statistically (ANOVA). Differences among treatments were treated for significance ($P<0.05$) using Tukey's test (Zar 1984).

Results and Discussion

Preference Tasting of Plants

Many types of plants are available on farms, but most are left unutilized. Of the 29 different terrestrial plants (natural and cultivated) given fresh to *T. rendalli* and *O. shiranus* in 200-m^2 ponds, 23 were eaten by *T. rendalli* and six by *O. shiranus*. The amount of dry matter consumed by *T. rendalli* was significantly higher ($P<0.01$) than that consumed by *O. shiranus* (Chikafumbwa et al. 1991). *T. rendalli* seems to be a voracious and nonselective feeder on plants, as also found by Junor (1969). Among the plants (*Cucurbita maxima*, *Tridax procumbens*, *Ipomoea batatas*, *Biden pilosa* and *Mucuna pruriens*) eaten, *T. rendalli* and *O. shiranus* preferred some more than others. Similar results were reported by Chifamba (1990).

On-farm Resources as Fishpond Inputs

Highest final weights and SGRs for *T. rendalli* (43.0 g; 0.52%$\cdot day^{-1}$) and *O. shiranus* (41.3 g; 0.42%$\cdot day^{-1}$) in monoculture were obtained in NG+MB, but were not significantly different ($P>0.05$) from MB or NG individually (Table 1). Similar results were obtained in a 50:50 polyculture for *T. rendalli* (46.9 g; 0.57%$\cdot day^{-1}$) and *O. shiranus* (41.9 g; 0.69%$\cdot day^{-1}$). Highest extrapolated net yields for *T. rendalli* and *O. shiranus* in monoculture (2,598 and 2,749 $kg \cdot ha^{-1} \cdot year^{-1}$, respectively) and in polyculture (3,015 $kg \cdot ha^{-1} \cdot year^{-1}$) were obtained with NG+MB.

Pruginin and Arad (1977) reported that fish yields, using indigenous tilapias

Table 1. Growth performance of *Tilapia rendalli* and *Oreochromis shiranus* in monoculture and 1:1 polyculture in 200-m^2 ponds fed napier grass (NG) (100 $kg \cdot ha^{-1} \cdot day^{-1}$ DM) and maize bran (MB) (3% fish body weight), from 5 January to 11 May 1989. Numbers in the same row having the same superscript are not significantly different (P>0.05); NG/MB = combination of napier grass and maize bran; net yield includes fry produced in a pond. The data are means of three replicates.

	Pond inputs			
	Napier grass	NG/MB	Maize bran	No input
a. *T. rendalli* monoculture				
Initial weight (g)	20.8	20.5	21.9	
Final weight (g)	35.5[a]	43.0[a]	34.8[a]	
Specific growth rates ($\% \cdot day^{-1}$)	0.42[a]	0.52[a]	0.37[a]	
Fish survival (%)	93	87	89	
Net yield ($kg \cdot ha^{-1} \cdot year^{-1}$)	2,303	2,598	1,585	
b. *O. shiranus* monoculture				
Initial weight (g)	22.9	24.1	23.5	
Final weight (g)	36.9[a]	41.3[a]	34.2[a]	
Specific growth rates ($\% \cdot day^{-1}$)	0.37[a]	0.42[a]	0.32[a]	
Net yield ($kg \cdot ha^{-1} \cdot year^{-1}$)	1,657	2,747	1,069	
c. *T. rendalli* and *O. shiranus* 1:1 polyculture				
Initial weight (g)				
T. rendalli	22.0	22.8	22.4	21.0
O. shiranus	21.1	17.4	19.0	18.6
Final weight (g)				
T. rendalli	41.7[a]	46.9[a]	41.4[a]	21.0[b]
O. shiranus	36.4[a]	41.9[a]	33.1[a]	22.5[a]
Specific growth rates ($\% \cdot day^{-1}$)				
T. rendalli	0.51[a]	0.57[a]	0.49[a]	0.01[b]
O. shiranus	0.41[b]	0.69[a]	0.39[b]	0.15[c]
Fish survival (%)	78	88	90	73
Extrapolated net yield ($kg \cdot ha^{-1} \cdot year^{-1}$)	1,405	3,013	1,726	-183

Table 2. Labor required for cutting and carrying fresh napier grass (80% moisture) at 100 $kg \cdot ha^{-1} \cdot day^{-1}$ dry matter as fishpond inputs. Chopping was done using a hand-operated machine cutter at a rate of 10.4 person-hours$\cdot ha^{-1}$.

Person	N	Cutting and carrying (person-hours$\cdot ha^{-1}$) Mean (SD)	Range	Cutting (person-hours$\cdot ha^{-1}$) Mean (SD)	Range
A	49	58.1 (15.6)	16.7-92.6	19.7 (4.8)	12.9-32.8
B	20	59.5 (13.3)	31.6-78.1	18.1 (4.1)	11.5-26.0
C	25	44.4 (19.1)	11.1-72.6	15.3 (6.7)	5.7-27.3
C	11	33.3 (18.8)	73.5-12.6	17.1 (3.2)	12.3-24.2
D	11	55.5 (13.3)	24.1-70.4	18.8 (3.9)	11.7-25.9
E	19	48.0 (15.6)	22.7-70.4	13.3 (5.0)	5.0-24.2
F	17	59.6 (15.2)	19.6-79.4	19.8 (3.4)	15.3-26.9

N = number of days for cutting and carrying napier grass.
person-hours$\cdot ha^{-1}$ = calculated from weight of fresh grass required per hectare divided by weight of fresh grass cut and/or carried during a given period.

Table 3. Effect of whole, chopped and ground napier grass (100 kg·ha^{-1}·day^{-1} DM) on the growth of *Tilapia rendalli* (TR) and *Oreochromis shiranus* (OS), and on water quality in 500-l concrete tanks. Numbers in the same column followed by the same letters are not significantly different (P>0,05).

Presentation method	Stocking data		Harvesting data						Water quality							
	Msw (g)	N/t	Mhw (g)	Wg/dy (%)	SGRs		Sv (%)	Eny	Ammonia (mg·l^{-1})		Conductivity (mmho·cm^{-1})		pH		Alkalinity (mg·l^{-1})	
					TR	OS			Initial	Final	Inititial	Final	Initial	Final	Initial	Final
Whole	10.05	12	14.40a	0.34	0.24a	0.29a	100	1,507	0.01	1.15a	145	440a	10.45	7.83b	39	169b
Chopped	9.75	12	15.05a	0.43	0.21a	0.36a	92	1,058	0.00	2.00a	144	570a	10.39	7.83b	40	243a
Ground	10.00	12	13.65a	0.29	0.11b	0.35a	66	839	0.01	0.95a	146	645a	10.30	7.93b	40	268a
No input	10.25	12	9.60b	-0.51	-	-0.001b	25	-	0.01	0.08b	145	160b	10.65	10.63a	39	63c

Msw=mean stocking weight.
Mhw=mean harvest weight.
N/t=number of fish per tank.
Wg/dy=weight in grams per day.
SGRs=specific growth rates (%·day^{-1}).
Sv=fish survival.
Eny=extrapolated net yield (kg·ha^{-1}·an^{-1}).

Table 4. Growth performance of *Tilapia rendalli* (TR) and *Oreochromis shiranus* (OS), survival and water quality for different rates of napier grass in 200-m² ponds, 5-m³ and 500-l concrete tanks. Numbers in the same column, in the same experimental unit, followed by the same letters are not significantly different (P>0.05).

Experimental units	App. rate ($kg \cdot ha^{-1} \cdot day^{-1}$ DM)	Stocking data		Harvest data						Eny	Water quality									
		Msw (g)	N/p-t	Mhw (g)	Wg/dy (%)	SGRs		Sv (%)	Fry (%)		Ammonia ($mg \cdot l^{-1}$)		Conductivity ($mmho \cdot cm^{-1}$)		pH		Alkalinity ($mg \cdot l^{-1}$)		DO_2 ($mg \cdot l^{-1}$)	
						TR	OS				Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
200-m² pond over 168 days	25	9.77	200	34.45b	1.5	0.67b	0.82b	83	15	476	0.45	0.75	23	45	7.0	6.7	15	19	7.4	3.8
(27 Oct. 1989 to 27 Feb. 1990)	50	8.84	200	43.55a	2.4	0.91a	0.98a	91	9	732	0.36	1.05	23	55	6.8	6.8	17	22	7.0	2.0
(a) TR and (b) OS	75	8.77	200	41.29a	2.2	0.87a	0.98a	81	8	583	0.42	1.30	23	71	6.8	6.7	16	24	7.2	1.1
	100	9.94	200	43.66a	2.0	0.77ab	0.98a	66	8	443	0.36	1.11	22	59	6.9	6.7	16	23	7.5	2.2
5-m³ tanks over 126 days	0	10.52	12	11.99c	0.1	0.09b	0.23c	75	0	0	-	0	100	101	10.5	10.6	40	43	7.7	7.9
(24 Jan. to 30 May 1989)	46	10.58	12	28.00a	1.3	0.73a	0.78b	100	0	1,216	-	0.88	104	398	10.0	7.7	38	160	6.8	0.7
(a) TR and (b) OS	92	10.94	12	31.26a	1.5	0.78a	1.01a	92	0	1,233	-	0.92	103	460	9.8	7.6	42	195	7.0	0.5
	138	10.72	12	32.54a	1.6	0.74a	0.99a	75	0	950	-	1.01	102	478	9.6	7.5	39	217	7.8	0.4
	184	10.89	12	23.94ab	0.9	0.28b	0.76b	75	0	486	-	1.33	105	535	9.5	7.4	43	265	7.3	0.4
	278	10.75	12	17.00bc	0.5	-	0.38c	8	0	0	-	2.80	106	580	9.6	7.3	40	310	7.3	0.4
500-l tanks over 70 days	10	15.00	12	15.40a	0.03	0.01c		100	0	250	-	0.75	70	156	10.2	9.0	13	37	6.4	4.4
(12 Jan. to 29 May 1991)	20	15.90	12	17.30a	0.12	0.12b		96	0	-	-	0.68	72	155	10.4	9.5	13	35	7.0	3.9
(a) TR and (b) OS	40	14.60	12	17.50a	0.28	0.26a		100	0	1,815	-	0.92	69	185	10.0	9.7	12	43	6.7	2.5
	60	15.80	12	18.90a	0.28	0.26a		96	0	1,012	-	1.22	71	276	9.8	8.5	13	67	6.5	1.8
	80	15.80	12	15.90a	0.01	0.02c		71	0	-	-	1.10	71	290	9.9	8.4	12	65	6.5	1.9

App=application rate.
Msw=mean stocking weight.
Mhw=mean harvest weight.
Wg/dy=weight in grams per day.
N/p-t=number of fish per pond or tank.
SGRs=specific growth rates ($\% \cdot day^{-1}$).
Sv=fish survival.
Eny=extrapolated net yield ($kg \cdot ha^{-1} \cdot year^{-1}$).

Table 5. Growth performance of *Tilapia rendalli* (TR) and *Oreochromis shiranus* (OS) stocked at different ratios at 1 fish·m^{-2} in 200-m^2 ponds fed 50 kg·ha^{-1}·day^{-1} DM napier grass over 168 days (from 27 October 1989 to 27 February 1990). Numbers in the same row having the same superscript are not significantly different (P>0.05). The data are means of three replicates.

Stocking ratios TR:OS (%)	75:25	50:50	25:75	100:0
Initial weight (g)				
T. rendalli	9.55	8.70	9.63	8.40
O. shiranus	9.87	8.97	8.73	-
Final weight (g)				
T. rendalli	36.75^a	40.23^a	38.06^a	35.66
O. shiranus	56.18^a	46.87^a	38.06^a	-
Specific growth rates				
T. rendalli (%·day^{-1})	0.80^a	0.91^a	0.82^b	0.86
O. shiranus (%·day^{-1})	1.03^a	0.98^a	0.87^a	-
Fish survival (%)	86	97	85	76
Extrapolated net yields (kg·ha^{-1}·year^{-1})	593^b	729^a	534^b	410^c

without feeding or fertilization, were about 300 kg·ha^{-1}·year^{-1} in Malaŵi. Noble and Costa-Pierce (1992) found that aquaculture ponds in Zomba district in 1989-1990 had total yields of 900 kg·ha^{-1}·year^{-1}. Use of green vegetation (napier grass) and widely available agricultural by-product (maize bran) have here demonstrated a significant increase in fish yields. Even higher yields (>3,000 kg·ha^{-1}·year^{-1}) have been obtained in a polyculture fed with a combination of maize bran and napier grass. These results are promising and suggest further investigations on the polyculture of different fish species with different combinations of the low cost fishpond inputs that are available on farms in Malaŵi.

Application of fresh napier grass to fishponds required 33-60 person-hours·ha^{-1}·day^{-1} for cutting and carrying. Cutting alone required 13-20 person-hours·ha^{-1}·day^{-1}. Chopping required additional 10 person-hours·ha^{-1}·day^{-1} at an application rate of 100 kg·ha^{-1}·day^{-1}·DM (Table 2). Results obtained in this study suggested high person-hours·ha^{-1}·day^{-1} requirement to feed fresh napier grass to fishponds. Investigations were conducted on presentation methods and application rates to explore the possibility of reducing person-hours·ha^{-1}·day^{-1} in feeding fishponds. Results obtained indicated 50% reduction in application rate (50 kg·ha^{-1}·day^{-1} DM) and could be presented whole and effectively reduce the person-hours·ha^{-1}·day^{-1} by 50% for cutting and carrying, and exclude additional the person-hours·ha^{-1}·day^{-1} requirement for chopping.

Presentation Methods of Green Vegetation

Fish final weight and SGRs were not significantly different (P>0.05) among whole, chopped and ground grass treatments (Table 3). However, fish survival was high (100%) when fed with whole grass, but low (66%) with ground grass.

Extrapolated net yield was 1,507 $kg \cdot ha^{-1} \cdot year^{-1}$ with whole grass.

Water quality parameters (pH, ammonia and conductivity) were not significantly different ($P>0.05$) among whole, chopped and ground grass treatments (Table 3). However, alkalinity and total hardness were significantly higher ($P<0.05$) with chopped and ground than with whole grass.

This study indicated that green grasses should be presented whole to fishponds. Chopping or grinding does not bring significant advantages in terms of fish growth and water quality, and requires extra effort. However, presentation of such feeds still requires further research. This study was done in small concrete tanks (500-l) and more work is needed in pond systems.

Application Rates of Green Vegetation

The application rates for low quality vegetation in fishponds was recommended at 100 $kg \cdot ha^{-1} \cdot day^{-1}$ DM (Edwards 1987b). In these studies, in tanks and ponds, highest final weight, SGRs, fish survival and net yields (*T. rendalli* and *O. shiranus*) were obtained at approximately 50 $kg \cdot ha^{-1} \cdot day^{-1}$ DM. Increasing application rates beyond 50 $kg \cdot ha^{-1} \cdot day^{-1}$ DM gave higher mortalities, decreased SGRs and net yields, and lowered water quality, principally by increasing ammonia concentrations and lowering DOs to critical levels (Table 4).

Tilapia Stocking Ratios

Final weights and SGRs for *O. shiranus* were significantly higher ($P<0.05$) with increasing *T. rendalli* to *O. shiranus* ratios. *T. rendalli* growth was relatively stable with different stocking ratios (Table 5). Net yield was highest at 50:50 stocking ratio. The increase in net production at a 50:50 ratio was probably due to better utilization of different food niches in the pond.

Conclusions and Recommendations

These studies demonstrated that use of green vegetation and agricultural byproducts (maize bran) in fishponds, singly or in combination, can significantly increase tilapia growth and production among resource-poor farmers. These inputs are commonly available on farms and require labor input to produce fish in ponds.

A wide range of green vegetation could effectively be utilized as a direct feed or fertilizer for tilapia in fishponds. Application rates of green vegetation should not exceed 50 $kg \cdot ha^{-1} \cdot day^{-1}$ DM for optimum tilapia growth and production, and to avoid destroying water quality. However, further studies on the use of green vegetation are required, because the present studies were conducted using indigenous tilapias (*T. rendalli* and *O. shiranus*) and there are other fish species which are grown or of interest among farmers.

Acknowledgements

This work was supported by the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), GmbH, Eschborn, Germany, through the International Center for Living Aquatic Resources Management (ICLARM). I thank Dr. Barry A. Costa-Pierce for his assistance in editing this manuscript and for his constructive comments. I also thank Prof. Peter Edwards of the Asian Institute of Technology, Bangkok, Thailand, for his advice on the initial design of some experiments. The Malaŵi Department of

Fisheries provided some research facilities at the National Aquaculture Center. Financial support to attend the conference was provided to the author by the International Foundation for Science (IFS), Stockholm, Sweden, under Grant No. A/1776-1.

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Waste Vegetable Leaves as Feeds for Juvenile *Oreochromis shiranus* and *Tilapia rendalli* in Mono- and Polyculture[a]

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CHIMATIRO, S.K. and B.A. COSTA-PIERCE. 1996. Waste vegetable leaves as feeds for juvenile *Oreochromis shiranus* and *Tilapia rendalli* in mono- and polyculture, p. 183-192. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Mixed sexes of *Oreochromis shiranus* and *Tilapia rendalli* were stocked in 5-m^3 concrete tanks in the ratios of 0:100, 25:75, 100:0 and 75:25, at the rate of 6 fish·m^{-2}. *O. shiranus* and *T. rendalli* were stocked at mean body weights (±SD) of 12±0.4 g and 12±0.7 g, respectively. Cabbage and pumpkin leaves were added to the tanks at a rate chosen to simulate pond conditions (50 kg dry matter [DM]·ha^{-1}·day^{-1}) as bundles tied with wire, fully submerged at a depth of about 30 cm. Maize bran was the control, added at 50 kg DM·ha^{-1}·day^{-1}. Cumulative treatment effects on fish growth and water quality parameters were explained using a nonparametric ranking order test and multivariate analysis.

Highest survival rate (98.4%) was obtained in 100:0 TR:OS ratio. Highest net yields (2,122 kg·ha^{-1}·134 days) were obtained in 0:100 TR:OS fed with maize bran followed by 25:75 TR:OS fed with cabbage leaves (1,516 kg·ha^{-1}·134 days). Two-way analysis of variance (ANOVA) indicated that pond inputs had significant effects ($P<0.001$) on water quality (DO, chlorophyll *a*, Secchi disk visibility [SDV], total alkalinity and conductivity). Pond inputs also had significant effects on specific growth rate (SGR) ($P<0.05$) and weight gain ($P<0.001$). Species stocking ratios had significant effects ($P<0.05$) on survival rates and fry production. Pumpkin leaves had significant effects on chlorophyll *a*, SDV and DO, while maize bran had significantly increased alkalinity ($P<0.05$).

In vegetable leaf treatments, best weight gains were obtained in monoculture of *T. rendalli* (225 g and 325 g, in cabbage and pumpkin leaves, respectively). *O. shiranus* monoculture performed poorly in vegetable leaf treatments, but did better in polyculture with maize bran. Vegetable leaves have good potential for being a pond input for small-scale, resource-poor farmers in Malaŵi. However, to achieve an optimal compromise between weight gain and survival, a 25:75 TR:OS polyculture might be the best combination.

Introduction

The use of terrestrial and aquatic vegetation as fish feeds has been reported by a number of workers (e.g., Pullin 1986; Edwards 1987; Edwards et al. 1988; Hasan et al. 1990). Fish yields of about 5-6 t·ha^{-1}·$year^{-1}$ are possible with vegetation as the sole pond input (Edwards 1987).

In Malaŵi, the majority of fish farmers grow a polyculture of the predominantly herbivorous *Tilapia rendalli* and microphagous *Oreochromis shiranus*. Waste vegetable leaves are used routinely as fishpond inputs, especially during the hot, rainy season when maize bran, the most common pond input, is scarce (Chikafumbwa 1990). Maize bran is diverted to household use during this period to stretch the family's food supply (Costa-Pierce et al. 1991; ICLARM and GTZ 1991).

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Cabbages and pumpkins are the most important cultivated vegetables in Malaŵi. For cabbages, large quantities of vegetable wastes are generated, with waste or insect-damaged leaves comprising up to 45-49% of the total yields (Chimatiro 1992). Whereas feeding of vegetable wastes to fish in ponds is practised widely in Malaŵi, little work has been conducted to investigate their use as feeds or fertilizers to fish. Our objective was to determine impacts on yields and water quality of different tilapia polycultures in tanks fed with vegetable wastes.

Materials and Methods

The experiment was done during the hot season in Malaŵi over 134 days (from 18 September 1990 to 30 January 1991), in 5-m^3, 1-m deep concrete tanks. Treatments were assigned randomly to 12 duplicate tanks (Table 1). *T. rendalli* (TR) and *O. shiranus* (OS) of mixed sexes were stocked by number at four ratios (25:75, 0:100, 100:0 and 75:25), at 6 fish·m^{-2} with mean body weight (MBW±SD) of 12±0.4 g and 12±0.7 g, respectively.

Cabbage and pumpkin leaves were grown on fishpond dikes. Proximate analyses of cabbage and pumpkin leaves were done to determine dry matter, crude protein, crude fiber, ether extract and total phosphorus. Maize bran was bought from villages around the National Aquaculture Centre (NAC), Domasi.

Tanks were filled with water from the Domasi River to 1-m mark and regularly topped up to replace evaporative losses. Ten water quality parameters were measured at 0600-0800 hours in all tanks at intervals, using the instruments and methods detailed in Table 2. Minimum and maximum temperatures were recorded daily. All feedings were done once a day at 1400 hours, five days a week (Monday to Friday). Cabbage and pumpkin leaves were added at a rate chosen to simulate pond conditions (50 kg dry matter [DM]·ha^{-1}·day^{-1}) as bundles tied with wire, fully submerged at a depth of about 30 cm. At each addition of new material, the remnants from the previous addition were left in the tank water. Maize bran was the control, added at 50 kg DM·ha^{-1}·day^{-1}.

Fish growth was evaluated fortnightly. All fish in each tank were netted, sedated with benzocaine (Ross and Ross 1984), then weighed individually to the nearest 1 g. Lengths (total and standard) were measured to 0.1 cm. Mortalities were recorded daily.

At harvest, all remaining fish were individually weighed and measured; tanks were drained and the accumulated sediments collected and weighed. Fry produced during the experiment were collected, counted and weighed for each treatment.

In order to explain the cumulative treatment effects on fish growth and water quality parameters, a nonparametric ranking order test was used (Siegel 1956; Alder and Roessler 1972). Where there were more than two variables, rank multiple correlation was measured by Kendall's coefficient of concordance (W_c) (Zar 1984). To find out whether the calculated W_c was significant, W_c was converted to Friedman χ^2. If significant concordance occurred for two groups of data, a multigroup coefficient of concordance was calculated (Schucany and Frawley 1973).

Results

Proximate analyses of inputs are shown in Table 3. Overall nutritional quality of inputs was very poor; the highest crude protein concentration was in pumpkin leaves (5.4%).

Table 1. Treatments and application rates of vegetable leaves and maize bran. All inputs were applied to 5-m^3 tanks. Wet weights of inputs were as in Table 3. TR = *Tilapia rendalli*; OS = *Oreochromis shiranus*.

Treatments						
TR:OS ratios (by numbers)	Inputs	Application rates ($g \cdot day^{-1}$)			Number of fish per m^3 tank	
		Cabbage	Pumpkin	Maize bran	TR	OS
25:75	Cabbage	292	-	-	8	22
0:100	Cabbage	292	-	-	0	30
100:0	Cabbage	292	-	-	30	0
75:25	Cabbage	292	-	-	22	8
25:75	Pumpkin	-	233	-	8	22
0:100	Pumpkin	-	233	-	0	30
100:0	Pumpkin	-	233	-	30	0
75:25	Pumpkin	-	233	-	22	8
25:75	Maize bran	-	-	39	8	22
0:100	Maize bran	-	-	39	0	30
100:0	Maize bran	-	-	39	30	0
75:25	Maize bran	-	-	39	22	8

Table 2. Water quality parameters measured: methods and instruments used.

Parameter	Units	Instrument	Frequency
Total alkalinity	$mg \cdot l^{-1}$ as CaCO3	Hach digital titrator	fortnightly
pH	-	Hach one meter	weekly
Dissolved oxygen	$mg \cdot l^{-1}$	YSI meter	fortnightly
Conductivity	$\mu mho \cdot cm^{-1}$	WTW LF 91 meter	fortnightly
Chlorophyll *a*	$mg \cdot l^{-1}$	Spectronic 20D spectrometer	fortnightly
Secchi disk visibility	cm	Code 1062 disk	weekly
Temperature (min/max)	°C	Brannan thermometer	daily
Ammonia	$mg \cdot l^{-1}$	Hach DREL/5	once/month

Table 3. Proximate analysis (%) of maize bran (Kadongola 1990), cabbage (*Brassica oleracea* var. *capitata*) and pumpkin leaves (*Cucurbita maxima*).

Parameter	Cabbage	Pumpkin	Maize bran
Dry matter	9.9	17.9	93.0
Crude protein	2.3	5.3	2.1
Crude fiber	5.1	3.0	8.0
Ether extracts	5.1	3.0	13.8
Phosphorus	0.2	0.1	

Table 4 summarizes fish yield parameters and fry production for the three inputs and stocking ratios. Parametric, two-way ANOVAs indicated that different inputs had significant effects on specific growth rates (SGRs) and total adult weight gain. Highest weight gains and SGRs were with maize bran for all stocking ratios. Species ratios had significant effects on adult survival and fry production. Kendall's coefficient of concordance with ties (W_c) for the 12 treatments (species x feeds) for seven fish yield parameters (total weight gain, net weight gains, survival, fry production, SGRs, net yields and FCRs) was significant ($P<0.05$) (Table 5).

Mean water quality is summarized in Table 6. Inputs had significant effects on pH, dissolved oxygen (DO), total alkalinity, Secchi disk visibility (SDV) and chlorophyll *a* (chl) concentrations. Pumpkin leaves gave significantly higher mean chl, lower SDVs and higher DOs. Maize bran significantly increased alkalinity. Overall water quality as indicated by the sum of group ranks (ΣR_i) was best in 25:75 TR:OS tanks fed with cabbage leaves, followed by 100% OS fed with pumpkin leaves. The best water quality was achieved with pumpkin leaves, followed by cabbage leaves and maize bran (Table 7). Kendall's coefficient of concordance with ties (W_c) for the 12 treatments for eight water quality variables (pH, DO, chlorophyll *a*, SDV, total alkalinity, ammonia and total phosphorus) was not significant (Table 7).

Discussion

In the cabbage and pumpkin leaf treatments, weight gains were highest in TR monocultures. Monocultures gave best weight gains when fed pumpkin leaves. For both species, highest percentage weight gains were achieved with 25% polycultures for all inputs. Best survivals were at 25:75 species combinations. Higher fry production occurred with higher proportions of OS and with the cabbage and pumpkin leaf treatments.

Ayoade et al. (1986) and Rajadevan and Schramm (1989) reported that cabbage leaves had 8.9% and 13.4% fiber contents, respectively, whereas Platt (1962) found that pumpkin leaves had 0.8%. Therefore, in tilapia ponds, pumpkin leaves may act more as direct feed, and cabbage leaves as a fertilizer to the pond environment.

Overall weight gains were higher with maize bran. Although its crude protein content was lowest of the three inputs, it had 93% DM composed to 18% DM for pumpkin leaves.

From the water quality data, pumpkin leaves gave a higher production of natural foods as shown by the higher pH values, DOs, chl, and lower SDVs (Tables 6 and 7). This may be due to

Table 4. Yield characteristics for various ratios of *Tilapia rendalli* and *Oreochromis shiranus* in 5-m^3 tanks for 134 days. Weight gain is for adults only. Total weight gain includes fry. Two-way significance levels are indicated by asterisks (*=5%, **=1% and ***=0.1%).

Variable	Treatments: (Feeds and species ratios)												Significance levels
	Cabbage				Pumpkin				Maize bran				
	25:75	0:100	100:0	75:25	25:75	0:100	100:0	75:25	25:75	0:100	100:0	75:25	
Average initial weight (g)	12.27	12.67	11.97	12.14	11.84	12.17	11.95	11.84	12.07	11.65	12.5	12.05	
range (g)	(10-15)	(10-15)	(10-15)	(10-15)	(10-15)	(10-15)	(10-15)	(10-15)	(10-15)	(10-15)	(10-15)	(10-15)	
Average final weight (g)	18.17	15.35	20.86	21.09	20.24	21.76	22.8	23.2	25.07	22.83	23.86	25.61	
range (g)	(15-25)	(11-23)	(14-28)	(17-32)	(17-29)	(13-32)	(16-35)	(18-32)	(14-35)	(11-36)	(15-34)	(19-36)	
Weight gain (g)	158	65	225	121	232	266	325	-8	364	312	317	355	Inputs***
Survival rate (%)	94.7	95.0	95.0	71.9	97.8	95.0	98.4	54.9	97.8	95.0	96.7	87.1	Ratios*
Fry production:-													
Total (g)	600	451	114	354	481	456	4	576	261	749	280	135	Ratios*
% of total production	79	87	34	75	68	63	1	101	42	71	47	28	
Total weight gain (g)	758	516	339	475	713	722	329	568	625	1,061	597	490	
Mean net yield: - $kg \cdot ha^{-1} \cdot 134$ days	1,516	1,032	678	950	1,426	1,444	658	1,136	1,250	2,122	1,194	980	
SGR (%/day)	0.36	0.16	0.40	0.47	0.43	0.45	0.49	0.47	0.53	0.51	0.48	0.61	Inputs*
FCR	3.6	5.3	8.0	5.7	3.9	3.9	8.5	4.9	4.5	2.6	4.7	5.7	
Sediment yield (kg)	14.1	14.7	14.7	16.2	16.2	13.5	26	34.6	12.2	8.9	20.9	19.1	

Table 5. Harvest data for *Tilapia rendalli* (TR) and *Oreochromis shiranus* (OS). Performance is expressed by ranking. First row (not numbered) gives the combination ratios of TR:OS. Rows 2, 5, 8, 9, 14 and 23 are group ranks for effects of stocking ratios on fish performance holding feed constant, e.g. Row 2, are ranks for effects of combination ratios on total weight gain (TR+OS) where fish were fed on cabbage, pumpkin leaf and maize bran (madeya), respectively. Rows 3, 6, 10, 12, 15, 19, 21 and 24 are ranks for the interactions between combination ratios and feeds, e.g. Row 2 data are ranks for the combined effect of combination ratios and feeds on total weight gain (TR+OS). Rows 1, 5, 7, 11, 13, 16, 17, 18, 20 and 22 are the actual data in grams (g); $kg \cdot ha^{-1}$ per 134 days and/or percentage (%); FCRs have been calculated taking into consideration the weight of fry produced. (ΣR_j) is a summation of six-rank groups in rows 10, 12, 15, 19, 21 and 25. # = Treatments tied for ranks, hence given the average of the tied ranks (Friedman 1937). Kendall's coefficient of concordance, with ties (W_c) is 0.33 (Kendall 1962; Zar 1984). W_c is significant at < 0.025 because: $-\chi^2_r = M(n-1) = 22.011$ is greater than χ^2 0.05, 11 = 19.675 where χ^2_r is Friedman Chi-square (Friedman 1937); M = number of groups (6); n=number of entities (group and total ranks minus rows 8, 9) ranked (12); and (R_j) is the rank for (ΣR_j)s.

Row	Results	TR:OS Feed	Treatments											
			25:75 Cabb.	0:100 Cabb.	100:0 Cabb.	75:25 Cabb.	25:75 Pumpk.	0:100 Pumpk.	100:0 Pumpk.	75:25 Pumpk.	25:75 Madeya	0:100 Madeya	100:0 Madeya	75:25 Madeya
1	Total	TR+OS	158	65	225	121	232	216	325	-8	364	312	317	355
2	weight	Group ranks	2	4	1	3	3	2	1	4	1	4	3	2
3	gain	Total ranks	9	11	8	10	7	6	3	12	1	5	4	2
	(g)	TR+OS												
4		+ fry	758	516	339	475	713	722	329	568	625	1.061	597	490
5		Group ranks	1	2	4	3	2	1	4	3	2	1	3	4
6		Total ranks	2	8	11	10	4	3	12	7	5	1	6	9
7	Total	TR+OS	135.5	94.4	135.8	130.4	159.2	160	200.7	89.3	303.6	197.8	172.6	205.9
8	wet	Group ranks (TR)	1	-	2	3	1	-	2	3	1	-	3	2
9	weight	Group ranks (OS)	2	3	-	1	3	2	-	1	2	3	-	1
10	gain	Total ranks (TR+OS)	9	11	8	10	7	6	3	12	1	4	5	2
	(%)													
11	Survival	TR+OS	94.7	95	95	71.9	97.8	95	98.4	54.4	97.8	95	96.7	87.1
12	(%)	Total ranks	9	6.5#	6.5#	11	2.5#	6.5#	1	12	2.5#	6.5#	4	10
13	Fry	(g)	600	451	114	354	481	456	4	576	261	749	280	135
14	production	Group ranks	1	2	4	3	2	3	4	1	3	1	2	4
15		Total ranks	2	6	11	7	4	5	12	3	9	1	8	10
16	SGR	TR	0.46	-	0.40	0.41	0.50	-	0.49	0.40	0.50	-	0.48	0.50
17	($\% \cdot day^{-1}$)	OS	0.25	0.16	-	0.52	0.36	0.45	-	0.54	0.56	0.51	-	0.71
18		TR+OS	0.36	0.16	0.40	0.47	0.43	0.45	0.49	0.47	0.53	0.51	0.48	0.61
19		Total ranks	11	12	10	6.5#	9	8	4	6.5#	2	3	5	1
20	Net yield	TR+OS	1,516	1,032	678	960	1,426	1,444	658	1,136	1,250	2,122	1,194	980
21	($kg \cdot ha^{-1} \cdot 134$ d)	Total ranks	2	8	11	10	4	3	12	7	5	1	6	9
22	FCR	TR+OS	3.6	5.3	8.0	5.7	3.9	3.9	8.5	4.9	4.5	2.6	4.7	5.7
23		Group ranks	1	2	4	3	2	1	4	3	2	1	3	4
24		Total ranks	2	8	11	9.5#	3.5#	3.5#	12	7	5	1	6	9.5#
25	Sum of group ranks	(ΣR_j)	35	51.5	57.5	54	30	32	44	47.5	24.5	16.5	34	41.5
26	Overall ranks	(R_j)	6	10	12	11	3	4	8	9	2	1	5	7

Table 6. Overall mean water quality parameters in 5-m^{-3} tanks for 134 days. Two-way significance levels are indicated with stars (*<5%, **<1% and ***<0.1%). Figures in parentheses are ranges of the means. TR = *Tilapia rendalli*; OS = *Oreochromis shiranus*. NS = no significance.

Water quality parameter	Treatments: (Feeds and species combination ratios, TR:OS)												Significance level
	Cabbage leaves				Pumpkin leaves				Maize bran				
	25:75	0:100	100:0	75:25	25:75	0:100	100:0	75:25	25:75	0:100	100:0	75:25	
pH (unit)	8 (7.1-9.8)	8.2 (7.3-9.7)	8.5 (7.3-9.3)	8.3 (7.5-9.5)	9.1 (7.8-10.2)	8.9 (8.5-9.6)	9.2 (7.8-9.9)	9.3 (7.8-9.8)	8.2 (6.0-9.6)	8.1 (7.3-9.5)	8.2 (7.5-9.3)	8.4 (7.6-9.9)	Inputs*
Dissolved oxygen ($mg \cdot l^{-1}$)	3.9 (1.3-9.0)	4.1 (1.3-8.8)	4.4 (1.6-9.3)	3.6 (1.3-8.7)	6.1 (4.0-9.9)	4.3 (1.5-9.1)	6.2 (1.9-10.9)	6.5 (2.6-9.5)	2.6 (1.0-9.1)	2.9 (1.3-8.8)	2.6 (0.9-9.3)	3.1 (1.1-9.3)	Inputs***
Chlorophyll *a* ($mg \cdot l^{-1}$)	15.6 (5.8-29.0)	9.7 (4.1-26.0)	8.0 (0.8-20.0)	9.7 (0.8-15.5)	15.5 (3.4-39.2)	20.1 (1.0-45.4)	17.4 (2.0-43.8)	17.7 (5.6-32.0)	10.7 (2.2-19.1)	5.9 (1.8-11.0)	6.2 (2.1-9.1)	5.7 (0.9-12.9)	Ratios***
Secchi disk visibility (cm)	55.0 (31->100)	59.6 (29->100)	60.5 (28->100)	59.6 (26->100)	53.9 (24->100)	37.4 (14->100)	53.1 (25->100)	49.0 (26->100)	62.1 (29->100)	70.0 (35->100)	68.9 (38->100)	68.0 (35->100)	Inputs*
Total alkalinity ($mg \cdot l^{-1}$)	75.2 (28-120)	69.5 (28-113)	63.9 (30-90)	69.1 (31-105)	57.4 (28-88)	68.6 (28-99)	60.7 (29-89)	61.3 (29-84)	84.3 (31-130)	83.8 (33-126)	82.3 (26-124)	80.7 (29-84)	Inputs***
Ammonia ($mg \cdot l^{-1}$)	0.55 (0.29-0.83)	0.504 (0.09-0.9)	0.503 (0.07-0.87)	0.756 (0.07-1.48)	0.528 (0.07-1.1)	0.783 (0.07-1.4)	0.544 (0.08-0.93)	0.622 (0.071.2)	0.53 (0.06-0.86)	0.431 (0.07-0.67)	0.552 (0.08-0.95)	0.569 (0.06-1.1)	NS
Total phosphorus ($mg \cdot l^{-1}$)	2.592 (0.9-5.4)	2.308 (0.8-2.8)	2.242 (0.9-3.3)	2.175 (0.9-4.4)	2.092 (1.2-2.8)	4.591 (0.9-9.6)	2.075 (0.8-4.3)	2.508 (0.8-5.1)	1.9 (1.0-2.3)	2.2 (0.8-4.8)	2.025 (0.9-5.7)	2.175 (0.8-3.7)	NS
Conductivity ($\mu mho \cdot cm^{-1}$)	154.2 (63-229)	141.5 (59-225)	139.8 (58-217)	145.5 (64-219)	127.8 (57-149)	148.3 (63-188)	133.5 (67-174)	133.6 (63-157)	173.3 (63-251)	161.3 (63-188)	175.8 (56-245)	162.1 (62-220)	Inputs***

Table 7. Overall mean and rank orders for water quality parameters. pH values of 6.0-8.0 were assigned a rank of 1; every 0.1 pH unit <6 or >8 were assigned a rank of 2, etc. *Treatments that obtained equal scores thereby tied for ranks, hence given the average of the tied ranks (Friedman 1937): Group 1 treatments tied for rank 3; Group 2 treatments tied for ranks 11 and 12, hence given 11.5; Group 3 treatments tied for ranks 7 and 8, hence given 7.5; Group 4 treatments tied for ranks 6 and 7, hence given 6.5; Group 7 treatments tied for ranks 7 and 8, hence given 7.5; (ΣR_j) is the sum of Groups 2, 3, 4, 5, 6, 7 and 8 (Group 1 is excluded); Kendall's coefficient of concordance, with ties (W_c) is 0.065 (Kendall 1962; Zar 1984); W_c is not significant at > 0.05 because $\chi^2_r = M(n-1)W = 5.72 < \chi^2\ 0.05,\ 11 = 19.675$ where χ^2_r is Friedman χ^2 (Friedman 1937); M = number of groups (8); n = number of entities ranked (12); and (R_j) is the rank for (ΣR_j)s.

Treatment																			
Species ratios TR:OS	Type of feed	pH Group 1		DO ($mg \cdot l^{-1}$) Group 2		Chlorophyll *a* ($mg \cdot l^{-1}$) Group 3		SDV (cm) Group 4		Alkalinity ($mg \cdot l^{-1}$) Group 5		Ammonia ($mg \cdot l^{-1}$) Group 6		Orthophosphate ($mg \cdot l^{-1}$) Group 7		Conductivity ($mg \cdot l^{-1}$) Group 8		Sum of group ranks (ΣR_j)	Overall ranks (R_j)
25:75	Cabbage	8.0	1	3.9	7	15.6	4	55.0	5	75.2	5	0.550	7	2.592	2	154.2	5	36	1
0:100	Cabbage	8.2	3*	4.1	6	9.7	7.5*	59.6	6.5*	69.5	6	0.504	3	2.308	4	141.5	8	44	3
100:0	Cabbage	8.5	6	4.4	4	8.0	9	60.5	8	63.9	9	0.503	2	2.242	5	139.8	9	52	7
75:25	Cabbage	8.3	4	3.6	8	9.7	7.5*	59.6	6.5*	69.1	7	0.756	11	2.175	7.5*	145.5	7	58.5	11.5*
25:75	Pumpkin	9.1	8	6.1	3	15.5	5	53.9	4	57.4	12	0.528	4	2.092	9	127.8	12	57	9
0:100	Pumpkin	8.9	7	4.3	5	20.1	1	37.4	1	68.6	8	0.783	12	4.591	1	148.3	6	41	2
100:0	Pumpkin	9.2	9	6.2	2	17.4	3	53.1	3	60.7	11	0.544	6	2.075	10	133.5	11	55	8
75:25	Pumpkin	9.3	10	6.5	1	17.7	2	49	2	61.3	10	0.622	10	2.508	3	133.6	10	48	4.5*
25:75	Madeya	8.2	3*	2.6	11.5*	10.7	6	62.1	9	84.3	1	0.530	5	1.900	12	173.3	2	49.5	6
0:100	Madeya	8.1	2	2.9	10	5.9	11	70.0	12	83.8	2	0.431	1	2.200	6	161.3	4	48	4.5*
100:0	Madeya	8.2	3*	2.6	11.5*	6.2	10	68.9	11	82.3	3	0.552	8	2.025	11	175.8	1	58.5	11.5*
75:25	Madeya	8.4	5	3.1	9	5.7	12	68	10	80.7	4	0.569	7	2.175	7.5*	162.1	3	57.5	10

their fertilizing effect from high total phosphorus (0.09%) and crude protein (5.4%) (Table 3).

When treatment parameters were subjected to parametric analyses, most of the results were inconclusive. ANOVA showed no significant difference among treatments. However, ranking order tests demonstrated significant effects on fish yield parameters. Kendall (1962) suggested that the best estimate of the true ranking of N treatments is given, when W_c is significant, by the order of the sums of ranks, ΣR_i. Using this, the best fish growth here was achieved with maize bran and 100% OS, and with maize bran and 25:75 TR:OS. These treatments gave the lowest ΣR_i values of 16.5 and 24.5, respectively, followed by that for 25:75 TR:OS with pumpkin leaves (ΣR_i=30). A ranking order test also showed that pumpkin leaves had the most influence on water quality.

Overall, the best weight gains were obtained in TR monoculture, and the best weight gains in monocultures of both TR and OS were achieved with pumpkin leaves. This suggests that there is little synergism in TR:OS polycultures. However, to achieve an optimal compromise between weight gain and survival, a 25:75 TR:OS polyculture might be the best combination. For resource-poor farmers, pumpkin leaves may form a good substitute for maize bran. Because fry production was higher for vegetable treatments, feeding of such vegetable waste may help alleviate fingerling shortages among fish farmers. Further research is needed to test whether these indications from tank experiments hold true in farm ponds.

Acknowledgements

This work was supported by the International Center for Living Aquatic Resources Management (ICLARM) with funds from the German Bundesministerium für Wirtschaftliche Zusammenarbeit (BMZ) and Gesellschaft für Technische Zusammenarbeit (GTZ), GmbH. Thanks are also due to Mr. Silence Sonthe for his untiring technical support during the experiments. Travel expenses were supported by Malaŵi-German Fisheries and Aquaculture Development (MAGFAD) Project.

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Effects of the Varying Protein-energy Levels on Food Consumption, Growth and Body Composition of *Sarotherodon melanotheron* (Rüppel, 1852)

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Abstract

The effects of varying protein-energy levels were studied on males of *Sarotherodon melanotheron* (7.5 g) kept in aquaria with the following results:

- the feed-energy level significantly affected food consumption while crude protein levels did not;
- the highest weight gains, feed conversion ratios and protein efficiency ratios were reported from individuals fed with a compound feed containing 30% crude proteins contributing about 1,700 kJ·100 g of feed^{-1}. The optimum protein-energy ratio for this species was found to be 17 mg protein·kJ^{-1}. Increased or decreased values resulted in poorer growth performances; and
- body fat levels are directly related to diet lipid levels and are inversely related to protein levels.

Introduction

Sarotherodon melanotheron is a species of lagoon tilapia much appreciated by Ivorian consumers. Its potential for aquaculture has already been reported (Pillay 1965; Pauly 1976; Silvalingam 1979; Legendre 1983; Albaret 1987; Legendre et al. 1989). Several studies were conducted by the Abidjan Oceanographic Research Center (CRO) to develop the aquaculture of this species in Ivorian lagoon environments. Preliminary results (Legendre 1983, 1986; Cissé 1986) showed high variability in feed conversion ratios depending on the compound feed used and the culture system. This study investigated different dietary protein-energy ratios and their effects on the food consumption, growth and body composition of the fish.

Materials and Methods

A 45-day experiment was conducted at the Layo Station (Côte d'Ivoire) in a series of rectangular 175-l aquaria with recirculated water using an EHEIM 2013 biofilter. Water was recirculated at a rate of 5-6 l·min^{-1}. Physicochemical parameters were measured giving the following values: water temperature (28±1°C); oxygen concentration (5.5-6.3 mg·l^{-1}); pH (6.2-7.5); and N-ammonia and nitrite concentrations maintained below 0.008 and 0.15 mg·l^{-1}, respectively.

The experiment followed a 3x2 factorial model with feeding treatments containing three crude energy levels (1,600x1,700 kJ·100 g of feed^{-1}) for two levels of crude proteins (20 and 30%). The actual protein levels for each treatment after chemical analysis are

presented in Table 1. The energy levels in all treatments were estimated using the following coefficients: 5.5 Kcal·day^{-1} for proteins, 9.1 Kcal·day^{-1} for lipids and 4.1 Kcal·day^{-1} for carbohydrates (Jauncey and Ross 1982). Energy sources and proteins were essentially of the same quality in all treatments. Ground-nut oil levels varied while cod liver oil was kept at constant levels to reduce changing palatability among treatments. The feed was mixed, moisturized (20% water) and extruded through the 1-mm orifices of an Alexanderwerk AGM-8876 micro-pelleter. The moist pelleted feed was steam-dried at 50°C to achieve a moisture content ≤10% before placing it in airtight plastic bags and stored in an air-conditioned room at 20-22°C until utilization.

The fish used in this experiment were young male *S. melanotheron* (mean individual weight: 7.5 g) from two pairs of siblings. Fish previously receiving a feed containing 45% proteins were starved for two days before the beginning of the experiment. Six replicated, experimental groups were set up with 30 individuals per aquarium. Each batch was given a test feed distributed manually twice daily, six days a week. The daily feeding rate was determined at

Table 1. Composition of the experimental feed (% of dry matter).

Composition	Groups, feed (% DM)					
	A	B	C	D	E	F
Local fish meal[a]	22.22	22.22	22.22	38.89	38.89	38.89
Soy cake	16.71	16.71	16.71	19.00	19.00	19.00
Groundnut oil	3.40	7.10	10.90	0.00	3.80	7.50
Cod liver oil	3.00	3.00	3.00	3.00	3.00	3.00
Maize meal	10.80	15.00	22.90	7.20	13.80	24.25
Wheat bran	38.37	30.47	18.77	26.41	16.61	1.86
Binder (cassava meal residues)	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin mix[b]	1.50	1.50	1.50	1.50	1.50	1.50
Vitamin C	0.50	0.50	0.50	0.50	0.50	0.50
Minerals[c]	0.50	0.50	0.50	0.50	0.50	0.50
	Analysis[d] (% DM)					
Ash	10.81	11.50	12.90	13.31	13.52	15.15
Crude fiber	8.11	7.02	6.32	6.32	4.83	4.33
Crude proteins (Nx6.25)	21.50	22.00	20.98	31.07	30.75	30.45
Fat	8.11	10.10	12.40	5.02	6.80	7.50
Nitrogen-free extract	51.47	49.38	47.40	44.28	44.10	42.57
	Values					
Crude energy (kJ·100 g^{-1})	1.61	1.74	1.84	1.62	1.72	1.76
mg protein·kJ^{-1}	13.3	12.7	11.4	19.2	17.9	17.3

[a]Tuna residues from local canning industries.
[b]mg·kg^{-1} of feed - Vit. E: 25,000; Vit. B1: 5,000; Vit. B2: 6,000; Vit. B6: 5,000; Vit. PP: 40,000; Vit. B12: 4; AP: 16,000; Vit. K: 5,000; Folic acid: 1,000; and Choline chloride: 250.
[c]mg·kg^{-1} of feed - Cobalt: 25; Iron: 22,000; Iodine: 2,500; Manganese: 13,500; Copper: 1,500; Zinc: 7,500; and Selenium: 45.
[d]Analyses done at the Central Laboratory for Animal Nutrition (LACENA).

10% of the total fish biomass. The feed left unconsumed was recovered by siphoning after each feeding. Daily mean consumption was determined by subtracting the dry weight of the excess feed from the quantity of feed distributed. The feeding rate was adjusted each week according to the fish biomass found in each aquarium.

The feed conversion ratios (FCR), defined as the ingested dry feed/wet weight gain ratio, were estimated. The protein efficiency coefficient (PER = wet weight gain/ingested proteins) and the energy efficiency (wet weight gain/100 Kcal ingested) were estimated based on initial and final mean weights. ANOVA and Duncan's Multiple Range Test (1955) were used for the interpretation of the results.

Results

Food Consumption

In *S. melanotheron*, food consumption was significantly affected ($P<0.05$) by feed energy level. In effect, food intake decreased as the calorific content of the feed increased. A change in protein content from 20 to 30% did not have any significant effect ($P>0.05$) on the quantity of feed consumed (Table 2).

Weight Gain, FCR and PER

Results are summarized in Table 3. At the end of the experiment, all batches fed with a 20% crude protein diet clearly showed poorer growth performance compared to batches fed with a 30% protein diet, irrespective of the feed energy level.

FCR were generally high, especially in batches fed with a 20% protein diet. The PER also varied. Batch E gave the best results but, in contrast to FCR, there was no clear difference between batches fed with a relatively high protein diet. In this experiment, individuals fed with a 30% crude protein diet contributing about 1,700 kJ·100 g of feed^{-1} showed the best performance. Under our experimental conditions, 17 mg protein· kJ^{-1} was found to be the optimum protein/energy ratio for *S. melanotheron*. Increased or decreased values resulted in poorer growth performance.

Table 2. Mean food consumption for *Sarotherodon melanotheron* over 45 days of experimentation[a].

Batch	Daily consumption·100 g of fish^{-1}		
	Dry feed ingested (g)	Crude energy (kJ)	Protein (g)
A	5.85 a	94.4 b	1.25 b c
B	5.01 b	86.9 d	1.10 c d
C	4.78 c	87.9 d	1.00 d
D	5.98 a	96.6 a	1.85 a
E	5.34 b	91.9 c	1.64 a b
F	4.99 c	87.6 d	1.51 b

[a]Values with the same letters are not statistically different ($P>0.05$).

Body Composition of the Experimental Fish

After 45 days of experiment, the overall body composition of the fish was clearly affected by feed quality, particularly in body fat and water content which varied in opposite directions (Table 4). For proteins, the highest level (16.3%) was found in fish fed with a 30% protein diet and the lowest (14.8%) in fish fed with a 20% protein diet.

Discussion

These results show that low protein diet was less efficient. Although fish were fed twice a day, they seemed to be fed to satiation considering the quantities

Table 3. Effect of the different feeds on the growth perfomance of *Sarotherodon melanotheron* after 45 days of experimention[a].

Batch	Weight gain (%)			FCR	PER
	Weight	Protein	Energy		
A	355 d	225 cd	240 d	3.89 c	1.19 c
B	339 c d	244 c	278 d	3.18 b	1.42 b
C	198 e	189 d	363 b c	5.25 d	0.91 c
D	470 b	374 a	310 c d	1.80 a	1.79 a
E	535 a	411 a	414 a b	1.54 a	2.11 a
F	380 c	312 b	467 a	2.22 a	1.48 b

[a]Values with the same letters are not statistically different (P>0.05).

Table 4. Body composition of *Sarotherodon melanotheron* after 45 days of experimention[a].

Batch	Body composition (% wet weight)		
	Water	Crude proteins	Crude lipids
A	75.10 a	15.60 a b	8.40 b
B	74.25 c	15.15 a b	10.30 a
C	73.95 d	14.80 b	10.85 a
D	74.47 b c	16.12 a	7.87 c
E	74.72 b	16.30 a	8.65 b
F	73.55 cd	16.10 a	10.40 a

[a]Values with the same letters are not statistically different (P>0.05).

of feed freely consumed (Table 2). However, the quantities ingested varied from batches to batches. The differences observed are most probably due to the effects of the protein-energy ratio than the lipid-carbohydrate ratio in the feed. Our results support those of Shell (1969), Winfree and Stickney (1981) and Jauncey (1982) on *Oreochromis mossambicus*, *O. aureus* and *O. niloticus*, respectively, in that tilapia regulate their food consumption according to energy intake not protein intake.

As Alliot et al. (1979) indicated, the daily quantities of proteins ingested under our experimental conditions may be insufficient to respond to growth requirements, and this may be accentuated by the high levels of carbohydrates in the feed. Palmer and Ryman (1972) have shown that hyperglycemia in trouts caused a fall in the serum free amino acids, producing changes in nutrient absorption. Our results also confirm the observations made by these authors on the importance of lipids as a source of energy in fish.

From the viewpoint of body composition, the feed energy levels did not seem to have any effect on the accumulation of fat. In fact, body composition seems to be directly related to the crude lipid levels in the feed. In effect, batches B and C were found to store more fat than batches D and E. In general, batches fed with a low protein diet

showed the highest crude lipid levels, which can only be explained by an important imbalance in the feed.

This study has certainly not assessed all the nutritional requirements that must be considered to improve the growth performance of *S. melanotheron* but it has shown some important factors that must be taken into consideration when developing experiments in which the effects of natural productivity are added to those of the feeds that are given.

Acknowledgements

This study was supported by a grant from the International Foundation for Science (IFS), research grant no. A/700-2. I wish to thank Dr. Coulibaly of the Central Laboratory for Animal Nutrition in Abidjan (LACENA) and his team, as well as Dr. Odi, for their assistance in the chemical and statistical analyses of the numerous data. My gratitude also goes to Dr. Luquet for the relevance of his comments.

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Feeding Cottonseed Cake to Tilapia (*Oreochromis niloticus*) in Earthen Ponds with Catfish (*Clarias gariepinus*) as Police-Fish[a]

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MIDDENDORP, A.J. 1996. Feeding cottonseed cake to tilapia (*Oreochromis niloticus*) in earthen ponds with catfish (*Clarias gariepinus*) as police-fish, p. 198-203. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

The effect of cottonseed cake as a supplementary feed was tested in tilapia-catfish polyculture ponds, which received dry cattle manure as a basic treatment. Cottonseed cake is relatively expensive while old, dry cattle manure is freely available from the corrals left by pastoralists. Three treatments were tested in six earthen ponds of 525 m^2 each: (A) daily application of dry cattle manure at 266 $kg \cdot ha^{-1} \cdot day^{-1}$; (B) daily manure + cottonseed cake at a nominal daily rate of 3% of tilapia biomass; and (C) daily manure + cottonseed cake at 6% of tilapia biomass. Stocking rates per pond were 250 male Nile tilapia, *Oreochromis niloticus* (mean weight 222 g); 150 female tilapia (202 g); 30 "large" North African catfish, *Clarias gariepinus* (198 g); and 30 "small" catfish (52 g). Feed was given six days per week, and the culture period was 100 days. Feeding rates were adjusted monthly. Average extrapolated annual pond production including recruits was: (A) -0.4 $t \cdot ha^{-1} \cdot year^{-1}$; (B) 4.9 $t \cdot ha^{-1} \cdot year^{-1}$; and (C) 6.5 $t \cdot ha^{-1} \cdot year^{-1}$. Best growth was observed in Treatment C (male tilapia: 0.9 $g \cdot day^{-1}$ and large catfish: 6.9 $g \cdot day^{-1}$), but production and growth in Treatments B and C were not different ($P>0.05$). The mean production of tilapia fingerlings in Treatments B (1,539 $kg \cdot ha^{-1} \cdot year^{-1}$) and C (1,829 $kg \cdot ha^{-1} \cdot year^{-1}$) were not significantly higher than in Treatment A (468 $kg \cdot ha^{-1} \cdot year^{-1}$), where catfish predation was probably more intense ($P>0.10$).

Introduction

In northern Cameroon, the two most important by-products available in large quantities as fishpond inputs are brewery waste and cottonseed cake meal. Brewery waste has been used successfully (Middendorp 1995a) but it is bulky, watery and acidic. The more expensive cottonseed cake is a dry product, which stores well and is easy to handle. It is used primarily as cattle feed during the dry season. Jackson et al. (1982) marked cottonseed cake meal as a superior source of plant protein for tilapias, compared to copra cake, soybean meal or groundnut cake.

An experiment was set up to evaluate the utility of locally available inputs, notably old, dry cattle manure, which is freely available from the temporary corrals when the pastoralists have left after the rainy season, and to investigate cottonseed cake as a supplementary feed. An attempt was also made to control the reproduction of

[a]A more detailed account of this work has been published; see Middendorp 1995b.

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Nile tilapia (*Oreochromis niloticus*) stocked as postfingerlings, by polyculture with the omnivorous African catfish (*Clarias gariepinus*) as a potential predator: the "police-fish concept."

The experiment was conducted at the Lagdo Fisheries Station, near Garoua, northern Cameroon. The station is located in an irrigation scheme of about 1,200 ha (1993). Water is received by gravity from the Lagdo dam (700 km^2), which filled up completely in 1988. Rearing tilapia postfingerlings to harvest weights of 300 to 350 g is necessary at the Lagdo Fisheries Station, in order to obtain competitive market prices. Wild tilapia (*Sarotherodon galilaeus*) of this size are caught in huge quantities from the Lagdo reservoir (estimated annual catch 8,000 to 10,000 t, 70% of which is *S. galilaeus*), thereby depressing the market price for smaller (cultured) tilapia.

Materials and Methods

Facilities and Fish

Six 525-m^2 earthen ponds were stocked with Nile tilapia and catfish at the onset of the rainy season of 1991. The pond bottoms had been previously cleared of mud. Stocking rates (average weights) per pond were: 250 male tilapia (222 g), 150 female tilapia (202 g), 30 "large" catfish (198 g) and 30 "small" catfish (52 g). The overall fish densities were: 7,619 tilapia·ha^{-1} and 1,143 catfish·ha^{-1}. All tilapia came from the same batch of broodstock and had been raised in three parallel ponds on the same diet. The numbers of tilapia stocked were limited by their availability. The catfish came from two different batches (Middendorp 1993). The large catfish were stocked on the same day as the tilapias, as 20% of the number of female tilapias. Small catfish were added after 40 days (also as 20%) in a secondary attempt to control the apparent large numbers of swim-up tilapia fry in all ponds.

Experimental Procedure and Feeding

Three treatments were tested in duplicate. Treatment A was a daily application of dry cattle manure (bucket of 20 l, weighing about 14 kg), which corresponded to 266 kg·ha^{-1}·day^{-1}. Treatments B and C comprised this same manure application plus cottonseed cake meal at nominally 3% (B) and 6% (C) of the tilapia biomass per day. Feeding was done six days per week, in the afternoon. The cottonseed cake meal was allowed to moisten during the day with a little water. The percentage composition of the commercial pre-pressed solvent-extracted cottonseed meal, as used in this experiment, was assumed to be similar to that reported by Jackson et al. (1982): moisture 8.2, protein 42.7, ether extract 1.0, ash 8.2, fiber 12.6, N-free extract 27.3, available lysine 92 and free gossypol 0.03. Its phosphorous content was assumed to about 1.36% of dry matter (Göhl 1975 in Jauncey and Ross 1982).

Feeding began on 2 May 1991, after five days of acclimatization. Samples of tilapia were weighed and measured every five weeks (sample size: 10%) and feeding rates were adjusted accordingly. The last feeding was on 8 August 1991 and the ponds were drained and harvested in the following days. Total experiment time, which was calculated from the first to the last feeding day, was 100 days for the male and female tilapias and for the large catfish. However, rearing time for the small catfish was only 55 days (assuming five days of nonfeeding right after stocking).

Pond oxygen and turbidity (Secchi disk) were measured in all ponds once every week at 0800 hours. On the last day of the trial, about 15 minutes after feeding, two tilapias per pond were killed to examine gut contents.

Data Analysis

Fish were batch-weighed and counted at stocking and at harvest. Average initial (W_0) and final weight (W_t) were calculated. Growth was expressed as daily growth rate (DGR: $g \cdot day^{-1}$) and as specific growth rate (SGR: $\%BW \cdot day^{-1}$), calculated as:

$$SGR = \frac{(\ln W_t - \ln W_0)}{t} x100$$

Fish production per pond was defined as the total yield minus the total stocking weight. Extrapolated pond production ($t \cdot ha^{-1}$) was adjusted for the shorter rearing period of the small catfish.

Two different feed conversion ratios (FCR) were calculated: the total quantity of cottonseed cake meal given to the pond, divided (1) by the combined production of the tilapia and catfish originally stocked, but not including their fingerlings; and (2) by the total pond production of tilapia, catfish and their fingerlings.

At harvest, 20 female and 20 male tilapias from each pond were measured individually to the nearest 0.5 cm (standard length, SL) and weighed (W). Condition factor (CF) was calculated as by Pitcher and Hart (1982):

$$CF = W / L^3$$

Means and coefficients of variation (CV) were computed. Differences between means were tested by Tukey's multiple range test ($P<0.05$). The general model tested was: $Y=m+Tr_{i=3}+e_{ij}$, with W_0, W_t, DGR, SGR, Production, FCR and R as variables (Tr=treatment factor; and j=2: number of pond replicates). The sampling results at harvest were analyzed as: $Y=m+Tr_{i=3}+Po_{j=2}+e_{ijk}$, with SL, W, and CF as variables (Po=pond factor; j=2: number of pond replicates; and k=25: number of fish sampled).

Results

Mean pond temperature varied from 28.0°C in the morning to 31.1°C in the afternoon. Secchi disk readings and oxygen measurements are presented in Table 1. Overall mortality of stocked tilapia was less than 1%. Tilapia fingerlings were numerous. All catfish were recovered, plus a few catfish fingerlings that were found in four of the six ponds.

Total weights of fish at stocking and harvest are shown in Table 2. Daily growth rates (DGR) and specific growth rates (SGR) were significantly different between Treatment A and both Treatments B and C for all types of fish. Growth rates were not different between Treatments B and C, except for DGRs of the small catfish. Within treatments, growth of male tilapia was not significantly better than growth of female tilapia (Fig. 1). Both small and large catfish grew significantly faster than the tilapias and the SGRs of small catfish in Treatments B and C were significantly higher than that of large catfish.

Mean fish production in kilogram per pond and per type of fish is presented in Table 3. Extrapolated annual pond production, including fingerling yield, was: Treatment

Table 1. Mean Secchi disk depths and dissolved oxygen at 0800 hours. Treatment means within the same time period, and with different superscripts are significantly different (Tukey's test, P=0.05).

Treatment	A		B		C	
	Secchi (cm)	Oxygen ($mg \cdot l^{-1}$)	Secchi (cm)	Oxygen ($mg \cdot l^{-1}$)	Secchi (cm)	Oxygen ($mg \cdot l^{-1}$)
May, June	21.1	13.4	19.6	11.3	21.8	10.9
July	19.4^a	10.6	15.0^b	6.4	15.0^b	7.1

Table 2. Production parameters (two replicates per treatment). Pond size: 525 m^2. Rearing time: 100 days, except for small catfish (55 days). Treatment means with different superscripts are significantly different (Tukey's test, P=0.05). See text for details of Treatments A, B and C.

Treatment	A	B	C
Tilapia males (250 fish per pond)			
Initial weight (g)	224.0	226.3	216.2
Harvest weight (g)	184.8^a	284.1	304.4^b
DGR ($g \cdot day^{-1}$)	-0.4^a	0.6^b	0.9^b
SGR ($\%BW \cdot day^{-1}$)	-0.2^a	0.2^b	0.3^b
Tilapia females (150 fish per pond)			
Initial weight (g)	201.8	200.7	204.0
Harvest weight (g)	148.3^a	214.7^b	241.9^b
DGR ($g \cdot day^{-1}$)	-0.5^a	0.1^b	0.4^b
SGR ($\%BW \cdot day^{-1}$)	-0.3^a	0.1^b	0.2^b
Large catfish (30 fish per pond)			
Initial weight (g)	195.0	200.0	198.3
Harvest weight (g)	328.3^a	756.7^b	887.8^b
DGR ($g \cdot day^{-1}$)	1.3^a	5.6^b	6.9^b
SGR ($\%BW \cdot day^{-1}$)	0.5^a	1.3^b	1.5^b
Small catfish (30 fish per pond)			
Initial weight (g)	48.3	56.7	51.7
Harvest weight (g)	91.9^a	308.3^b	393.3^b
DGR ($g \cdot day^{-1}$)	0.7^a	2.5^b	3.4^b
SGR ($\%BW \cdot day^{-1}$)	0.9	1.7	2.0

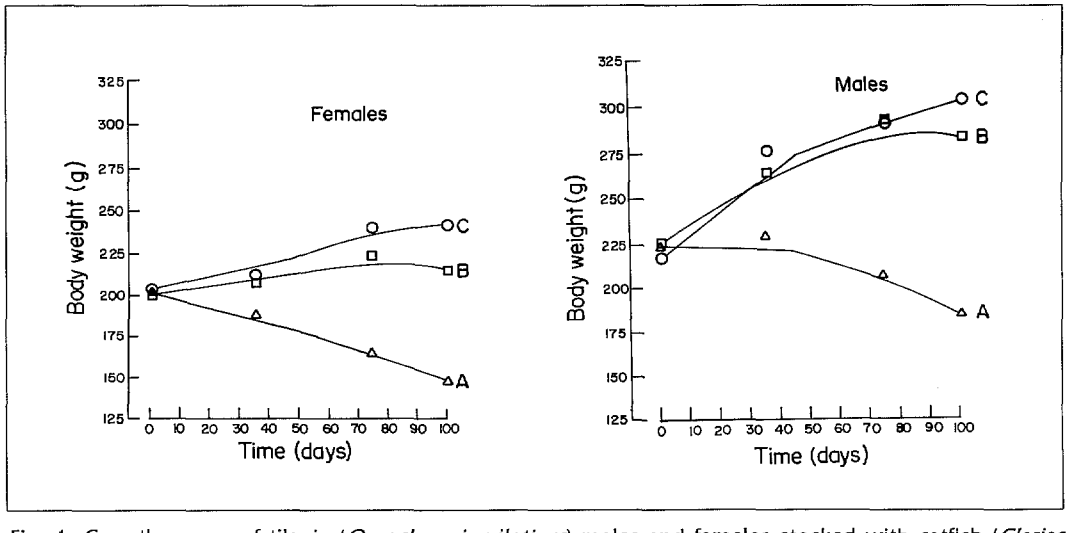

Fig. 1. Growth curves of tilapia (*Oreochromis niloticus*) males and females stocked with catfish (*Clarias gariepinus*) in 525-m^2 experimental ponds. The plotted points are means of fish from duplicate ponds for each treatment: (A) dry manure only; (B) dry manure and cottonseed cake fed at 3% biomass per day; and (C) dry manure and cottonseed cake fed at 6% biomass per day.

Table 3. Mean fish production in kilogram per pond of 525 m^2. Tma=stocked male tilapia, *Oreochromis niloticus*; Tfm=stocked female tilapia; Clg=stocked large catfish, *Clarias gariepinus*; Csm=stocked small catfish; Tf=tilapia fingerlings; and Cf=catfish fingerlings. CSC is the total amount of cottonseed cake fed per pond. FCR1 is calculated over the stocked fish production only; FCR2 is calculated over total pond production. Means with different superscripts are significantly different (Tukey's test, P<0.05).

Treatment	Tma·	Tfm	Clg	Csm	Tf	Cf	Stock*	Total**	CSC	FCR1	FCR2
A	-11.1[a]	-8.1[a]	4.0[a]	1.2[a]	6.7	0.1[a]	-14.0[a]	-7.2[a]	-	-	-
CV%	-7.2	-5.3	24.7	35.4	3.0	58.2	-18.9	-33.4			
B	14.2[b]	1.6[b]	16.7[b]	7.6[b]	22.1	0.5[b]	40.0[b]	62.7[b]	245.5	6.4	4.0
CV%	59.0	66.1	14.4	15.9	4.9	102.2	32.5	23.3	4.4	28.4	19.1
C	23.1[b]	4.8[c]	20.7[b]	10.3[b]	26.3	0.5[b]	58.9[b]	85.7[b]	499.2	8.5	6.0
CV%	4.6	5.2	11.0	6.2	73.1	141.4	1.4	22.6	1.7	3.1	24.3

*Combined production of the originally stocked tilapia and catfish, not including their fingerlings.
**Total pond production of tilapia and catfish plus their fingerlings.

(A) -0.4 $t \cdot ha^{-1} \cdot year^{-1}$; (B) 4.9 $t \cdot ha^{-1} \cdot year^{-1}$; and (C) 6.5 $t \cdot ha^{-1} \cdot year^{-1}$. Average male tilapia production represented an increase of 25% (B) and 42% (C) of the total weight of male tilapia at stocking, compared to an increase of 278% (B) and 345% (C) of large catfish stocking weight. Small catfish production even increased by 447% (B) and 644% (C). Average tilapia fingerling yields in Treatments B (1,539 $kg \cdot ha^{-1} \cdot year^{-1}$) and C (1,829 $kg \cdot ha^{-1} \cdot year^{-1}$) were nearly four times the average fingerling yield in Treatment A (468 $kg \cdot ha^{-1} \cdot year^{-1}$), although the differences were not significant due to high variation in Treatment C (P>0.10). Mean feed conversion rate of cottonseed cake meal calculated over the total pond production was 4.0 (B) and 6.0 (C) (P>0.05).

Tilapia standard length (SL), tilapia weight (W) and condition factors (CF) at harvest were significantly different between Treatment A and Treatments B and C (Table 4). The poor condition of female tilapia in Treatment A, which continued to reproduce while losing weight, was visible to the naked eye.

Tilapias from all treatments had fully filled intestines and stomachs at dissection. However, in Treatment A the gut contents consisted of a greyish muddy substance compared to the dark green algal mass found in Treatments B and C. Some cottonseed cake meal was also found, but the quantity was insignificant compared to the ingested algae.

Discussion

The rationale of manuring all fishponds at a relatively high rate was to establish a basic level of pond productivity against which the supplementary effect of cottonseed cake could be compared. However, application of old, dry cattle manure alone led to a significant decrease in tilapia body weight. Fish were observed browsing through the manure, but apparently did not gain anything from it. The old cattle manure, although applied at a relatively high rate, did not introduce sufficient nutrients into the pond in order to even maintain the total fish biomass. It was presumably poor in nutrients because of leaching during the rainy season.

Cottonseed cake is both high in nitrogen and phosphorous, and supports dense algal blooms via decomposition or via fish feces. Fish fed vigorously when cottonseed cake was applied. The moistened cake leached small milky "clouds" in the water.

Table 4. Standard length and weight of male and female tilapias (n=20), and the condition factor (W/SL^3). Means within treatments, with different superscripts, are significantly different (Tukey's test, P=0.05).

Treatment	Females			Males		
	Length (SL; cm)	Weight (g)	Condition factor	Length (SL; cm)	Weight (g)	Condition factor
A	17.7^a	160.7^a	0.029^a	18.6^b	198.2^b	0.031^b
B	18.8^a	213.1^a	0.032^a	21.1^b	298.6^b	0.032^a
C	19.6^a	249.5^a	0.033^a	21.3^b	312.0^b	0.032^b

It took about five weeks for the algal blooms to develop (even longer before differences showed in Secchi disk readings), occurring a little sooner in Treatment C. Once established, the bloom was maintained until the end of the experiment.

However, concerning the utility of cottonseed cake-induced algal blooms as nutrient resource for Nile tilapia, it has been pointed out that if the examined tilapia guts were filled with green algae instead of blue-green algae, the tilapia would then have derived very little nutritional benefit from these (R.S.V. Pullin, pers. comm.). The algae had not been examined, but the water was dark green, suggesting blue-green dominance. Hence, most fish growths in Treatments B and C were probably derived from the cottonseed cake.

Considerably lower quantities of tilapia fry were harvested from Treatment A probably due to catfish predation, although possibly the reproductive output of the tilapia was also lower in this treatment. Catfish clearly grew much better in Treatments B and C, where they had access to the supplemental cottonseed cake meal and where they were therefore probably less piscivorous and not effective in controlling tilapia recruitment.

Acknowledgements

This experiment was conducted as part of the applied research program of the Lagdo Fish Farming Project (1987-1992) under the authority of the Mission d'études pour l'aménagement de la vallée supérieure de la Bénoué (MEAVSB). The project was supported by the Dutch government (DGIS) in a bilateral cooperation agreement with the government of Cameroon and executed by HASKONING Consultants (Nijmegen, the Netherlands). Special thanks are due to Mr. Hamayadji Kombo, Fisheries Station Manager, and his staff.

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Measurement of the Apparent Digestibility Coefficients for *Oreochromis niloticus* of Agro-industrial By-products Available in Côte d'Ivoire

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Abstract

The adaptation of fish to a new feed was monitored for the first week of experiment by measuring variations in the apparent digestibility coefficient (ADC) of dry matter, nitrogen and organic matter. Three series of experiments were conducted on nine different feeds and one reference feed. Each feed was distributed to two batches of animals. Each batch comprised 15 fish (87±4 g) for the first experiment, and 20 fish (177±3 g) for the secohd and third experiments. Feces were collected every 12 hours (0800 and 1700) before each distribution of feed. The influence of the day/night cycle on digestibility was measured by comparing feces excreted by day with those excreted by night. ADC values obtained during the first day of distribution were significantly lower than those obtained over the following days. There was no difference between day and night in the ADCs of nitrogen and organic matter, except for the first meal. However, a slight difference between day and night in the ADC of dry matter does persist beyond the second day. To determine the ADC of a feed, the first collection of feces must occur at least 24 hours after the first distribution of feed. If fish are fed several times per day, it is best to use the aggregate of feces excreted in each 24-hour period to estimate a practical value for the different ADCs.

The ADCs of dry matter, proteins (nitrogen) and organic matter were determined for several by-products available in Côte d'Ivoire: fish meal, maize meal, cassava meal, copra cake, soy cake and cotton cake.

Introduction

The use of agricultural by-products in fish feed requires a good knowledge of the quality of these by-products. From a nutritional viewpoint, the digestibility of the nutrients in a by-product is an essential criterion. The results of digestibility studies are therefore an essential tool, not only for researchers but also for manufacturers of fish feed.

Digestibility measurements rely on the use of an indigestible marker in the feed. Digestibility studies have caused many controversies over the type of marker used (Bowen 1978; Lied et al. 1982; De Silva and Perera 1983; Tacon and Rodrigues 1984) or the choice of collection method for feces (Smith and Lowell 1973; Austreng 1978; Windell et al. 1978; Cho et al. 1982; Cho et al. 1985; Vens Cappell 1985; Spyridakis et al. 1989). In contrast, relatively few studies have focused on the time needed for the fish to adapt to the test feed and on the diurnal or infradiurnal variations of digestibility (De la Noüe et al. 1980; De Silva et al. 1990).

The objective of this study was therefore to define the adaptation time of *Oreochromis niloticus* to a marked feed,

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and to test the effects of the day/night cycle on the digestibility of dry matter, organic matter and proteins (nitrogen). This study paves the way for the practical use of the digestibility of different agricultural by-products available in Côte d'Ivoire.

Materials and Methods

Digestibility was measured by the apparent digestibility coefficient (ADC) using the "by incorporation" method (Cho et al. 1985). The value of this method has been shown for a related species, *Oreochromis aureus* (De Silva et al. 1990). Feces were collected using a decanter system similar to the "Guelph system" (Cho et al. 1982).

Three series of experiments were conducted at the Centre de Recherches Océanologiques (Abidjan, Côte d'Ivoire) on 10 feeds: a reference feed (Table 1) and nine feed mixes (reference feed 70%: test by-products 30%). The ratio of reference feed to test by-product was confirmed by analyzing the reference feed marker, chromium oxide, in the mix. The overall composition of the different by-products tested is given in Table 2. The by-products were:

- three fish meals: one imported (Chile type) and two different samples of a meal manufactured locally from the residues of tuna canning industries;
- three cakes: soy, cotton and copra; and
- maize and cassava meals.

Each feed was distributed to two batches of fish selected randomly. The reference feed was tested during each experiment. Each batch comprised 15 fish (87±4 g) for the first experiment, and 20 fish (177±3 g) for the second and third experiments. In the laboratory, the fish were placed in 110-l cylindroconical tanks with a recirculated water system and were maintained naturally at a temperature of 28 to 30°C. The photoperiod remained natural, with 12 hours of day and 12 hours of night.

Table 1. Composition of the reference feed.

Ingredients	Percentages
Fish meal (imported)	37.62
Maize	53.13
Cod liver oil	3
Mineral premix	1
Vitamins	2
Ca propionate	0.25
Binder (CMC)	0.5
Chromium oxide	2
Dicalcium phosphate	0.5

The fish were fed ad libitum twice a day (0800 and 1700). Feces were collected every 12 hours (before each meal) for seven days, beginning with the second distribution of the test feeds. The samples obtained were deep-frozen and freeze-dried for analysis.

The effects of the day/night cycle on meal digestibility were measured by comparing all feces excreted by day to all those excreted by night.

The following analytical techniques were used for feeds and feces:

- dry matter: 105°C, 24 hours;
- ash: 550°C, 24 hours;
- crude proteins: Kjeldahl nitrogen total (catalyst, $CuSO_4$ and K_2SO_4); and
- Cr_2O_3: oxidation as per Stevenson and de Langen (1960), then determination by atomic absorption (Lied et al. 1982).

The ADCs of each component (dry matter, organic matter or proteins) and dry matter of the test by-products were calculated for each feed using the formulae of Cho et al. (1985). For the ADCs of proteins (or organic matter) in the test ingredients, the formula used was:

$$Dp_I = P_I/(a * P_M) * 100 * (Dp_M - Dp_R) + Dp_R$$

where

P_I, P_M: protein concentrations in the ingredient and mix;

Dp_R, Dp_I, Dp_M: ADCs of the proteins for the reference feed, ingredient and mix; and

Table 2. Overall composition (% of dry matter) of the different by-products tested. *Local 1* and *Local 2* are two different samples of fish meals manufactured locally from the residues of tuna canning industries.

	Crude proteins	Fat	Ash	Raw fibers	NEA
Fish meals					
Import	62.3	8.5	16.7	-	12.6
Local 1	62.9	3.7	24.3	-	9.1
Local 2	56.5	5.7	29.8	-	8.1
Cakes					
Soy	50.5	1.5	7.4	6.7	33.9
Cotton	43.9	0.7	7.8	15.8	31.8
Copra	23.8	2.0	6.9	19.3	48.0
Miscellaneous					
Maize meal	12.2	4.0	4.0	2.3	77.5
Cassava meal	3.0	0.2	0.2	7.9	88.7

a: percentage of the ingredient in the mix.

The statistical analysis of the results was done through an analysis of variance and the Duncan multiple range test (SAS 1988). The significance level used was 0.05.

Results and Discussion

For the reference feed, an apparent increase in ADCs was observed for dry matter, organic matter or proteins when measured from feces collected during the first 24 hours (Fig. 1). Beyond that, ADC values stabilized and did not show any clear difference between day-collected and night-collected feces.

The analysis of variance conducted on all values obtained for each feed did not show a day/night effect on the digestibility of dry matter, organic matter or proteins that was independent of the day of feces collection (Table 3). The multiple range test on the different ADCs calculated from samples of each batch indicated that only results from the first collection (the first 12 hours) were different for organic matter or proteins (Fig. 2). Beyond that, even though differences persisted for dry matter, no clear tendency was identifiable.

The time of adaptation of the fish to the new feed was therefore limited to the 12 hours following the first distribution. This adaptation time is substantially shorter than for the trout *Oncorhynchus mykiss* (Possompes 1973; De la Noüe et al. 1980). It is, however, in line with the differences observed between gastric evacuations, 36 hours for trout (Windell and Norris 1969) and eight hours for *O. niloticus* (Ross and Jauncey 1981). Beyond that, no clear trend appears in the digestibility variations linked to the day/night cycle. This supports the results obtained for *O. aureus* (De Silva et al. 1990).

Using the mean values obtained for the different feeds, the ADCs of dry matter, organic matter and proteins were calculated for the test by-products available in Côte d'Ivoire (Table 4). Several measurements were made on the same fish meal samples. Results showed consistency between measurements of ADCs for dry matter and proteins at weekly intervals (experiments a and b) and measurements at five-month intervals

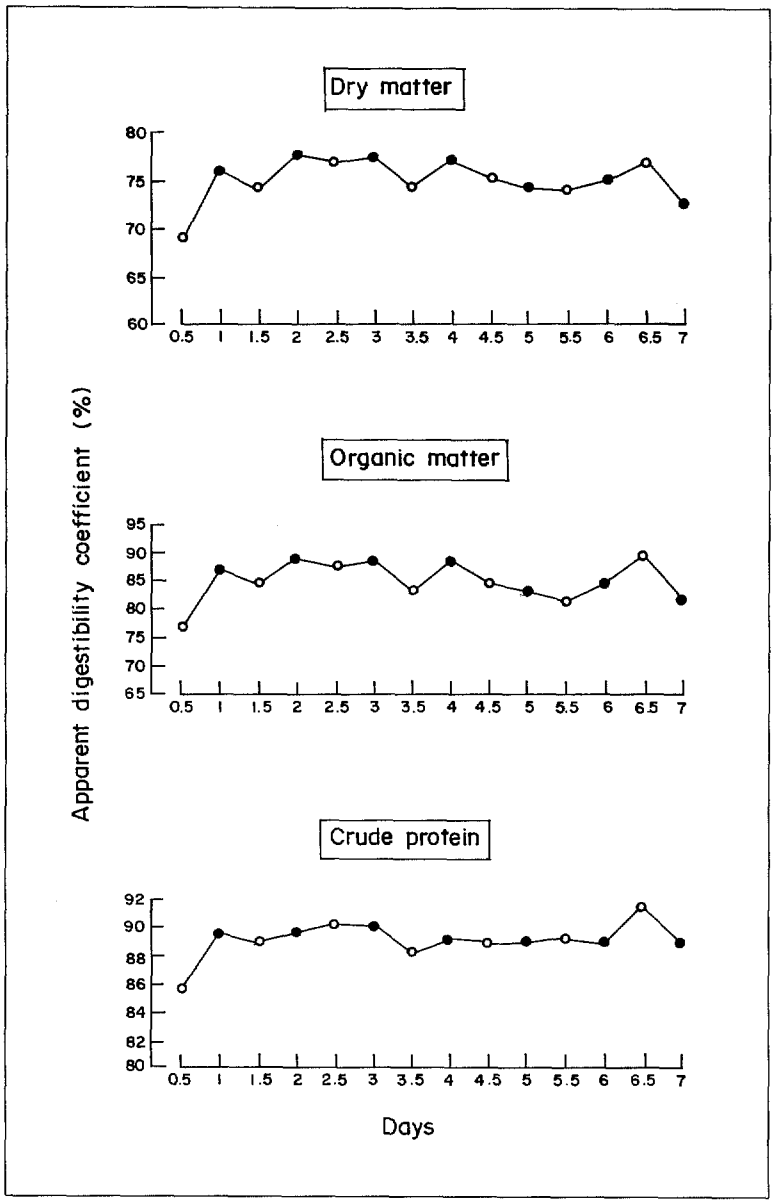

Fig. 1. Changes in the apparent digestibility coefficients (ADCs) of dry matter, organic matter and proteins for *Oreochromis niloticus* with the number of days for fecal collection, following the first distribution of reference feed (day o, night •).

Table 3. Analysis of variance for the different apparent digestibility coefficients (ADCs): all feeds and all observations aggregated.

Source of variation	df	Sum of squares	Mean square	F	Prob (f>F)
Dry matter					
Day	7	852.38	121.77	18.1	0.0001
Hour	1	274.75	274.75	40.84	0.0001
Interaction Day X Hour	7	359.59	51.37	7.64	0.0001
Diet nested with trial	12	3,780.7	315.06	46.84	0.0001
Error	302	2,031.5	6.73		
Organic matter					
Day	6	235.05	39.18	7.42	0.0001
Hour	1	28.98	28.98	5.49	0.0201
Interaction Day X Hour	6	96.02	16	3.03	0.0073
Diet nested with trial	9	3,521.09	391.23	74.09	0.0001
Error	212	1,119.52	5.28		
Proteins					
Day	7	91.58	13.08	4.97	0.0001
Hour	1	13.41	13.41	5.1	0.0247
Interaction Day X Hour	7	69.49	9.93	3.78	0.0006
Diet nested with trial	12	765.49	63.79	24.26	0.0001
Error	302	794.17	2.63		

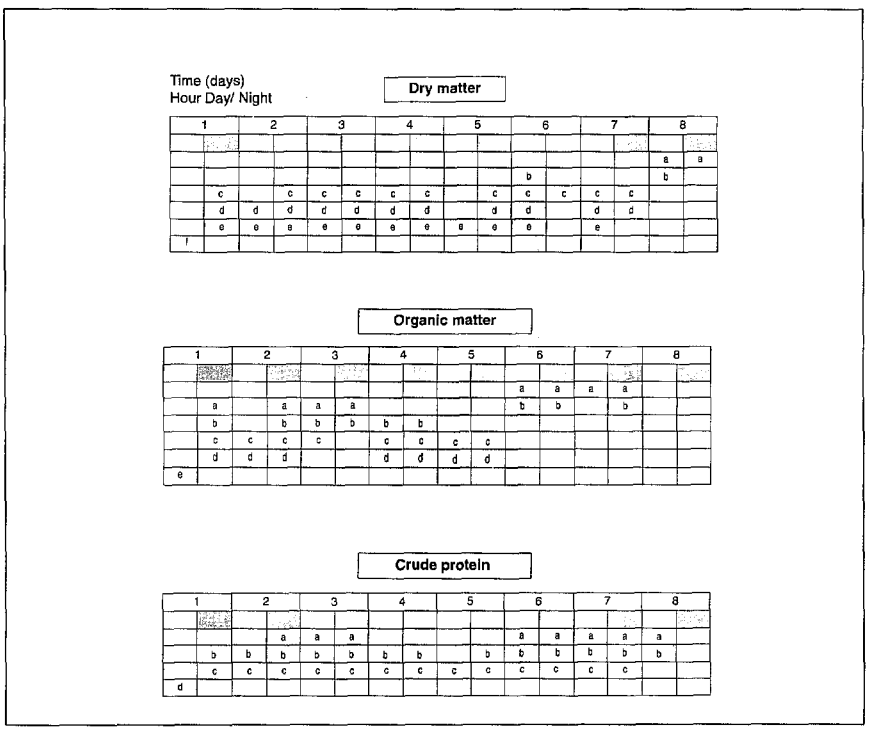

Fig. 2. Duncan multiple range test (α = 5%) for the different apparent digestibility coefficients (ADCs). Each sampling (at a particular day and at a particular hour) is associated with a group of letters. The groups of values that are not significantly different are represented by identical letters.

(experiment c). Low ADC values for the "Local 2" meal resulted essentially from the particularly high ash content of this sample.

The high ADC values for organic matter in the fish meals can be linked to an increase in the digestibility of the organic matter of the reference feed in the mix. The organic matter of maize and cassava meals, two compounds very rich in starch, is well-digested (ADC>75%). The cassava meals is pre-cooked during manufacture. This process increases the digestibility of starch (Bergot and Brèque 1983) and could be the cause of the high value of the apparent digestibility coefficient. However, in this case, the maize did not undergo any particular pretreatment. The ADC of organic matter for maize in *O. niloticus* is higher than in trout or carp (Steffens 1989) and is similar to the ADC of the energy obtained with the same species by Hanley (1987) or for the channel catfish, *Ictalurus punctatus* (Wilson and Poe 1985).

Conclusion

Tilapias adapt to a new feed rapidly. Twelve hours after the first meal, the feces collected reflect the new diet. The absence of day/night variations implies that feces can be collected less frequently. It is, however, essential to collect all feces every 24 hours to avoid excessive variability in the results, especially for dry matter.

The tilapia seems to utilize the starch in feeds like maize or cassava meals. This should be confirmed by a study of its aptitude for using such nonprotein sources of energy.

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Table 4. ADCs of dry matter, organic matter and proteins for the different by-products available in Côte d'Ivoire. The results were obtained from three experimental tests at different times (experiments a, b and c). For the comparison of results, the same fish meals were tested in two separate tests. *Local 1* and *Local 2* are two different samples of fish meals manufactured locally from the residues of tuna canning industries. Values are means and standard errors (given in parentheses).

Ingredients	Exp.	Dry matter	Organic matter	Crude proteins
Fish meals				
Imported	b	92.24 (±2.08)	108.18 (±3.53)	94.38 (±0.92)
	c	93.02 (±1.93)	-	95.78 (±0.74)
Local 1	a	82.49 (±2.94)	98.13 (±4.16)	89.49 (±0.90)
	b	87.59 (±1.49)	102.23 (±2.99)	91.62 (±0.78)
Local 2	c	63.56 (±3.21)	-	82.42 (±0.90)
Cakes				
Soy	a	76.27 (±7.62)	77.48 (±2.13)	93.12 (±1.40)
Cotton	a	67.67 (±3.98)	68.82 (±2.38)	89.65 (±0.73)
Copra	a	54.44 (±5.36)	55.71 (±2.54)	81.22 (±1.82)
Miscellaneous				
Maize meal	b	76.66 (±2.24)	76.17 (±1.79)	89.45 (±3.22)
Cassava meal	b	83.32 (±2.32)	82.15 (±1.94)	74.35 (±30.93)

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Models for Estimating the Food Consumption of Tilapias[a]

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PALOMARES, M.L. and D. PAULY. 1996. Models for estimating the food consumption of tilapias, p. 211-222. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Two approaches are presented for the estimation of food consumption in wild and farmed tilapias. The first which relies on diurnal changes in stomach contents is illustrated with three examples pertaining to Nile tilapia, *Oreochromis niloticus*. The second is a multiple regression model which allows estimation of daily ration (R_d) in tilapia (genera *Oreochromis, Sarotherodon* and *Tilapia*). The model was derived from empirical estimates of R_d in 55 populations of marine and freshwater fishes from temperate and tropical habitats, including 16 populations of (mainly) African tilapias. This model allows estimation of R_d for different food types in natural and captive tilapias, and should be particularly useful for parametrization of pond dynamics models. The mathematical and conceptual relationships between these two approaches are discussed.

Introduction

Estimation of food consumption, or more precisely of "daily" ration (R_d) is even more useful when applied to farmed fish than to wild fish. In aquaculture, estimates of ration can be used to devise appropriate feeding schedules, and to integrate fish into pond dynamics models. Ration estimates for wild fish populations enable fisheries biologists and ecologists to establish trophic links between an investigated population and its prey and predators, such investigations often culminating in ecosystem models (see, e.g., Palomares et al. 1993).

The approach most commonly used to deal with food consumption in aquaculture research is to report "food conversion ratios" (FCR), where:

$$FCR = \frac{\text{food provided during an experiment}}{\text{growth during that same experiment}} \quad ...1)$$

Although perhaps appropriate for farmers, FCR estimates are not optimal in the context of research, for two major reasons: usually not all the food provided is consumed by the experimental fish; and the denominator and numerator of equation (1)

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are usually not based on the same units, i.e., "growth" is usually expressed as wet weight (i.e., the difference between the weight of fish harvested and those stocked), whereas "food" is commonly expressed as dry weight, thus often leading to FCR values <1.

Hopkins (1992) proposed the routine use of the von Bertalanffy growth function (VBGF) in aquaculture, instead of the absolute or per cent growth rates commonly used. Similarly, we propose here that aquaculturists use daily ration (R_d) instead of or at least in addition to equation (1) to report the results of feeding experiments. One reason for this is that those two concepts (the VBGF and R_d) are closely linked, and hence allow for a consistent mathematical treatment of one's result, and comparisons with studies on the trophic dynamics of wild fish populations.

The VBGF takes for length the form:

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right) \qquad ...2)$$

and for weight

$$W_t = W_\infty\left(1 - e^{-K(t-t_0)}\right)^b \qquad ...3)$$

where L_t, W_t are the length and respective weight at age t; K is a growth constant; t_0 the arbitrary and usually negative "age" at which size is zero; and b is the exponent of a length-weight relationship of the form $W = aL^b$. Numerous well-established methods exist for estimating these parameters from various input data (Gulland 1983; Pauly 1984). Given the VBGF, growth rate (dw/dt, i.e., as $g \cdot day^{-1}$) can be expressed as the first derivative of equation (3) in terms of weight, i.e.,

$$dw/dt = 3KW_t((W_\infty/W_t)^{1/b}-1) \qquad ...4)$$

The relationship between food consumption (R_d) and growth rate is then captured through the food conversion efficiency K_1 of Ivlev (1945) defined by:

$$K_1 = (dw/dt)/R_d \qquad ...5)$$

(It will be noted that $K_1 = 1/FCR$ when the latter is based on food actually ingested, expressed in the same units as growth rate).

The link between K_1 —and hence ration— and the VBGF is given by:

$$K_{1(t)} = 1-(W_t/W_\infty)^\beta \qquad ...6)$$

where W_t and W_∞ are as defined above and ß is a parameter whose value is obtained from equations (5) and (6) and one or several estimates of R_d, pertaining to given values of W_t and W_∞ (Pauly 1986; Silvert and Pauly 1987).

We present below two methods for estimation of R_d, of which the second relied extensively on the application of equations (1) to (6) to various published datasets (Table 1).

Method I: Estimation of Daily Ration from Changes In Stomach Contents

In herbivores with well-established feeding rhythms, stomach contents rapidly increase during the feeding period, then decrease when feeding stops. Thus, changes in the fullness of stomach contents sampled over a 24-hour cycle can be used to infer feeding rate and feeding periods, and hence ration. However, such analyses must take account of the effect of evacuation during the feeding period, which invalidates linear models such as applied to tilapias by Moriarty and Moriarty (1973) or Getachew (1987). Sainsbury (1986) developed a parsimonious model to describe such dynamics and this was implemented as a computer program called MAXIMS by Jarre et al. (1990, 1991). In its simplest form (one feeding period per day, feeding rate independent of stomach contents), the model used to represent the nonfeeding period is:

$$dS/dt = -E \cdot S \qquad ...7)$$

Table 1. Estimates of ration (R_d) and related statistics of 55 species/populations of freshwater and brackishwater fishes (adapted from Palomares 1991).

No.	Family	Species	Location	W_t (g)	T (°C)	A	h	p	R_d (day^{-1})	Remarks
1	Clupeidae	*Alosa pseudoharengus*	Lake Michigan, USA	40	20.0	2.32	0	0	1.39	R_d back-calculated from K_1; K_1 from bioenergetic model of Stewart and Binkowski (1986)
2	Salmonidae	*Oncorhynchus kisutch*	Lake Ontario, Canada	1,000	13.0	2.40	0	0	24.20	R_d back-calculated from K_1; K_1 from growth and feeding experiments of Niimi (1981)
3	Salmonidae	*Oncorhynchus kisutch*	Oregon, USA	90	13.0	2.40	0	0	1.50	R_d from MAXIMS using 24-hour cycle from Brodeur and Pearcy (1987)
4	Salmonidae	*Salmo trutta*	Brandstrup Brook, Norway	39	15.5	2.21	0	0	0.62	R_d back-calculated from K_1; K_1 from estimates of population consumption and production by Rasmussen (1986)
5	Salmonidae	*Salmo trutta*	Dartmoor Stream, UK	51	9.0	2.21	0	0	0.93	R_d from MAXIMS using 24-hour cycle from Chaston (1969)
6	Salmonidae	*Salmo trutta*	France	20	16.0	2.21	0	0	2.40	R_d from MAXIMS using 24-hour cycle from Neveu (1980) in experimental canals
7	Esocidae	*Esox lucius*	UK	100	15.0	1.50	0	0	1.34	R_d back-calculated from K_1; K_1 from tank growth and feeding experiments by Hart and Connellan (1984)
8	Esocidae	*Esox lucius*	River Frome, UK	840	15.0	1.50	0	0	18.70	R_d back-calculated from K_1; K_1 from growth and feeding experiments by Mann (1982) in canals
9	Esocidae	*Esox lucius* ♀ + ♂	Lake Windermere, UK	4,600	7.0	1.50	0	0	32.20	K_1 from population estimates of consumption and production by Johnson (1966a, 1966b)
10	Esocidae	*Esox lucius* ♀	Lake Windermere, UK	5,000	7.0	1.50	0	0	19.70	K_1 from population estimates-of consumption and production by Johnson (1966a, 1966b)
11	Characidae	*Alestes baremoze*	Lake Chad, Africa	112	25.8	2.52	0	0	1.59	R_d from MAXIMS using 24-hour cycle from Lauzanne (1969)
12	Characidae	*Colossoma macropomum*	Amazonia, Brazil	200	24.5	2.37	0	1	2.51	K_1 from FCR from feeding experiments by Merola and Pagán-Font in (1988) in ponds
13	Cyprinidae	*Catla catla*	Pakistan	300	31.7	1.94	0	1	2.92	K_1 from FCR from polyculture feeding experiments by Nadeem-Sheri (1984) in ponds
14	Cyprinidae	*Cirrhinus mrigala*	Pakistan	220	30.8	1.51	0	1	3.70	K_1 from FCR from polyculture feeding experiments by Nadeem-Sheri (1984) in ponds
15	Cyprinidae	*Labeo rohita*	Pakistan	430	30.8	1.98	0	1	6.33	K_1 from FCR from polyculture feeding experiments by Nadeem-Sheri (1984) in ponds
16	Cyprinidae	*Mirogrex terraesanctae*	Lake Kinneret, Israel	12	14.0	1.41	0	0	0.50	R_d from aquarium experiments by Gophen and Threlkeld (1989)
17	Cyprinidae	*Rutilus rutilus*	Lake Pareloup, France	118	9.0	1.49	1	0	4.40	R_d from MAXIMS using 24-hour cycle from Arias-Gonzalez and Richeux (1990)
18	Cyprinidae	*Rutilus rutilus*	Lake Sovdeborgssjon, Sweden	48	14.0	1.49	0	1	0.50	R_d from Persson (1982, 1983)
19	Cyprinidae	*Rutilus rutilus*	Garonne River, France	67	12.4	1.49	1	0	3.53	R_d from MAXIMS using 24-hour cycle from Palomares (1991)

continued

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Table 1 (continued)

No.	Family	Species	Location	W_t (g)	T (°C)	A	h	p	R_d (day^{-1})	Remarks
20	Ictaluridae	*Ictalurus nebulosus*	Garonne River, France	198	12.4	1.34	0	0	7.13	R_d from MAXIMS using 24-hour cycle from Palomares (1991)
21	Clariidae	*Clarias gariepinus*	Africa	40	25.0	1.26	0	0	0.68	K_1 from laboratory experiments by Hogendoorn (1983)
22	Clariidae	*Clarias gariepinus*	Lake Malawi, Africa	1000	22.5	1.26	0	0	4.78	R_d from Willoughby and Tweddle (1978)
23	Gasterosteidae	*Gasterosteus aculeatus*	Lake Frongoch, Wales	0.3	10.0	1.69	0	0	0.0038	R_d from MAXIMS using 24-hour cycle from Allen and Wooton (1984)
24	Centropomidae	*Lates niloticus*	Lake Chad, Africa	665	25.0	1.09	0	0	11.20	K_1 from growth and feeding experiments by Hamblyn (1966) in aquaria
25	Centropomidae	*Lates strappersii*	Lake Tanganyika, Africa	264	26.0	2.40	0	0	4.36	R_d from M. Pearce (pers. comm.)
26	Serranidae	*Dicentrarchus labrax*	Thau Lagoon, France	4	15.0	1.76	0	0	0.43	R_d from MAXIMS using 24-hour cycle obtained from tank experiments by Palomares (1991)
27	Serranidae	*Dicentrarchus labrax*	Marseille, France	5	15.0	1.76	0	1	0.098	K_1 from growth and feeding experiments by Alliot and Pastoureaud (1979) in tanks
28	Centrarchidae	*Lepomis gulosus*	USA	100	27.0	1.49	0	0	4.00	R_d from Hunt (1960)
29	Centrarchidae	*Micropterus salmoides*	Minnesota, USA	186	27.0	1.69	0	0	4.50	R_d from Rice and Cochran (1984)
30	Centrarchidae	*Micropterus salmoides*	USA	486	27.0	1.69	0	0	13.8	R_d from Hunt (1960) and Lewis et al. (1974)
31	Percidae	*Perca flavescens*	Lake Washington, USA	115	10.0	1.64	0	0	1.70	R_d from MAXIMS using 24-hour cycle from Costa (1979)
32	Percidae	*Perca fluviatilis*	Lake Botjörn, Sweden	46	9.0	1.94	0	0	0.670	K_1 from population estimates of consumption and production by Nyberg (1979)
33	Percidae	*Perca fluviatilis*	Lake Ladoga, Russia	230	15.4	1.94	0	0	3.68	R_d from Federova and Drozzhina (1982)
34	Percidae	*Perca fluviatilis*	Lake Vitalampa, Sweden	90	9.0	1.94	0	0	1.01	K_1 from population estimates of consumption and production by Nyberg (1979)
35	Percidae	*Perca fluviatilis*	Loch Leven, Scotland	200	16.5	1.94	0	0	8.52	R_d from cage experiments by Thorpe (1977a, 1977b)
36	Sciaenidae	*Plogioscion squamosissimus*	Lake Janauaca, Brazil	43	27.0	0.91	0	0	3.00	R_d from MAXIMS using 24-hour feeding cycle by Worthmann (1982)
37	Cichlidae	*Haplochromis nigripinnis*	Lake George, Africa	5	30.0	0.76	1	0	0.220	R_d from MAXIMS using 24-hour feeding cycle from Moriarty and Moriarty (1973)
38	Cichlidae	*Oreochromis alcalicus*	Lake Nakuru, Africa	3	20.5	1.32	1	0	0.393	K_1 from laboratory experiments by Vareschi and Jacobs (1984)
39	Cichlidae	*Oreochromis aureus*	Virgin Islands, USA	200	25.0	1.20	0	1	3.92	K_1 from FCR obtained for cage-reared fish from Hargreaves et al. (1988)
40	Cichlidae	*Oreochromis mossambicus*	Africa	4	27.0	1.17	0	1	0.0684	K_1 from FCR for tank-reared fish from Jauncey (1982) and Micha et al. (1988)

continued

Table 1 (continued)

No.	Family	Species	Location	W_t (g)	T (°C)	A	h	p	R_d (day^{-1})	Remarks
41	Cichlidae	*Oreochromis mossambicus*	Parakrama Samudra, Sri Lanka	19	27.0	1.17	1	0	6.00	R_d from Hofer and Schiemer (1983)
42	Cichlidae	*Oreochromis niloticus*	Africa	11	26.0	1.28	0	1	0.289	K_1 from FCR for aquarium-reared fish
43	Cichlidae	*Oreochromis niloticus*	Bouaké, Côte d'Ivoire	82	28.5	1.28	0	1	1.27	K_1 from estimates of growth and feeding for cage-reared fish from Boidy (1984)
44	Cichlidae	*Oreochromis niloticus*	Lake Awasa, Africa	265	24.5	1.28	1	0	39.30	R_d from MAXIMS using 24-hour feeding cycle by Getachew (1987)
45	Cichlidae	*Oreochromis niloticus*	Lake George, Africa	213	30.0	1.28	1	0	9.50	R_d from MAXIMS using 24-hour feeding cycle by Moriarty and Moriarty (1973)
46	Cichlidae	*Oreochromis niloticus*	Lake Kossou, Africa	102	28.5	1.28	0	1	0.837	K_1 from FCR for cage-reared fish from Coche (1977)
47	Cichlidae	*Oreochromis niloticus*	Lake Turkana, Africa	137	30.0	1.28	1	0	9.30	R_d from MAXIMS using 24-hour feeding cycle by Harbott (1976)
48	Cichlidae	*Oreochromis niloticus*	Liège, Belgium	2	26.0	1.28	1	0	0.910	K_1 from FCR for tank-reared fish from Appler (1985)
49	Cichlidae	*Oreochromis niloticus*	Thailand	7	27.0	1.28	0	1	0.263	K_1 from FCR for tank-reared fish from Wee and Wang (1987)
50	Cichlidae	*Oreochromis niloticus*	Thailand	45	32.0	1.28	0	1	0.892	K_1 from FCR for tank-reared fish from Edwards et al. (1985)
51	Cichlidae	*Sarotherodon galilaeus*	Lake Chad, Africa	603	25.8	1.56	1	0	23.70	R_d from Lauzanne (1977)
52	Cichlidae	*Tilapia rendalli*	Africa	10	26.0	1.48	0	1	0.262	K_1 from FCR for pond-reared fish from Micha et al. (1988)
53	Cichlidae	*Tilapia zillii*	Africa	33	27.5	1.65	1	0	7.50	K_1 from growth and feeding experiments by Legner and Murray (1981) in aquaria and Saeed and Ziebell (1986) in tanks
54	Mugilidae	*Mugil cephalus*	Thau Lagoon, France	4	15.0	2.55	1	0	0.580	R_d from MAXIMS using 24-hour feeding cycle under semi-controlled conditions from Palomares (1991)
55	Mugilidae	*Mugil cephalus*	Crystal River, Florida	597	23.0	2.55	0	0	15.60	R_d from MAXIMS using 24-hour feeding cycle from Collins (1981)

where S is the stomach content at time t expressed as $S=S_o \exp(-E\cdot(t-t_0))$; E the instantaneous evacuation rate (in $hour^{-1}$); S_o the stomach content at the beginning of the feeding period; and t_0 the time at the beginning of the interval in question. Stomach contents are continuously pushed along the intestine whether the fish actively feeds or not. Thus, for the feeding period we have:

$$dS/dt = J - E\cdot S \qquad \textbf{...8)}$$

where:

$$S = (S_o\cdot\exp(-E\cdot(t-t_0))) + (J/E\cdot(1-\exp(-E\cdot(t-t_0))) \qquad \textbf{...9)}$$

where J is the ingestion rate (in units of weight per hour) and where the first term of equation (9) represents the amount evacuated from the stomach. The second term incorporates into the evacuation term (represented by S) the amount of newly ingested food that is being simultaneously evacuated from the stomach. Daily ration is then estimated by integrating equation (8) over the feeding period.

Fig. 1 presents three applications of this model to three lake populations of Nile tilapia, *Oreochromis niloticus*. As might be seen, the model not only led to ration estimates (Table 1), but also allowed the identification of very similar feeding rhythms in these three tilapia populations.

Method II

An alternative approach to estimating ration is to derive a predictive model, based on a number of previous, suitably standardized estimates of ration and a suite of appropriate predictor variables.

Based on earlier models we developed for marine fishes (Palomares and Pauly 1989; Pauly 1989; Jarre et al. 1991), we hypothesized the following variables to be related to ration:

- body weight;
- environmental temperature (Régier et al. 1990);
- aspect ratio of the caudal fin, as an index of activity level (Pauly 1989; Palomares and Pauly 1989; Sambilay 1990; Fig. 2);
- taxonomic status (v=0 for tilapias, and v=1 for nontilapias); and
- food type, expressed via dummy variables (i.e., taking only the values 0 and 1).

We distinguished three types of fish based on food types, labelled h (for herbivorous, and including three cases of detritivorous fish) and p (for those fed generally unspecified pellets); these variables imply a third type of fish, i.e., those that are carnivores, when h=0 and p=0.

Table 1 presents the 55 cases assembled for this exercise. From this, a model was obtained of the form:

$$\log_{10}R_d = -1.80 + 0.7816\log_{10}W_t + 0.5714\log_{10}T + 0.4248\log_{10}A - 0.1960v - 0.4030p + 0.3445h \qquad \textbf{...10)}$$

where W_t is the weight (in g) of the fish; T the mean annual water temperature (°C); and A the aspect ratio of the caudal fin, all other (dummy) variables being as defined above.

This model explains 89% of the variation of the dataset in Table 1 (df =48) with all except two partial slopes significantly different from zero at the 5% level (Table 2).

Aspect ratio (A) was found to have no significant effect on R_d for this dataset, although this was shown to have an important effect on relative food consumption of marine fish populations (Palomares and Pauly 1989; Pauly 1989; Palomares 1991). We explain this by the overall similarity of A values of the freshwater fish in Table 1. The dummy variable accounting for taxonomic status was also found to have no significant effect on R_d. This is not surprising: once food type, weight and temperature are accounted for, there is no reason why fish should differ in their food consumption. Indeed, this confirms the results of Palomares (1991) who found no difference

Table 2. Summary statistics of multivariate model for prediction of ration in freshwater fishes (equation [10]; R^2=0.886; F=62.44; df=54).

Symbol	Partial slope associated with:	Estimate	Prob.	Contrib. to total R^2 (%)
W_t	Body weight ($\log_{10}$ g)	0.7816	0.000	83.78
T	Water temperature ($\log_{10}$ °C)	0.5714	0.020	1.85
A	Aspect ratio of caudal fin	0.4248	0.236	0.02
t	Not being a tilapia (dummy)	-0.1957	0.106	1.44
h	Being a herbivore (dummy)	0.3445	0.006	2.22
p	Being fed with pellets (dummy)	-0.4028	0.001	10.69

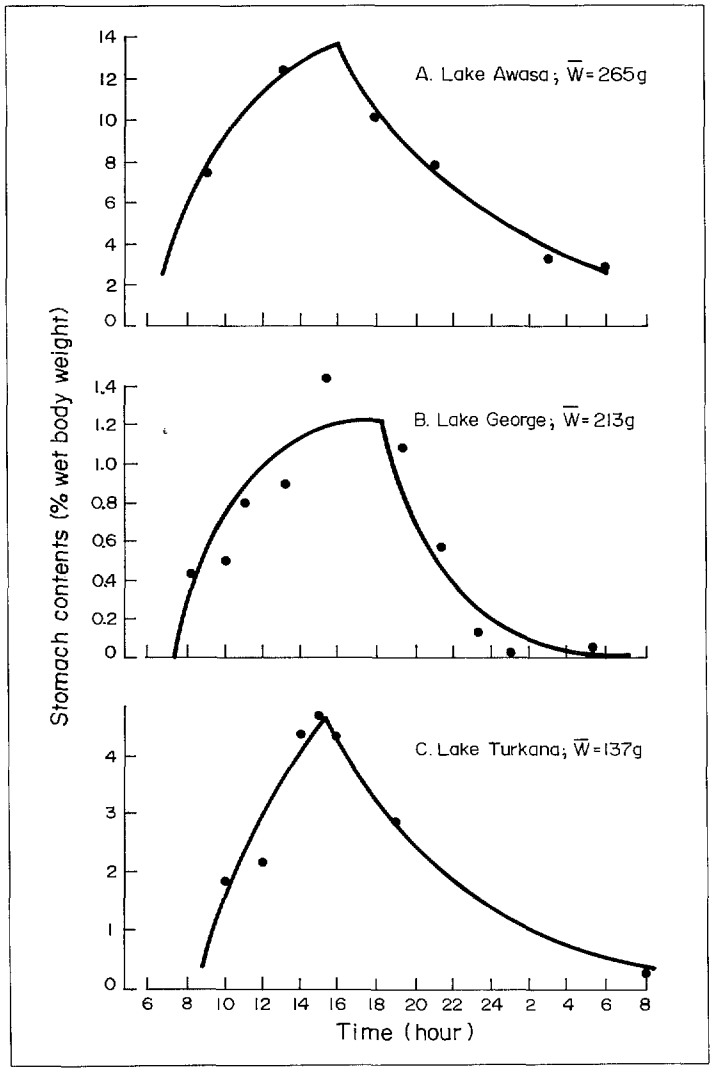

Fig. 1. Examples of populations of *Oreochromis niloticus* for which data on stomach contents over a 24-hour period were used to obtain estimates of daily rations using the MAXIMS software of Jarre et al. (1990). Adapted from Palomares (1991) based on data in (A) Getachew (1987); (B) Moriarty and Moriarty (1973); and (C) Harbott (1976).

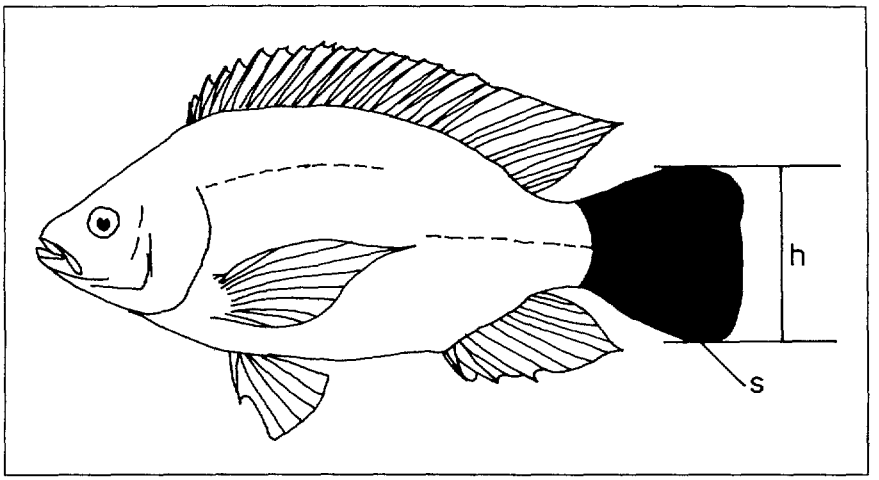

Fig. 2. The Nile tilapia, *Oreochromis niloticus*, and the definition of the aspect ratio of its caudal fin: $A=h^2/s$, where h is the height of the fin and s its surface area (in black). In this case, A=1.28 (see also Table 1).

in the food consumption of marine and freshwater fishes, once the above factors (and the aspect ratios) were accounted for.

Re-expressing equation (10) as a model for use exclusively with tilapias was achieved here by solving this equation for the mean aspect ratio value in the tilapia of Table 1 (A=1.32) and dropping the variable v. This led to:

$$\log_{10}R_d = -1.75+0.782\log_{10}W_t+0.571\log_{10}T -0.403p + 0.344h \quad ...11)$$

Fig. 3 shows a plot of predicted over observed log R_d values from equation (10) for all fishes in Table 1 except the tilapia and for equation (11), referring to tilapias only. The even distribution of the points around the 1:1 line indicates that these models are robust and that the (log) transformations used were appropriate for the data in Table 1.

Discussion

That the two methods presented above are straightforward to apply in an aquaculture context hardly needs emphasizing. Indeed, even Method I, which does require field data, requires one 24-hour cycle (or preferably two or three cycles) of samples for estimates of R_d to be obtained, whose reliability, moreover, will increase rapidly as the time intervals between successive samples are reduced (those illustrated here in Fig. 1 are actually based on too few data points for the estimates of R_d to be very reliable; at least 16 points, with 1.5 hours between them should have been used).

However, the relationships between R_d and weight that will emerge will be different, depending on whether one uses a series of predictions from equation (11) or a single estimate of ration extrapolated to a range of weights using equations (1) to (6). This is here illustrated with a hypothetical example.

For herbivorous tilapias living at T=26°C, equation (11) predicts values of R_d varying with weight as shown on line A of Fig. 3; at 100 g, this corresponds to a ration of 9.25 $g \cdot day^{-1}$.

If those tilapias have the growth parameters W_∞=200 g, K=1.0 $year^{-1}$ (t_0=0) and b=3, they will have, at a weight of 100 g, a growth rate of 0.214 $g \cdot day^{-1}$ (see equation [4]), and hence a value of K_1=0.0337 (see equation [5]). Hence solving equation (6) for ß will give 0.0377. Turning equation (5) into a model for predicting R_d from dw/dt and K_1 gives:

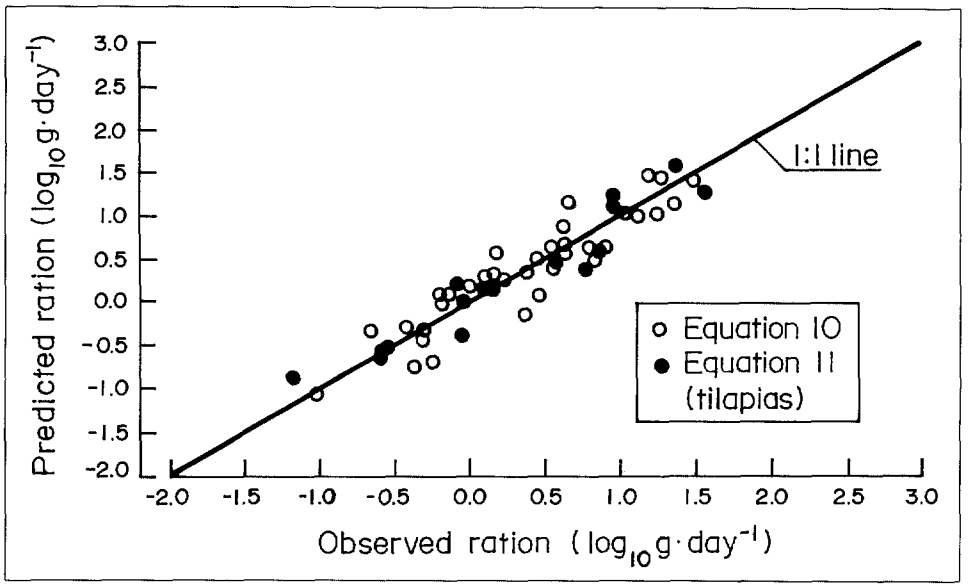

Fig. 3. Plot of observed *vs.* predicted log R_d estimates obtained using equation (10) for all fishes and equation (11) for the tilapias. Notice the even distribution of the points around the 1:1 line.

$$R_d = (dw/dt)/K_1 \qquad \text{...12)}$$

Solved for different weights, and with the parameters above leads to line B in Fig. 4.

As expected, these two lines cross at 100 g; more importantly they almost completely overlap over the entire range of weights considered, showing that equation (11) has a slope associated with weight which appropriately mimics the behavior of equations (1) to (6) pertaining to change of R_d due to individual growth.

This demonstrates that the two models presented here to estimate ration in tilapias are not only easy to apply—even in the context of aquaculture—but also provide results that are mutually compatible, hence increasing our confidence in them.

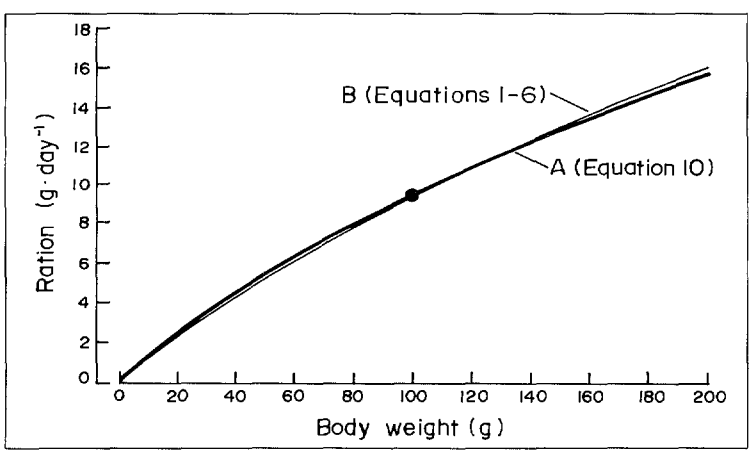

Fig. 4. Predicted rations for herbivorous tilapias kept at 26°C; A: from equation (10); and B: from equations (1) to (6), assuming that A=B at 100 g body weight. Note the nearly complete overlap of both curves.

Acknowledgement

The first author thanks Dr. Jacques Moreau of ENSAT for his support of the thesis work upon which this contribution is based.

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SESSION III. REPRODUCTION AND GENETICS

Studies on the Effect of Manipulating Hapa Size on Broodstock Conditioning of *Oreochromis niloticus* in Fertilized Earthen Ponds

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Abstract

The study was carried out to determine the effect of hapa size on seed production during the female conditioning period of *Oreochromis niloticus* in fertilized earthen ponds. An equal biomass of female broodfish (average weight 84 g) was stocked in three hapas of different sizes: 10.78 m^2 (low density); 5.29 m^2 (medium density); and 2.72 m^2 (high density). Stocked biomass ($kg \cdot m^{-2}$) was 0.6-0.8; 1.2-1.4; and 2.3-2.5 for low, medium and high stocking density treatments, respectively. Females were conditioned for a period of 10 days and males for five days. Broodfish were allowed to spawn naturally for a period of five days in 40-m^2 hapas. The hapa depth was 0.9 m and the total hapa volume was 36 m^3: water depth was 0.75 m and water volume in the hapa was 30 m^3. No significant differences ($P>0.05$) were observed in seed production. However, production exceeded 30 seeds·kg total female biomass stocked·day^{-1} and 100 seeds·kg female spawned·day^{-1}. The number of seed produced per m^2 of conditioning hapa area was inversely related to hapa size. Yields of 147 (±14); 299 (±34) and 575 (±60) seed·m^{-2}·conditioning hapa·day^{-1} were obtained from low, medium and high stocking densities, respectively. There were no significant differences ($P>0.05$) in the gonadosomatic indices (GSI) among the three stocking densities, but GSI increased over the duration of the experiment. Water quality deteriorated during days 45 to 60 of the experimental period, when early morning dissolved oxygen concentrations fell below 0.5 ppm. This coincided with a drop in seed production.

Introduction

Low fecundity of commercially important *Oreochromis* species means that large numbers of broodfish must be kept. The optimization of stocking density of broodfish is therefore of economic importance, especially because of the density-dependent nature of their spawning behavior. The importance of broodfish density in the control of spawning in *Oreochromis* spp. is well established. The breeding territory required by the male for making and defending a "nest" has been used as a criterion in estimating optimal stocking densities (e.g., Uchida and King 1962; Fishelson 1966; Balarin and Haller 1982). Manipulation of density has also been used to inhibit breeding

and to counter its negative effects on the growth of stocked fish (Mair and Little 1991).

The poor productivity of many *Oreochromis* breeding systems can be related to the difficulties of managing a population of asynchronously breeding fish and harvesting the seed efficiently. Frequent harvesting of seed from mouthbrooding tilapias (Verdegem and McGinty 1987) or exchange of broodfish (Lovshin and Ibrahim 1988) is known to improve seed production and this is enhanced if both practices are combined and female fish are "conditioned" by holding in single sex groups at densities that inhibit spawning behavior (Little 1990). However, keeping all-female groups does not by itself prevent ovulation (Silverman 1978) and high density appears to be an important factor in restraining females from breeding until they are released at a lower density with males. The maintenance of adequate water quality at high fish densities has practical limitations depending upon the type of system in which the fish are to be conditioned. Little (1990) held broodfish at densities between 1.2 and 5.0 $kg \cdot m^{-2}$ (10-25 $fish \cdot m^{-2}$) in nylon net hapas and in tanks with a recirculated water supply, before release, with males, for spawning.

This study was carried out to investigate the effect of stocking density during conditioning in hapas on the subsequent reproductive performance of female *Oreochromis niloticus* broodfish.

Materials and Methods

Broodstock production and the experiment described were carried out in the same earthen pond (1,740 m^2) on the campus of the Asian Institute of Technology (AIT), 50 km north of Bangkok, Pathum Thani, Thailand. Pure *Oreochromis niloticus* (Chitralada strain) were obtained as seed from natural spawning females held in large nylon hapas. After artificial incubation, early nursing in hapas and growout in a fertilized earthen pond, they were sexed and used as broodfish. Initial mean body weights of the female and male broodstock (seven months old) were 84 g (range 50-120 g) and 93 g (range 64-140 g), respectively. Nylon net hapas (mesh 1 mm: 20 grade) were suspended in the same pond for conditioning an equal biomass of female broodstock (Table 1), conditioning the male broodfish used in all treatments (40 m^2 x 1 m) and natural spawning (40 m^2 x 9 m).

Each treatment was replicated six times; five conditioning and spawning cycles were run during a 75-day experimental period. Females and males were conditioned for periods of 10 and five days, respectively.

Table 1. Hapa size (length x width x height) used for each treatment during the conditioning period of female broodstock *(Oreochromis niloticus)*.

Treatment	Size of hapa (m)	Area of hapa (m^2)	Stocking density ($kg \cdot m^2$)
Low density	3.3 x 3.3 x 0.9	10.89	0.6 - 0.8
Medium density	2.3 x 2.3 x 0.9	5.29	1.2 - 1.4
High density	1.65 x 1.65 x 0.9	2.72	2.3 - 2.5

After conditioning, females from each replicate were stocked in a 40-m^2 hapa, together with males, at a sex ratio of 1:1. The broodstock were allowed to spawn naturally for a period of five days.

After the five-day spawning period, seed was harvested and measured using methods described by Little et al. (this vol.).

Broodstock biomass was maintained at initial levels by killing four females from each replicate every 15 days to minimize the difference between the initial and the final total biomass in each treatment. Two females were killed after every conditioning period and the other two after every spawning period. The latter comprised one female that had spawned and one female that had not spawned. The gonadosomatic index (GSI) of these females was determined as:

$$\text{GSI (\%)} = \frac{\text{Weight of gonads (g)}}{\text{Total body weight (g)}} \times 100$$

Floating catfish pellets (30% crude protein; Charoen Pokphand, Bangkok) were fed to fish in the conditioning and spawning hapas three times per day at 0900, 1300 and 1600 hours according to appetite. Urea and triple superphosphate (TSP) were applied weekly at a rate of 34.4 and 17.2 $kg \cdot ha^{-1}$, respectively. These inorganic fertilizers were dissolved in water before addition to the pond. Water quality was monitored every ninth and fourth day during the conditioning and spawning periods, respectively. The parameters measured were dissolved oxygen (DO) at 0600 and 1800 hours, pH and temperature. Analysis of variance was used to test for differences between treatment means of reproductive parameters.

Results

Seed production increased during the first 45 days of the experimental period and declined during days 46 to 75 (Fig. 1). Depending on harvest date, seed production varied from 35 to 141 $seed \cdot m^{-2} \cdot$spawning $hapa \cdot day^{-1}$. Total seed production in terms of stocked or spawned female biomass

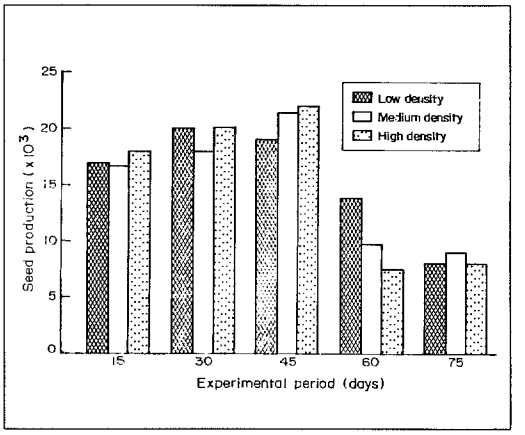

Fig. 1. Total number of seed produced from *Oreochromis niloticus* broodstock in net hapas with three stocking densities during a 75-day experimental period.

($seed \cdot kg^{-1} \cdot$female $stocked \cdot day^{-1}$) or unit area ($seed \cdot m^{-2} \cdot$spawning $hapa \cdot day^{-1}$) were not significantly different between treatments ($P<0.05$). Seed yields per unit area of conditioning net were improved using small conditioning hapas (Table 2).

Use of conditioned males improved seed production by almost 40% (92 $seed \cdot m^{-2} \cdot$spawning $hapa \cdot day^{-1}$ compared to 66 $seed \cdot m^{-2} \cdot$spawning $hapa \cdot day^{-1}$).

The mean GSI (%) showed an increasing trend throughout the experimental period (Table 3). The differences in GSI were not significant ($P>0.05$) among the three treatments. The mean GSI of spawned females were lower than those of unspawned females (Table 4).

The general trend observed was that DO levels at dawn were higher at the bottom of the hapa (75-cm depth) than at 15-cm depth below the water surface. In the conditioning hapas, the highest DO was recorded on day 44 (mean = 1.67 and 11.8 $mg \cdot l^{-1}$) for readings taken at dawn and in

the evening, respectively. After day 45, the DO declined in all the treatments such that at day 59 DO levels (expressed as means of bottom and surface readings) in the spawning hapas were less than 1.0 $mg \cdot l^{-1}$ in the low density treatment and outside hapas and less than 0.5 $mg \cdot l^{-1}$ in the medium and high density treatments. The relationship between DO levels in the breeding hapas and seed production was that the greatest seed production coincided with the lowest DO level. Water temperature varied between 26 and 31°C, and pH readings between 7.27 and 8.15, and the variation of both was density independent.

densities were used in large-sized hapas, since water quality is easier to maintain in large than in small hapas.

Differences in seed production occurred over time and this is a common phenomenon and a major limitation of hatchery systems for *Oreochromis* spp. (Lee 1979; Mires 1982). In small and large spawning units, decline in seed output has often been associated with changes in the environment and loss of broodstock condition. The main cause of decline in seed production over the latter part of this experiment was correlated with low early morning DO, which may have restricted spawning activity and/

Table 2. Mean seed production (±SE) from *Oreochromis niloticus* broodstock at three stocking densities during conditioning.

	Low density	Medium density	High density
Seed·m^{-2} spawning[a] hapa·day^{-1}	80 (±7)	79 (±9)	78 (±8)
Seed·m^{-2} conditioning[b] hapa·day^{-1}	147 (±14)	299 (±34)	575 (±60)
Seed·kg^{-1}·total female stocked·day^{-1}	30 (±3)	31 (±4)	33 (±5)
Seed·kg^{-1}·total female spawned·day^{-1}	101 (±7)	117 (±5)	109 (±4)

[a]Based on a 5-day spawning period, 40-m^2 hapa.
[b]Based on a 10-day female conditioning period (high = total 5-day seed harvest/27.2; medium = total 5-day seed harvest/52.9; low = total 5-day seed harvest/108.9).

Discussion

The reproductive performance of these female broodstock did not show any relationship with conditioning density. These observations support the hypothesis that tilapia broodstock can be stocked at high densities during the conditioning period with consequent savings in broodstock management costs. It can be expected that similar results would be obtained if these

or an ability by the females to mouthbrood successfully (Little 1990). It is also postulated that those females that spawned during the first 45 days did not spawn again in the following 20-30 days, thus reducing seed production. Earlier studies on *O. niloticus*, carried out at AIT, reported spawning intervals ranging from 25 to 35 days, depending on the crude protein level in the diet. The shortest interval was observed in females fed a diet of 20% crude protein

Table 3. Mean gonadosomatic index (±SE) of female *Oreochromis niloticus* after a 10-day conditioning period in nylon net hapas at three stocking densities.

	Day				
	10	25	40	55	70
GSI	%	%	%	%	%
Low density	3.42 (±0.52)	3.89 (±0.55)	4.09 (±0.43)	4.26 (±0.28)	4.65 (±0.24)
Medium density	3.23 (±0.48)	3.56 (±0.42)	3.80 (±0.36)	4.20 (±0.35)	4.70 (±0.33)
High density	3.43 (±0.37)	3.69 (±0.29)	3.87 (±0.44)	3.87 (±0.27)	4.08 (±0.26)

Table 4. Gonadosomatic indices (GSI) (means and standard errors) of spawned and unspawned female *Oreochromis niloticus* killed after different experimental periods. Each entry represents a mean of 18 fish (2 fish per replicate x 6 replicates x 3 treatments = 36, of which half were spawned and half unspawned).

Period (days)	GSI spawned females Mean ± SE	GSI unspawned females Mean ± SE
15	1.0 ± 0.09	2.1 ± 0.63
30	1.2 ± 0.07	2.4 ± 0.46
45	1.5 ± 0.25	2.8 ± 0.39
60	1.6 ± 0.23	3.1 ± 0.36
75	1.7 ± 0.15	3.5 ± 0.49

and the longest with 50% crude protein (Tuan 1986).

The increase with time in GSI (Tables 3 and 4) and the decline in egg production on days 60 and 75 (Fig. 1) suggest that reproductive efficiency declined as the fish grew. It is known that individual clutch size in tilapias increases with age and size of the fish (Siraj et al. 1983; Rana 1986), but these results suggest that the proportion of ripe eggs in the ovary that could be spawned successfully is reduced. The number of seed harvested from the mouth of females relates not only to the number of eggs in their ovaries, but also to the increased time required to spawn and to fertilize a larger clutch of eggs in a densely stocked spawning arena.

The differences in GSI of spawned and unspawned females support earlier findings that efficiency may be improved and seed production increased only if spawned females are exchanged (Little 1990) or if females are selectively exchanged when judged visually to be ripe (Little et al., this vol.).

Acknowledgements

We are greatly indebted to ICLARM and the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH for funding the study through a postgraduate scholarship grant awarded to the first author. The Malaŵi-Germany Fisheries and Aquaculture Development (MAGFAD) Project is also thanked for sponsoring the first author to attend the symposium. We are also grateful to the Overseas Development Administration of the United Kingdom for the partial funding of the study provided through the Fry Production grant awarded to the second author and for seconding him at AIT. The following individuals are thanked for the

valuable comments they made to the original report: Dr. Donald Macintosh; Dr. Amararatne Yakupitiyage; and Ms. Maliwan Meewan.

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Significant Proportions of Unexpected Males in Progenies from Single Pair Matings with Sibling Sex Reversed Males of *Oreochromis niloticus*

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Abstract

The profitability of tilapia culture is directly influenced by the efficient control of its reproduction through artificial means. Thus, many studies have focused on gonadal differentiation and sex determination. In *Oreochromis niloticus*, XX sex reversed males (produced by hormone treatment and analysis of the sex ratio of their progenies from single pair matings) can be used as a reliable tool to analyze both the efficiency of masculinizing treatments and sex determination. Twenty-two broodfish from two sets of sibling sex reversed males were tested through 35 single pair matings by microscopic sexing of all 4,546 fry. The 35 crosses yielded progenies composed of a majority of females, but all-female progenies were observed in only five reproductions. The other progenies yielded sex ratios significantly different from 1:1, ranging from 65 to 99% female with an average of 85%. Three unexpected males from these crosses were further tested; their progenies also included unexpected males. These results cannot be explained by a simple monofactorial sex determining system. Environmental factors may be involved in the sex determining mechanisms of *O. niloticus*.

Introduction

Oreochromis niloticus is one of the two major cultured species of tilapias in the world (Pullin 1983). Its ability to reproduce early (Baroiller and Jalabert 1989) leads, in controlled environments, to rapid overpopulation with a tendency to stunting. The efficient control of tilapia reproduction through artificial means directly influences the profitability of its culture. Since growth potential is higher in male tilapias than in females (Hanson et al. 1983), three methods are usually proposed to produce all-male populations. These are manual sexing, hybridization, and hormonal sex reversal (see reviews of Guerrero 1982; Lovshin 1982; Wohlfarth and Hulata 1983; Pandian and Varadaraj 1987). On the average, manual sexing involves 2.7-10% error, and also requires the elimination of 50% of the population after two to three months of rearing (Baroiller and Jalabert 1989). Because of its efficiency and reliability, hormonal sex reversal is today the most widely used method among the three. However, since the metabolism and the effects on the environment of the degradation products of synthetic androgen are not

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fully understood in fish, this technique is not authorized in some countries. Hybridization between two parental species of aquaculture interest such as *Oreochromis niloticus* and *O. aureus* does not generally produce all-male populations (Majumdar and McAndrew 1983; Wohlfarth and Wedekind 1991).

The viability of the YY genotype in *O. niloticus*, as demonstrated by Baroiller (1988b), Baroiller and Jalabert (1989) and Scott et al. (1989), suggests a fourth, intraspecific approach. In *O. niloticus* where the male is heterogametic, a feminization treatment produces XY sex reversed females which, when crossed with an XY male, produce a new YY genotype. Each progeny from crosses between this homogametic YY male and any homogametic XX female is theoretically an all-male population.

However, these inter- and intraspecific crosses sometimes yield sex ratios which cannot be explained by a simple XX/XY monofactorial sex determining mechanism in *O. niloticus*. To explain these unexpected sex ratios, three other models have been proposed (see reviews of Avtalion and Don 1990; Mair et al. 1991; Wohlfarth and Wedekind 1991): a theory combining two alleles from an autosomal locus and two of the three sex chromosomes (Avtalion and Hammerman 1978; Hammerman and Avtalion 1979); a polygenic model (Majumdar and McAndrew 1983); and finally, a monofactorial sex determination with the possible presence of a rare autosomal gene, epistatic to major genes. However, these three theories do not explain all the results found in the literature.

In order to analyze sex determination in *O. niloticus*, two sets of sibling sex reversed males were produced and tested in single pair matings.

Materials and Methods

Animals

PRODUCTION OF TWO STOCKS OF SEX REVERSED MALES (FIG. 1)

A progeny of fry from *O. niloticus* of the "Bouake" strain (Baroiller et al, in press) was produced by crossing a male with one of the four females placed in the same 400-l aquarium. The progeny, removed after 10 days post-fertilization (PF), was exposed to a hormonal sex reversal treatment using 17α-methyltestosterone added to the feed at 30 $\mu g \cdot g^{-1}$ (Baroiller and Toguyeni, this vol.). Thirty males from the resulting all-male population were kept to analyze the sex ratio of their progenies in 400-l aquaria. Twenty of these males were bred with normal females. Thirty juveniles of each of these progenies were killed to determine the sex ratio of each population. Sexing by gonadal squash was done after 90 days PF when the histological characteristics of differentiation were already established for both sexes (Baroiller 1988a and b). The 13 broodfish with progenies showing no significant difference in sex ratio compared to a 1:1 population were eliminated (sex ratios ranging from 33.3 to 60% male). The seven other males with all-female progenies (30 females in the sample of 30 juveniles sexed in each progeny) were kept as sex reversed males. One of them was crossed again and its entire progeny was exposed to a hormonal sex reversal treatment using 11ß-OHΔ4 added to the feed at 30 $\mu g \cdot g^{-1}$ (Baroiller and Toguyeni, this vol.). The resulting all-male population was theoretically an exclusively XX sex reversed male population; 60 of them constituted Stock 1 and were identified

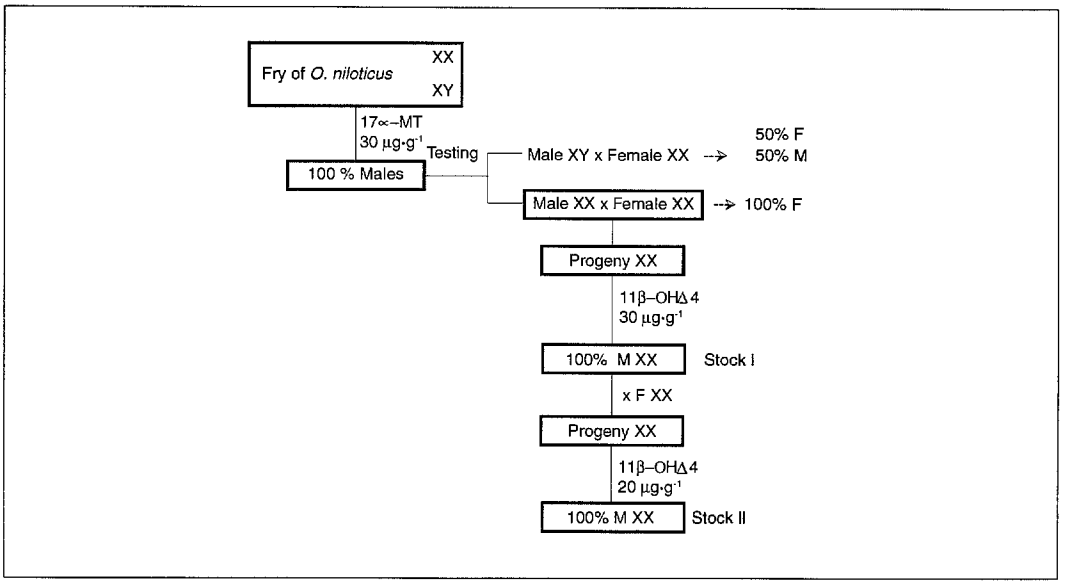

Fig. 1. Production of two stocks of sex reversed males of *Oreochromis niloticus*.

by a letter. The progeny of one of them, sex reversed male J, was exposed to a hormonal sex reversal treatment using 11ß-OHΔ4 at 20 $\mu g \cdot g^{-1}$ (Baroiller and Toguyeni, this vol.). The resulting sex reversed all-male population constituted Stock 2, each broodfish being identified by a number.

REPRODUCTIONS OF SEX REVERSED MALES AND SEXING OF THEIR PROGENIES

Thirteen "sex reversed male" broodfish from Stock 1 and nine sex reversed males from Stock 2 were crossed in single pair matings with one or several females in 400-l aquaria. The water in the spawning aquaria was filtered permanently and maintained at 27°C using a thermoregulation device. Each animal was tagged in the dorsal musculature. Reproduction was detected by the onset of maternal mouthbrooding characterized by dilation of the female's mouth. As soon as this particular characteristic was observed, all other individuals were removed to leave the female mouthbrooding in the aquarium. Five days after hatching, i.e., nine days PF, the fry were removed from the mother's mouth. The entire progeny (or a sample of at least 100 fry from the batch), identified by its fertilization date and the respective tags on the parents, were transferred to 200-l aquaria. The fry were fed ad libitum six times a day, seven days a week, with a first-feeding salmonid feed (Aqualim) distributed by automatic feeder during a 12-hour photoperiod. The water, aerated and filtered, was kept at 28±1.5°C. After three weeks of rearing in aquaria, when the gonadal differentiation was already well established (Baroiller 1988a and b), fry were transferred to external 1.5-m^3 tanks until sexing and were given the same feed. After 60-90 days PF, when the histological characteristics of the male and female differentiation were already established (Baroiller 1988a and b), all fry were sexed by examination of the gonad squash at x125 magnification. The presence of previtellogenic or vitellogenic oocytes and the lobular configuration showed the female and male sexes, respectively.

Four sex reversed males from Stock 2 (XX4, XX9, XX17 and XX18) were each successively crossed with three or four different females.

Results

The sex ratios of 22 progenies from sex reversed males were determined by sexing all 2,429 fry produced, i.e., approximately 110 fish per batch. Four of them, i.e., 18% of the progenies, gave sex ratios of 0:1 (male:female). In the 18 other progenies, unexpected males based on a monofactorial sex determination were produced in proportions ranging from 1 to 40.5% (Table 1 and Fig. 1). Only one of these sex ratios (40.5% male) was not significantly different from a 1:1 ratio. Compared to the ratio of an "average" synthetic population of 5,299 fry from crosses of 42 normal broodfish of the same species (1.39:1), the 40.5% male showed a highly significant difference. The male frequency distribution in these progenies was unimodal, around 0-10% male.

Since the progeny of the sex reversed male is theoretically composed of 100% XX individuals, four of the naturally unexpected males from the XXI sex reversed male (Stock I) were kept until their sexual maturity to analyze the sex ratio of their progenies. Three unexpected males (D1, D2 and D3) naturally originating from XXI sex reversed males were bred with different females (Table 2). Their progenies, female for most of them included, however, 2.5-9.2% unexpected males. The sex ratios of the progenies of the D1, D2 and D3 males all differed significantly from the sex ratios of the progeny of the XXI sex reversed male. There was no significant difference between two of the three unexpected male progenies (D2 and D3).

The XX4 sex reversed male was successively bred three times with two females. There was no significant difference between the three sex ratios whether the progenies came from the same pair or only had the same sire (Table 3).

Four progenies were produced by crossing an XX9 sex reversed male with four different females. Three progenies (XX4 male x XX11, XX12 and XX16 females) out of four yielded sex ratios which did not differ significantly among themselves. In contrast, the cross with the XX10 female gave a progeny with a sex ratio differing significantly from that of the progenies from the XX11 and XX12 females.

The same applied to the sex ratios of progenies from XX18 sex reversed males as three of them did not show any significant difference (progenies from XX16, XX17 and XX20 females).

In the XX17 male, there was no significant difference between the proportions of unexpected males in two out of the three crosses (XX17 x XX15 and XX11 females). However, only the XX17 x XX11 cross produced an all-female population. The progeny from the same male crossed with the XX16 female gave 39.6% males. Therefore, the same XX17 male successively produced 0 and 39.6% unexpected males with two different females.

Finally, a diallel cross experiment was made involving two sex reversed males and two females (Table 4). A maternal influence was observed in the two progenies from XX9 sex reversed males. In contrast, a paternal influence was observed between the two progenies from the XX16 female.

Discussion

In species such as *O. niloticus* where the female is considered homogametic, the progeny from a sex reversed male is theoretically all-female, based on a

Table 1. Single pair matings of sex reversed males from Stocks 1 and 2 of *Oreochromis niloticus* with XX females.

Crosses (M x F)	No. of progeny at sexing	Survival (%)	No. of males	No. of females	Males (%)	χ^2 (level of significance)[a] 1:1	1.39:1
XXA x XXA	116	55	47	69	40.5	2.1 (NS)	6.9(**)
XX7 x XX7	179	89.5	63	116	35.2	8 (**)	
XX9 x XX10	146	65.2	50	96	34.2	7.4 (**)	
XXD x XXD	149	ND	38	111	25.5	18.9(***)	
XXE x XXE	141	47	36	105	25.5	17.9(***)	
XX10 x XX20	89	ND	21	68	23.6	13.3(***)	
XXF x XXF	199	ND	45	154	22.6	32.2 (***)	
XXG x XXG	152	ND	34	118	22.4	25.1 (***)	
XXH x XXH	27	ND	6	21	22.3	4.44 (*)	7.7(**)
XXI x XXI	175	54.2	35	140	20	34.5 (***)	
XX8 x XX8	85	85	15	70	17.6	19.8 (***)	
XXJ x XXJ	70	ND	11	59	15.7	18.6 (***)	
XXK x XXK	119	95.2	14	105	11.8	40.6 (***)	
XXL x XXL	76	ND	6	70	7.9	32.7 (***)	
XX18 x XX16	101	95.3	4	97	4	54.2 (***)	
XX17 x XX15	54	51.9	2	52	3.7	29.5 (***)	
XX4 x XX7	103	94.5	1	102	1	65.1 (***)	
XX1 x XX1	96	90.6	1	95	1	60.5 (***)	
XXC x XXC	74	66.1	0	74	0	74 (***)	
XXB x XXB	86	43	0	86	0	86 (***)	
XXM x XXM	32	ND	0	32	0	32 (***)	
XX11 x XX11	160	93.6	0	160	0	160 (***)	

[a]When χ^2 is lower than or close to the level of significance vis-à-vis a 1:1 population, sex ratios are tested against a 1.39:1 sex ratio which is the mean sex ratio (min.: 15.8% males; max.: 77.1%) from all 42 single pair matings of XY males, i.e., 5,299 sexed fry. Levels of significance were: *=p<0.05; **=p<0.01; and ***=p<0.001.
ND = No data on survival: the initial number of fry was not recorded.

Table 2. Crosses of a sex reversed male *Oreochromis niloticus* with three of the unexpected males found in its progeny: progenies from the same sex reversed male characterized with the same lower case letter (a, b or c) show significant differences (p=0.05) in their sex ratios; ** = p<0.01.

Crosses (M x F)	No. of progeny at sexing	Survival (%)	No. of males	No. of females	Males %	χ^2 (level of significance)	
Sire							
XXI x XXI	175	54.2	35	140	20		a b
Progenies							
D1 x XX21	317	92.9	8	309	2.5		a b
D2 x XX22	314	89.4	29	285	9.2	12.9(**)	a
D3 x XX23	102	86.4	8	94	7.8		b

Table 3. Successive matings of four sex reversed *Oreochoromis niloticus* males: progenies from the same sex reversed male characterized with the same lower case letter (a, b or c) show significant differences (p=0.05) in their sex ratios; NS = Non-significant; ***=p<0.001.

Crosses (M x F)		No. of progeny at sexing	Survival (%)	No. of males	No. of females	Males (%)	χ^2 (level of significance)
XX4	x XX5	101	84.2	4	97	4	
	x XX5	104	95.4	3	101	2.9	1.8 (NS)
	x XX7	103	94.5	1	102	1	
XX9	x XX10	146	65.2	50	96	34.2	a b
	x XX11	211	98.6	20	191	9.5	57.5 (***) a
	x XX12	97	94.2	2	95	2.1	b
	x XX16	21	19.6	3	18	14.3	
XX17	x XX15	54	51.9	2	52	3.7	a
	x XX11	99	93.4	0	99	0	58.7 (***) b
	x XX16	53	46.5	21	32	39.6	a b
XX18	x XX16	101	90.9	4	97	4	c
	x XX20	363	ND	3	363	0.8	40.9 (***) b
	x XX17	279	90.6	7	272	2.5	a
	x XX14	56	56	9	47	16.1	a b c

Table 4. Sex ratio (♂:♀ and % of males) in a diallel cross expirement involving two sex reversed males and two females: progenies from the same sex reversed male characterized with the same lower case (a, b or c) show significant differences (p=0.05) in their sex ratios.

	MXX17 ♂		MXX9	
FXX11	0:99 (0)	a b	20:191 (9.5)	a
♀				
FXX16	21:32 (39.6)	a	3:18 (14.3)	b

monofactorial system. Out of 35 crosses of sex reversed males conducted in the present study, only five progenies, i.e., 14.3%, correspond to the monofactorial theoretical model. In the 31 other progenies, 1-40.5% of unexpected males were observed. These males did not result from sexing errors during microscopic examination of gonads and showed normal fertility: three of them bred. For *O. niloticus*, three other studies have analyzed the sex ratios of progenies from sex reversed males. In the first study of eight male progenies produced by masculinizing treatment, four progenies were all-female, one showed a sex ratio not significantly different from 1:1 and three yielded unexpected high proportions of females in the range 65.2-81.8% (Jalabert et al. 1974). However, it is

impossible to attribute the paternity of these unexpected sex ratios to sex reversed males rather than to XY males, considering the large range of sex ratios that these XY males show in *O. niloticus* (Shelton et al. 1983; Wedekind 1987 cited by Wohlfarth and Wedekind 1991; Lester et al. 1989). In the second study (Calhoun and Shelton 1983), sex reversed males, produced by exposing the progenies of a sex reversed broodfish to a masculinizing treatment, were mass-bred (22 per experiment) with two batches of females and a sample of 100 fry was sexed. In one of the female batches, one progeny out of nine yielded a 99% female sex ratio. In the other batch, eight progenies out of nine yielded 87-99% females, and only one progeny (i.e., 11%) was a real all-female population and therefore corresponded to the monofactorial XX/XY theoretical model. As in the present study, one of the batches produced a majority of progenies with unexpected sex ratios. Finally, in the third study, four sex reversed males were identified by the sex ratio of their progenies in 11 single pair matings. Two progenies out of 11 yielded unexpected sex ratios, with 6.7 and 17.4% males. Nine other progenies were considered all-female. However, the number of sexed fish was still very low, with an average of 20 fry per batch (min.: 6; max.: 43), and low percentages of unexpected males may therefore have escaped notice.

These four different studies show sex ratios that cannot be explained by a monofactorial sex determination although they can constitute 50 (Calhoun and Shelton 1983) to 88% (this study) of the progenies. However, as the number of these unexpected proportions of males was often fairly low, they may have been masked, at least partially, in other studies based on a very limited sample size.

The percentage of unexpected males in successive progenies from the same sex reversed male differs from progenies to progenies; a paternal and maternal effect may be involved. A maternal influence is also suggested by the differences in the proportions of unexpected males observed in the mass breeding of sex reversed males depending on the batch of females used (Calhoun and Shelton 1983). In diallel crosses involving five pairs of broodfish of *O. niloticus*, there is no such maternal or paternal influence (Mair et al. 1991).

Therefore, deviations from the theoretical model may be found in high proportions and seem to be incompatible with rare autosomal factors. The conditions under which these reversals occur in relation to the expected phenotype have not yet been clearly established. The hypothesis of genetic contamination seems unlikely since Majumdar and McAndrew (1983) demonstrated the presence of unexpected sex ratios in isolated natural populations. More complex, polygenic genetic models could be involved. However, the absence of sex-specific genetic markers in tilapias renders difficult genetic analyses such as those conducted on platyfish (*Xiphophorus maculatus*) (Kallman 1984). The deviations observed could also result from the potential effects of external factors on differentiation. Depending on the genotype, one or more external factors could influence the phenotypic sex of the fry during the gonadal differentiation of sex. This hypothesis, far easier to test than a complex sex determining mechanism, could explain some unexpected results such as the presence of males in progenies from sex reversed males as well as the presence of a natural XY sex reversed female in *O. niloticus* (Scott et al. 1989; Mair et al. 1991). Consequently, a study on the effects of temperature on the sex ratio

was conducted on progenies from these sex reversed males (Baroiller et al., this vol.).

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Comparative Effects of a Natural Androgen, 11ß-Hydroxyandrostenedione, and a Synthetic Androgen, 17α-Methyltestosterone, on the Sex Ratios of *Oreochromis niloticus*

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BAROILLER, J.F. and A. TOGUYENI. 1996. Comparative effects of a natural androgen, 11ß-hydroxyandrostenedione, and a synthetic androgen, 17α-methyltestosterone, on the sex ratios of *Oreochromis niloticus*, p. 238-245. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Synthetic androgens are often considered to be more potent than natural androgens for sex reversal treatments in fish. Preliminary experiments on the natural androgen, 11ß-hydroxyandrostenedione (11ß-OHΔ4), recently identified in the gonads of fry of *O. niloticus* in the early stages of testicular ontogenesis, showed a high masculinizing potential. Fry of 10-14 days post-fertilization (dPF) were produced from single pair matings using untreated females and either normal or sex reversed males, to assess any significant deviation in sex ratio compared to the controls. The fry were then treated during a minimum period of 21 days either with the natural or synthetic androgen added to the feed at different concentrations. Complete sex reversal was achieved with both steroids. All-male populations were produced in eight groups treated with 10-35 µg 11ß-OHΔ4·g^{-1} of feed and in two groups treated with 17α-methyltestosterone (17α-MT) at 5 and 20 µg·g^{-1} of feed, respectively. There was no significant difference in the potency of the two androgens for concentrations higher or equal to 10 µg·g^{-1}. However, at 5 µg·g^{-1}, only 17α-MT produced 100% male populations whereas 11ß-OHΔ4 produced 88.9%. In contrast, the lowest concentration of 11ß-OHΔ4 (1 µg·g^{-1}) significantly deviated sex ratios whereas 17α-MT had no effect compared to the controls.

The natural androgen 11ß-OHΔ4 can therefore constitute an alternative treatment to synthetic steroids. Low concentration treatments can be optimized by increasing the duration of treatment.

Introduction

Today, with an annual global production of approximately 500,000 tons (Lazard 1990), tilapias (*Oreochromis*, *Sarotherodon* and *Tilapia*) constitute, with the cyprinids and salmonids, one of the three most important groups of freshwater fish for aquaculture. However, in culture conditions where competition

for food is high, the highly efficient reproduction of the species of *Oreochromis* (Baroiller and Jalabert 1989) and their early sexual maturity lead to overcrowding and stunting, resulting in limited economic yields for fish farms. A solution to this problem consists in producing monosex populations. All-male populations are preferred because their growth performance is better compared to females (Pruginin 1967; Hickling 1968; Hanson et al. 1983).

Currently, two techniques are used to produce monosex populations for fish culture (Baroiller and Jalabert 1989):

Manual sexing. Based on the sexual dimorphism observed in the urogenital papilla, this technique entails the elimination of all females, i.e., approximately half of the initial population as soon as possible (after a two to three month nursing period). Manual sexing, which is used in Africa, is time-consuming; it also requires qualified personnel, and includes 3-10% errors (Lazard 1980; Chervinski and Rothbard 1982). In addition, this technique requires rearing a population of fry for two to three months after which half (the females) will be eliminated. Although manual sexing is simple, it is expensive in terms of time and labor and implies the underutilization of farming infrastructures and lower feed productivity.

Hormonal sex reversal. This technique consists in masculinizing the entire population of fry by adding a steroid to the feed for a short period (Guerrero 1982; Hunter and Donaldson 1983; Pandian and Varadaraj 1987; Baroiller and Jalabert 1989). Hormonal sex reversal has been commonly used for several decades by a number of tilapia producing countries such as Israel, Taiwan and the Philippines. This technique requires the systematic treatment of every new population of fry. However, the use of hormones in the production of animals for human consumption is still prohibited in many countries (France and the United Kingdom, for example) which consider that the treatment and the effects of the synthetic steroid waste products are still insufficiently understood, especially their ecological impact.

In tilapias, as in all teleost fish, no definite physiological proof supports the hypothesis of Yamamoto (1969) that steroids are the natural inducers of differentiation (Adkins-Regan 1987). The modification of the natural process of sexual differentiation by exogenous steroids could be due to pharmacological effects (Reinboth 1970). In fact, few studies have investigated early steroidogenesis during the gonadal sex differentiation of gonochoristic fish (van den Hurk et al. 1982; Rothbard et al. 1987; Baroiller 1988a and b; Baroiller et al. 1988).

In *Oreochromis niloticus*, early steroidogenous potential in male and female gonads has been analyzed during the first three months of their life; this period covers the entire process of testicular and ovarian differentiation (Baroiller et al., in press). Testosterone can be synthesized by the gonads of both sexes whereas oestradiol is exclusively produced by the ovaries (Baroiller 1988b).

In contrast, certain androgens like 11ß-hydroxyandrostenedione (11ß-OHΔ4) and adrenosterone prove male sex-specific during the same period (Baroiller 1988a and b) and show masculinizing potentialities (Baroiller 1988b).

Artificial steroids are generally more potent in their masculinizing effects than natural androgens (Hunter and Donaldson 1983). The potency of 17α-methyltestosterone (17α-MT) is attributed to the presence of the 17α-methyl group which makes its elimination slower than in natural androgen like testosterone (Fagerlund and McBride 1978; Donaldson et al. 1979).

A study of the masculinizing potential of 11ß-OHΔ4 and 17α-MT was conducted in *O. niloticus* to compare the respective performances of both artificial and natural hormones; to test the hypothesis of a possible role of 11ß-OHΔ4 in the process of testicular differentiation; and also to study possible alternatives to traditional sex reversal treatments.

Materials and Methods

Animals Tested

Two types of male broodfish of *O. niloticus* of the "Bouake" strain (Baroiller 1988b) were used for the production of fry families: normal males (XY) and sex reversed males (XX). The latter came from two related families and yielded, for most of them, significant proportions of males in their progenies produced by single pair matings (Baroiller, this vol.).

Breeding

Single male broodfish were placed in 400-l aquaria with normal females at a sex ratio of 4:1. The water in the breeding aquaria was filtered and maintained at a constant temperature of 27°C. Each animal was identified by a mark inserted in the dorsal muscles. Reproduction was detected at the onset of maternal mouthbrooding behavior which is characterized by a dilation of the mouth. On the first day of incubation, all other individuals were removed to leave the mouthbrooding female on her own in the breeding aquaria. Five days after hatching, i.e., nine days post-fertilization (dPF), the fry were removed from the mouth. Each brood, identified by the fertilization date and the marks on the respective parents, was divided into two to five batches of at least 100 fry reared separately in 200-l aquaria.

Hormone Treatment

Steroids were administered through the feed. A first feeding salmonid feed (Aqualim) was impregnated with an alcohol solution containing steroids. Concentrations of 1 to 45 ug of steroids per gram of feed were tested. For the control batches, the same preparation was applied except that the feed did not contain any steroid.

Fry were fed ad libitum six times daily using an automatic feeder during the 12-hour photoperiod, seven days a week.

Fry at 10-15 dPF were treated thus for 45 and 21 days. To avoid a potential masculinizing effect from the temperature (Baroiller et al., in press), the water, which was filtered and aerated, was maintained at 28±1.5°C.

Nursing and Sexing the Fry

At the end of the treatment, fry older than 31 dPF were transferred to 1.5-m^3 outdoor tanks where they were fed the same diet until sexing was possible. At 60-90 dPF, when the histological differentiation of the male and female gonads had already taken place (Baroiller 1988a and b), all fry were sexed by microscopic examination of the gonad squash (x125). The presence of previtellogenic (auxocytosis) or vitellogenic oocytes, and the lobular configuration showed the female and male sexes, respectively.

A χ^2 test was used to compare the sex ratios of the batches that had received treatment and the control batches (α=0.05).

Results

After treatment and at the moment of sexing, there was no significant difference in the survival rate of the fry between the controls and the batches that underwent hormonal sex reversal, regardless of the treatment duration and the concentrations used (Table 1).

The microscopic examination of the gonad squashes of fry treated with 11ß-OHΔ4 (1,631 sexed animals) or 17α-MT (416 fry) did not show any hermaphrodite characteristics, sterility, or structural abnormality. The gonads of the individuals treated with 11ß-OHΔ4 were functional, irrespective of their genotype: functional sex reversed male XX and normal males were obtained and identified by progeny testing at the end of these treatments (Baroiller, this vol.).

Of the 17 batches treated with 11ß-OHΔ4, only one batch showed no deviation of sex ratio compared to the controls (Table 2). The progeny of the XY4 male underwent hormonal sex reversal only after treatment 15 dPF whereas the 13 other families received treatment after 10-14 dPF. Since sex reversal was produced in the four other batches also treated with 5 $\mu g \cdot g^{-1}$, this concentration did not seem to be the cause of absence of a deviation in sex ratio. This result could instead indicate a critical period of hormonal sensitivity.

All treatments using 11ß-OHΔ4 on fry of less than 15 dPF significantly affected the sex ratio towards the male sex compared to the controls, regardless of the concentration used (Table 2).

In contrast, 17α-MT had masculinizing effects only in a range of concentrations between 5 and 45 $\mu g \cdot g^{-1}$.

The 21-day treatment yielded all-male populations using hormonal concentrations between 10 and 35 $\mu g \cdot g^{-1}$ (11ß-OHΔ4) and at 5 $\mu g \cdot g^{-1}$ (17α-MT). With concentrations lower or equal to 5 µg of 11ß-OHΔ4 per gram of feed administered over a period of 21 days, the percentage of males was proportional to the concentration used (Fig. 1).

There was no significant difference in masculinizing potency between the two hormones for concentrations of 10-45 $\mu g \cdot g^{-1}$. With concentrations of 5 $\mu g \cdot g^{-1}$ and higher, significant differences were observed in the results of both treatments: using 5 $\mu g \cdot g^{-1}$, only 17α-MT yielded 100% male populations against a maximum of 88% with 11ß-OHΔ4. Inversely, 17α-MT was not effective if used at 1 $\mu g \cdot g^{-1}$, whereas 11ß-OHΔ4 used at the same concentration significantly altered the percentage of males compared to the controls.

Replicates (2-4) were conducted for four different concentrations of 11ß-OHΔ4 (5, 20, 30 and 35 $\mu g \cdot g^{-1}$) (Table 2). There was no significant difference in sex ratios between replicates of the same treatment.

Discussion

The hormones used for masculinization of fish are generally artificial molecules derived from testosterone: 17α-MT, 17α-ethynyltestosterone, dihydrotestosterone acetate and testosterone propionate. These synthetic androgens are considered more potent than natural androgens for the hormonal sex reversal of gonochoristic teleost fish species (Hunter and Donaldson 1983).

Many authors have also produced 100% male tilapia populations using these steroids, despite a great heterogeneity in the experimental conditions: especially concentrations varying from 10 to 240 $\mu g \cdot g^{-1}$ of feed (Baroiller and Jalabert 1989). The optimum concentrations usually suggested are 30 $\mu g \cdot g^{-1}$

Table 1. Survival of *Oreochromis niloticus* fry according to hormone treatment applied for sex reversal.

Treatment	Survival after treatment (%)	Number of batches tested	Survival at sexing (%)	Number of batches tested
11ß-OHΔ4	83.8	15	55.2	17
17α-MT	85.6	4	56.7	4
Control	79.6	12	50.2	14

Table 2. Sex reversal treatments administered to *Oreochromis niloticus* fry, the progeny of normal male (XY_n) or sex reversed male (XX_n) broodfish pairs.

	Treatment characteristics				Sexing characteristics			
Broodfish male type and no.	Steroid	Level in feed ($\mu g \cdot g^{-1}$)	Initial age of progeny (dPF)	Final Age (dPF)	Duration days	Number of males	Number of females	Males (%)
XY1	11ß-OHΔ4	35	12	57	45	81	0	100
	Control	0	12	57	45	45	55	45
XY2	11ß-OHΔ4	35	12	57	45	92	0	100
	Control	0	12	57	45	45	41	52
XX1	11ß-OHΔ4	35	12	57	45	128	0	100
	Control	0	12	57	45	64	56	53
XX2	11ß-OHΔ4	30	13	55	42	64	0	100
	11ß-OHΔ4	20	13	55	42	51	1	98
	Control	0	13	55	42	0	74	0
XX3	11ß-OHΔ4	30	14	42	28	129	0	100
	11ß-OHΔ4	30	14	35	21	168	0	100
	Control	0	14	42	28	47	69	41
XX4	11ß-OHΔ4	30	12	54	42	112	2	98
	11ß-OHΔ4	20	12	54	42	103	1	99
	Control	0	12	54	42	0	86	0
XX5	11ß-OHΔ4	20	11	32	21	74	0	100
	17α-MT	20	11	32	21	110	0	100
	Control	0	11	32	21	23	80	22
XX6	11ß-OHΔ4	10	10	31	21	100	0	100
	17α-MT	10	10	31	21	172	1	99
	Control	0	10	31	21	34	118	22
XX7	11ß-OHΔ4	5	10	31	21	24	3	89
	17α-MT	5	10	31	21	47	0	100
	Control	0	10	31	21	6	21	22
XX8	11ß-OHΔ4	5	11	32	21	79	30	73
	Control	0	11	32	21	28	96	23
XX9	11ß-OHΔ4	5	11	32	21	71	30	70
	Control	0	11	32	21	16	69	19
XY3	11ß-OHΔ4	5	11	32	21	46	20	70
	Control	0	11	32	21	15	70	18
XY4	11ß-OHΔ4	5	15	36	21	79	30	73
	Control	0	15	36	21	71	70	70
XX10	11ß-OHΔ4	1	13	34	21	41	72	36
	17α-MT	1	13	34	21	12	74	14
	Control	0	13	34	21	11	59	15

dPF=days post-fertilization.

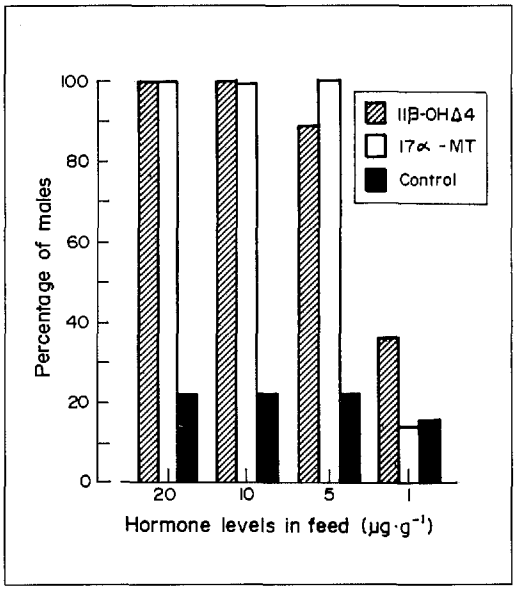

Fig. 1. Efficiency of hormonal sex reversal using different steroids and different concentrations in tilapia feed.

for 17α-MT and 60 $\mu g \cdot g^{-1}$ for ethynyltestosterone (Pandian and Varadaraj 1987; McGeachin et al. 1987; Rothbard et al. 1987; Baroiller and Jalabert 1989). For *O. mossambicus*, the minimum concentration for the production of all-male batches using 17α-MT is 5 $\mu g \cdot g^{-1}$ (Pandian and Varadaraj 1987). Other artificial androgens such as 17α-methyl-5-androsten-3ß-17ß-diol (Varadaraj and Pandian 1987) and mibolerone (Guerrero and Guerrero, this vol.) have also been used.

The present study indicates that in *O. niloticus*, the minimum concentration of 17α-MT yielding a 100% male population is also 5 $\mu g \cdot g^{-1}$ for a 21-day treatment period; there was no deviation in sex ratio with concentration of 1 $\mu g \cdot g^{-1}$.

As the steroidogenesis in the period of gonadal differentiation has not been extensively studied in teleost fish, particularly in tilapia (Baroiller 1988a and b; Baroiller et al. 1988), only a limited number of natural androgens have been tested as masculinizing agents. Among these, two 11-oxygenated derivatives, administered through the rearing water and through the feed, respectively, can modify the differentiation process in tilapia: adrenosterone causes a destruction of the ovarian structures in *O. niloticus* at a concentration of 5 $mg \cdot l^{-1}$ (Katz et al. 1976) and 11 ketotestosterone, at 200 $\mu g \cdot g^{-1}$, inhibits the formation of the ovarian cavity in *O. mossambicus* but does not prevent the formation of young oocytes that will subsequently degenerate (Nakamura 1981). However, considering the high levels used, the hypothesis of toxic or paradoxical effects cannot be discounted (Hunter and Donaldson 1983). In *O. niloticus*, the masculinizing effects of adrenosterone have also been demonstrated after treatments through the feed at 45 $\mu g \cdot g^{-1}$ (Baroiller 1988b).

Until then, 11ß-OHΔ4 had not been used in hormonal sex reversal treatment in tilapia. This natural androgen shows a masculinizing potency comparable to that of 17α-MT at concentrations of 10-35 $\mu g \cdot g^{-1}$. In addition, deviations of sex ratio are observed at concentrations lower than 5 $\mu g \cdot g^{-1}$, which is not the case for 17α-MT.

The microscopic examination of the gonads of fry treated for 60-90 days did not show any abnormality in the course of gonadal ontogenesis. Moreover, the functional reversal of the gonads was demonstrated by the identification of the sex reversed males after progeny testing of the individuals treated in the present study (Baroiller, this vol.).

The steroid 11ß-OHΔ4 was identified *in vitro* in three species of teleost fish in the early stages of differentiation: this 11-oxygenated steroid can be specifically synthesized by the testes of the rainbow trout *Oncorhynchus mykiss* (van den Hurk et al. 1982), the catfish *Clarias*

gariepinus (van den Hurk et al. 1989) and *O. niloticus* (Baroiller 1988b) during the early gonadal ontogenesis.

Administered through the feed (60 and 6 $\mu g \cdot g^{-1}$) and through the rearing water (300 $\mu g \cdot l^{-1}$), 11ß-OHΔ4 produces populations with high percentages of males, yielding 76-78% in the trout (van den Hurk and Lambert 1982; van den Hurk and van Oordt 1985) and 77% in the catfish (van den Hurk et al. 1989), respectively, against 48 and 50% males in the controls, respectively. In *C. gariepinus* (van den Hurk et al. 1989), 17α-MT significantly biases the sex ratio towards males (65%) at concentrations of 30 $\mu g \cdot l^{-1}$ and towards the female sex at 100 $\mu g \cdot l^{-1}$. In *Oncorhynchus mykiss*, testosterone derivatives may not be essential for testicular differentiation: testosterone and its 11-oxygenated derivative can be synthesized by the testis only stages beyond those at which 11ß-OHΔ4 has been identified (van den Hurk et al. 1982). Furthermore, a treatment using cyproterone acetate did not affect the sex ratio of the batches of trout (van den Hurk and van Oordt 1985) and tilapia fry (Hopkins et al. 1979). The androstenedione 11-oxygenated derivatives may be involved in some stages of testicular differentiation in these three species.

In *O. niloticus*, response to the hormone treatment is observed during a determined critical period. To be efficient, treatment must begin between nine and 13 dPF. Beyond this period, differentiation seems definitively according to the genotype; from then on, it could only be affected by exogenous steroid factors.

The 21-day treatment is therefore applied between 9-30 dPF. From a histological perspective, at 27°C, oogonia proliferation occurs in females between 20-28 dPF and is followed by their first meiotic prophase at 28-35 dPF (Baroiller 1988a and b). In males, a highly progressive multiplication of somatic cells and spermatogonia is observed during the same period (Baroiller 1988 a and b). Exogenous hormones are therefore administered to the fry before the establishment of these histological processes.

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Effects of High Rearing Temperatures on the Sex Ratio of Progeny from Sex Reversed Males of *Oreochromis niloticus*

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BAROILLER, J.F., A. FOSTIER, C. CAUTY, X. ROGNON and B. JALABERT. 1996. Effects of high rearing temperatures on the sex ratio of progeny from sex reversed males of *Oreochromis niloticus*, p. 246-256. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

In *Oreochromis niloticus*, hormonal sex reversal of an entire progeny from crosses between a sex reversed XX male (produced by steroid treatment) and a normal XX female, resulted in a progeny of sex reversed XX male siblings. The sex ratio of the progenies produced by single pair matings of 22 of these sex reversed males is only rarely all-female, as opposed to predictions of a monofactorial sex determination model. In order to assess a potential thermosensitive differentiation, progenies from these sex reversed males were submitted to high temperatures for 21 days. Nine to 13 day-old fry post-fertilization (PF) from 17 progenies were reared at high temperatures ranging from 30 to 36°C and to an average control temperature of 28°C, respectively. No significant difference in survival was observed between the two groups of fry with 76.2 and 74.2% survival rates at 28 and 30-36°C, respectively. All surviving fry (an average of about 100 per batch) were sexed by histological examination of tissue squashes at 60-90 dPF. High temperatures shifted the control sex ratios from 0 to 91%. As the low mortalities cannot account for these deviations, thermosensitivity is demonstrated in *O. niloticus*.

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Introduction

In controlled environments, the early and prolific breeding of tilapias (Baroiller and Jalabert 1989) results in rapid overpopulation with a tendency to stunting. The artificial control of tilapia reproduction is therefore essential to make its culture profitable. Since individual males show better growth rates than females (Pruginin 1967; Hickling 1968; Hanson et al. 1983), three approaches have been suggested to produce all-male populations: (1) manual sexing by examination of the urogenital papilla (Hickling 1963; Guerrero 1975); (2) hybridization (reviews of Lovshin 1982; Majumdar and McAndrew 1983; Wohlfarth and Hulata 1983); and (3) hormonal sex reversal (reviews of Guerrero 1982; Hunter and Donaldson 1983; Pandian and Varadaraj 1987). Manual sexing by examination of the urogenital papilla (2.7-10% errors) results in the elimination of half of the population after two to three months (Baroiller and Jalabert 1989). Although today, hormonal sex reversal is the most widely used of the three techniques because of its efficiency and reliability, it is often questioned. For example, the effects of the degradation products of synthetic steroids have not been studied sufficiently, especially for their ecological consequences. Hybridization using two parental species of culture interest like *Oreochromis niloticus* and *O. aureus* does not systematically produce 100% males (Majumdar and McAndrew 1983; Wohlfarth and Wedekind 1991).

A fourth, intraspecific approach was suggested by Yamamoto (1969) and has been used for the past ten years. This technique consists in producing homogametic male and female broodfish which in turn produce all-male progenies. In species like *O. aureus*, where the male is homogametic (ZZ), feminization followed by an analysis of the sex ratio of the progenies from the females produced by single pair matings are sufficient steps to produce such individuals (Hammerman and Avtalion 1979; Jensen and Shelton 1979). In species like *O. niloticus*, where the male is heterogametic, an additional step is required to produce a new, viable and fertile YY genotype (Baroiller 1988b; Baroiller and Jalabert 1989; Scott et al. 1989; Varadaraj 1989).

However, in most of these intra- and interspecific studies, some sex ratios are incompatible with a simple monofactorial sex determination, XX/XY or ZZ/ZW depending on the tilapia species (reviews of Mair et al. 1991a, 1991b; Wohlfarth and Wedekind 1991). To explain these unexpected sex ratios, two other models were suggested: one suggests combining two alleles from an autosomal locus and two of the three sex chromosomes (Avtalion and Hammerman 1978; Hammerman and Avtalion 1979); the other suggests a polygenic model (Majumdar and McAndrew 1983; Mair et al. 1987). However, these two theories cannot explain all the results found in the literature (Avtalion and Don 1990; Wohlfarth and Wedekind 1991).

However, in several species of invertebrates (Bacci 1965; Charnov and Bull 1977) and lower vertebrates (Conover 1984; Adkins-Regan 1987; Dournon et al. 1990), environmental factors can determine the phenotypic sex, independently of the genotypic sex determined during fertilization. Temperature is the major factor in the environmental sex determination of a majority of lower vertebrates (Bull 1983). In fish, the use of various hormone treatments inducing functional inversions artificially has shown a high level of plasticity in gonadal differentiation (Yamamoto 1969; Hunter and Donaldson 1983). Moreover, environmental factors (social and thermal) are known to influence sex determination in hermaphrodites (Harrington 1967, 1968, 1971; Reinboth

1975; Bruslé and Bruslé 1983; Chan and Yeung 1983). It is only in the last ten years that thermodependent sex determination has been identified in a gonochoristic fish (Conover and Kynard 1981; Conover 1984; Conover and Fleisher 1986; Conover and Heins 1987a, 1987b). In species where heterogamety cannot simply account for the varied sex ratios observed, environmental factors could be involved (Conover and Fleisher 1986; Chourrout 1988). Sibling sex reversed males of *O. niloticus* with a majority of progenies showing unexpected sex ratios, based on a classic monofactorial model, were recently produced (Baroiller, this vol.). Studies on the effect of high rearing temperatures on the sex ratio were consequently conducted on this type of progeny.

Materials and Methods

Animals

Seven sex reversed males produced by hormone treatment using a family of fry of *O. niloticus* of the "Bouake strain" (Baroiller et al., in press) were identified by their all-female individual progeny. One of these sex reversed males was bred again and a sex reversal treatment was applied to its entire progeny (Baroiller, this vol.). Nine of the sex reversed males thus produced were selected for breeding and placed individually with normal females at a sex ratio of 4:1 in 400-l aquaria. Most of the sex reversed males originating from the same stock produced theoretically unexpected males (Baroiller, this vol.). One of these males (male D3) naturally originating from a sex reversed male was used as a broodfish in the present experiment. The water temperature was kept at 27°C using a thermoregulating device. Each animal was identified by a tag inserted in its dorsal musculature. Reproduction was detected by the onset of maternal mouthbrooding behavior (eggs then fry) which is characterized by the dilation of the female's mouth. As soon as this particular characteristic was observed, all other individuals were removed leaving the female to incubate in the aquarium. Five days after hatching, i.e., nine days post-fertilization (dPF), fry were removed from the mother's mouth. Each progeny, identified by its fertilization date and the tags on the parents, was divided into two to five equal batches of at least 100 individuals. Each batch was reared separately in 200-l aquaria. The water in the aquaria was filtered, aerated and thermoregulated. Batches for exposure to high temperatures were placed in small 0.5-l plastic containers containing an air stone. These containers were left afloat in the aquaria for a few hours for temperature adjustment before releasing the fry which remained 21 days in the thermoregulated aquarium. This period corresponds to the optimum treatment duration to produce all-male populations by hormonal sex reversal with a natural androgen, 11ß-hydroxyandrostenedione (11ß-OHΔ4) (Baroiller and Toguyeni, this vol.). Fry were fed ad libitum six times a day and seven days a week using first-feeding salmonid feed (Aqualim) distributed by an automatic feeder during the 12-hour photoperiod. Seventeen sex reversed male progenies were used in the present study. After treatment, batches of one month-old fry were stocked individually in external 1.5-m^3 tanks where they were fed ad libitum six times a day and six days a week until sexing at 60-90 dPF.

Identification of the Phenotypic Sex

At a minimal age of two to three months, when the histological characteristics of the male and female differentiation are already established (Baroiller 1988a, 1988b), all fry from each batch were

dissected and their gonads examined by simple squash, using a 125X microscope. The presence of previtellogenic or vitellogenic oocytes and the lobular configuration revealed the ovary and the testis, respectively, and consequently the phenotypic sex.

Results

The mean survival rate (S) at the time of sexing did not differ significantly between the control batches reared at temperatures of 26-29°C (S = 76.2% for 1,879 sexed fry) and the batches subjected to higher temperatures ranging from 30 to 36°C (S = 74.2% for 2,880 fry). These survivals produced batches of an average of 100 individuals at the time of sexing.

For three families of fry MXX9 x FXX10, MXX9 x FXX11 and MXX7 x FXX7, two control batches were reared at a mean temperature of 28°C (Tables 1 and 2). No significant difference was observed in the sex ratio among batches of the same family.

Seventeen families of fry, each divided into several batches, were reared at two temperatures: 20 batches served as controls (temperatures of 26-29°C); and 32 batches were exposed to higher rearing temperatures ranging from 30 to 36°C (Table 3). Compared to controls, 16 of these families showed different sex ratios in at least one of the experimental batches (Tables 1 and 2, Fig. 1). These deviations in sex ratios cannot be attributed to differential mortalities related to high temperatures. The low mortality rate observed in most batches does not sufficiently explain these large deviations. In the XX17 x XX11 cross especially, the seven and six deaths observed in the control batch and in the batch exposed to 36°C, respectively, cannot explain the difference of 91% of males between the two populations of 99 and 100 fry previously identified. The sex ratios of progenies from sex reversed males of *O. niloticus* were therefore directly influenced by the high rearing temperatures.

No significant difference in sex ratios was observed between the batches exposed to 31°C and the control batch (Table 3). Among the sex ratios of six batches exposed to 32-33°C, five of them were significantly biased towards the males compared to the percentages observed in the control batches exposed to 27°C (Table 3). Between 34 and 36°C, 20 batches out of 23 showed significant changes in the percentage of males as compared to their respective control batches. For temperatures ≥ 32°C, proportions of males in experimental populations increased from 0 to 91% as compared to the control sex ratios.

The intensity of response was neither directly proportional to the temperature applied, nor to the percentage of males produced at the control temperature. Progenies differed significantly in their sensitivity to temperature, both vis-à-vis an effective minimal temperature and in the extent of deviation in sex ratios in identical culture conditions. In experiments using the same temperature of 36°C, changing male sex ratios (14.1 to 91%) were observed depending on the progenies (Tables 1 and 2). Strong paternal (XX9 x XX16/XX18 x XX16) and maternal (XX17 x XX15/XX17 x XX11) influences were observed on the thermosensitivity of the progenies (Table 3). However, the proportion of males increased with the temperature in nearly all experimental batches.

Out of all progenies used, only one family showed no deviation in sex ratio regardless of the temperature used. In contrast to all other experiments, temperature treatments were applied to fry from the XX4 x XX5 cross only after 15 dPF instead of 9-13 dPF. This result probably reflects a critical period of thermosensitivity. Beyond certain stages of differentiation,

Table 1. Characteristics of *Oreochromis niloticus* fry reared at different temperatures: for details of parental crosses, see text.

Parental cross (M x F)	T°C (± range)	Initial age (dPF)	Survival (no.)	Survival (%)	Males (no.)	Control (%)	χ^2 (level of significance)[a]
XX x XX	27±2	13	141	47	36	25.5	***
	35±2	13	67	22	56	83.6	62.05
XX0 x XX0	26±2.5	10	152	76	34	22.4	
	34±1.5	10	125	62.5	107	85.6	109.74 ***
	36±1.5	10	23	11.5	20	86.9	39.1 ***
XX1 x XX1	27±2	9	96	90.6	1	1	
	31±1.5	9	78	73.6	0	0	0.011
	36±2	9	80	25	20	25	21.61 ***
XX4 x XX7	29±1	10	103	94.5	1	1	
	31±1	10	109	100	2	1.8	0.002
	35±1.5	10	105	96.3	13	12.4	9.04 **
	36±1.5	10	54	49.5	15	27.8	24.96 ***
XX4 x XX5	28±1	15	101	84.2	4	4	
	34±1	15	110	91.7	9	8.2	0.97
	35±1	15	98	81.7	5	5.1	0.002
XX4 x XX5	28±1.5	9	104	95.4	3	2.9	
	33±1	9	101	92.7	14	13.9	6.73 **
	36±1.5	9	67	61.5	38	56.7	61.86 ***
XX7 x XX7	28±1.5	11	90	90	34	37.8	
	28±1.5	11	89	89	29	32.6	
	35±2	11	88	88	54	61.4	16.4 **
XX9 x XX10	28±1.5	11	78	69.6	27	34.6	
	28±1.5	11	68	60.7	23	33.8	
	34±2	11	79	70.5	41	51.9	6.63 *
XX9 x XX11	28±1.5	10	104	97.2	10	9.6	
	28±1	10	107	100	10	9.3	
	34±1.5	10	98	94.2	30	30.6	20.5 ***
	35±1.5	10	93	89.4	47	50.5	63.3 ***
XX9 x XX12	27±2	10	97	94.2	2	2.1	
	33±1.5	10	80	77.7	10	12.5	5.99 *
	36±1.5	10	94	91.3	20	21.3	15.46 ***
XX9 x XX16	28±1.5	10	21	-	3	14.3	
	36±1.5	10	44	-	29	65.9	13.6 ***
XX11 x XX5	27±2.5	11	119	94.4	5	4.2	
	34±2	11	119	94.4	4	3.4	0.000
	36±1.5	11	104	82.5	19	18.3	10.02 **
XX17 x XX15	27±2	10	54	51.9	2	3.7	
	32±1.5	10	92	88.5	18	19.6	5.06 *
	36±2	10	81	77.9	39	48.1	28.2 ***
XX17 x XX11	28±1.5	10	99	93.4	0	0	
	32±1.5	10	106	100	8	7.5	5.89 *
	36±1.5	10	100	94.3	91	91	162.35 ***
XX17 x XX16	28±1.5	10	53	-	21	39.6	
	32±1.5	10	99	-	66	66.7	9.24 **
XX18 x XX16	28±1.5	12	101	95.3	4	4	
	31±1.5	12	101	95.3	0	0	2.29
	32±1.5	12	93	87.7	1	1.2	0.66
	35±1	12	105	99.1	2	1.9	0.214
	36±2	12	67	63.2	22	32.8	23.5 ***
XXD3 x XX17	28±1.5	10	102	86.4	8	7.84	
	34±1.5	10	118	100	71	60.2	62.83 ***
	34±1.5	10	102	86.4	66	64.7	68.89 ***

[a]*$p<0.05$; **$p<0.01$; ***$p<0.001$.

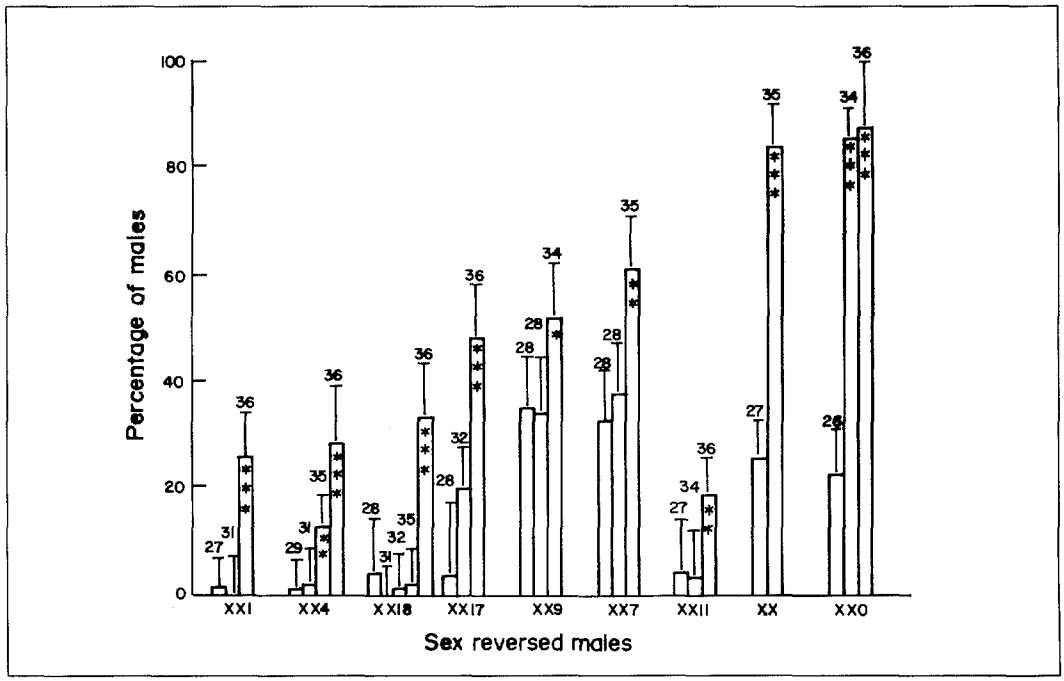

Fig. 1. Effects of temperature (27-36°C) on the percentages of males in the progeny of *Oreochromis niloticus* from various parental crosses: for details on parental crosses, see Table 1 and text; scale bars represent standard errors and asterisks represent levels of significant differences of the high temperature from lower temperatures in that group (*p<0.05; **p<0.01; ***p<0.001).

Table 2. Sex ratio responses in batches of *Oreochromis niloticus* fry reared at different temperatures.

Temperatures (°C)	No. batches tested	No. significant responses
31	3	0
32	4	3
33	2	2
34	7	6
35	6	4
36	10	10

Table 3. Parental effect on the sex ratios of *Oreochromis niloticus* fry reared at different temperatures: for details on parental crosses, see text.

Parental cross M x F	% Males		
	Control	at 36°C	Difference[a] (%)
XX4 x XX5	2.9	56.7	53.8
XX4 x XX7	1	27.8	26.8**
XX9 x XX12	2.1	21.3	19.2
XX9 x XX16	14.3	65.9	51.6***
XX17 x XX15	3.7	48.1	44.4
XX17 x XX11	0	91	91***
XX9 x XX16	14.3	65.9	51.6
XX18 x XX16	4	32.8	28.8**

[a]**p<0.01; ***p<0.001.

the gonadal sex may no longer be influenced by external factors such as temperature.

The progeny from the XX17 x XX11 cross, with a sex ratio corresponding to the expected sex ratio following a simple genetic model in a sex reversed male of *O. niloticus*, also showed a thermosensitive differentiation. Similarly, the progeny of an unexpected male (male D3) from a cross between a sex reversed male and a normal female also showed a thermosensitive differentiation.

Discussion

Very few studies have analyzed the environmental factors possibly involved in the sex determination of fish. Most studies have focused exclusively on hermaphrodite species. In these species, the influence of environmental factors on natural sex reversal has been demonstrated (reviews of Reinboth 1975; Chan and Yeung 1983). In *Rivulus marmoratus* especially, low temperatures produced primary males (Harrington 1967, 1968) and variations in the photoperiod resulted in the production of secondary males (Harrington 1971). In gonochoristic species, only the studies on *Menidia menidia* (Conover and Kynard 1981; Conover 1984; Conover and Fleisher 1986; Conover and Heins 1987a, 1987b) clearly demonstrated the effect of external factors. Based on samples taken from the wild, a species of the same genus, *M. peninsulae*, may also show thermosensitivity (Middaugh and Hemmer 1987). Studies on viviparous species such as *Poeciliopsis lucida* suggest an environmental influence on sex determination. However, the deviations observed could be due to the effects of temperature (Sullivan and Schultz 1986), or of pH (Rubin 1985) on the physiology of the maternal parent, and might not reflect a direct effect on the embryo. In the rainbow trout (*Oncorhynchus mykiss*), heat shocks of 25 or 29°C or longer exposures to 23°C do not significantly modify the sex ratio (Van den Hurk and Lambert 1982). In tilapias, potential thermosensitivity has been studied in three species. In *O. aureus*, *O. niloticus* and their hybrids, low survivals (≤ 26%) and the small average quantity of fry (23-28) produced in batches exposed to extreme temperatures (19.5 and 32°C) prevented differential mortality from being excluded as causal factor (Mair et al. 1990). In the same study, the sex ratios of two batches of *O. mossambicus* exposed to 19-20°C differed significantly from the sex ratios of their controls, with a bias towards the male sex. However, the survivals (10.6 and 35.3%) and the number of fry (18 and 41, respectively) from batches exposed to low temperature did not lead to obvious conclusions.

In view of the relatively high survivals and numbers of fry used in the present study, the hypothesis of a differential mortality cannot explain the deviations observed in the sex ratio of progenies from sex reversed males of *O. niloticus*. Temperature may therefore have an effect on sex determination in *O. niloticus*. The proportions of males within a single family are, independent of the rearing temperature (26-36°C), under high paternal and maternal influences. At 34°C and above, a clear environmental effect on the sex ratio shows thermosensitive differentiation in progenies from sex reversed males of *O. niloticus*. In *M. menidia*, the effect of temperature on the sex ratio is also under strong paternal influence; some progenies do not seem to be affected by the rearing temperatures; significant male and female percentages are observed at extreme temperatures (Conover and Heins 1987b). Sex determination in *M. menidia* is considered to be an intermediate step between an entirely genetic determination and an exclusively environmental determination (Conover and Heins 1987b). In reptiles showing environmental sex determination, an insignificant genetic base is found only in narrow ranges (sometimes only 2°C) around the temperature thresholds and only one sex is produced at extreme temperatures (Bull et al. 1982); sex determination in such situations thus depends entirely on the environment.

In *O. niloticus*, the environmental influence on sex determination can operate during a specific critical period of thermosensitivity: between 9 and 13 dPF. Beyond this range, sex differentiation is probably irreversible, at least vis-à-vis external factors, and would then correspond

to the genotype. A 21-day thermal treatment yields 91% maximum deviations if initiated before the 15th dPF. Additional experiments are, however, necessary to determine accurately the duration of the critical period. Histologically, two basic events in oogenesis occur between 20 and 35 dPF at 27°C: oogonia proliferate between 20 and 28, dPF followed by the first prophase between 28 to 35 dPF, or 756 to 945 degrees x days (Baroiller 1988a and b). During the same period, the highly progressive multiplication of the somatic and spermatogonia occurs in the testis (Baroiller 1988a and b). The chronology of these events depends on the rearing temperature and seems to be more accurately determined by a number of degrees x days than by an absolute age (this study). During a 21-day treatment beginning 10 dPF, high temperatures are applied before and at the onset of these events. In *M. menidia*, sensitivity to temperature also occurs during a precise critical period at the end of which gonadal sex differentiation takes place. The time of occurrence and the duration of this period depend upon the temperature (Conover and Kynard 1981; Conover and Heins 1987b). The same occurs in turtles (Yntema 1979; Pieau and Dorizzi 1981) and in alligators (Ferguson and Joanen 1982).

The chronological characteristics of this period as determined in the present study correspond, as in reptiles (Gutzke and Chymiy 1988) to the period of sensitivity to hormonal treatment in the same species (Baroiller and Toguyeni, this vol.). In *O. niloticus*, hormonal sex reversal treatments using an androgen identified in vitro and testis-specific at the early stages of its ontogenesis (Baroiller 1988a and b) can be used to determine a period of hormonal sensitivity: to be efficient, the treatment with 11ß-OHΔ4 should begin before 15 dPF (Baroiller and Toguyeni, this vol.) and should last 21 days. However, in thermosensitive turtle species such as *Emys orbicularis*, production of some steroids (estrogens), normally regulated via the genotype, would become thermodependent beyond certain critical temperature thresholds (Dorizzi et al. 1991). A thermosensitive factor may be involved in the regulation of the synthesis of enzymes that are specific to the estrogen production (Pieau et al. 1987). The level of these steroids would then determine the phenotypic sex. Such pattern may exist in most species with thermodependent sex determination (Zarborski et al. 1988).

Recent studies of *O. niloticus* show that this type of environmental sex determination is found in progenies from normal males and females (Baroiller et al., unpublished data).

In *O. niloticus*, sensitivity to temperature similar to that observed in *M. menidia* is described in the present study. The 34°C thermal threshold for thermosensitivity can be experienced by tilapias in the wild as well as in culture conditions (Denzer 1968; Philippart and Ruwet 1982). This characteristic in sex determination could explain part of the unexpected results described in the literature. Environmental sex determination could be far more common than initially believed (Conover 1984; Zaborski et al. 1988). In the platyfish (*Xiphophorus maculatus*) in which genetic determination is well-established, unexpected sex ratios have also been observed, including some within a population. Long and complex studies have shown autosomal interactions in species possessing several sex-specific markers (Kallman 1984). In view of these experimental difficulties, these analyses cannot be reasonably conducted on all species presenting unexplained sex ratios. The present study suggests that a potential thermosensitivity in the species studied should first be investigated before undertaking further studies.

Acknowledgements

We would like to thank D. Chourrout for reviewing the manuscript and for his valuable suggestions. This study was conducted under the Groupement de coopération scientifique sur les bases biologiques de l'aquaculture (GCS/BBA) by the "Aquaculture tropicale" study group.

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Cold Tolerance in Maternal Mouthbrooding Tilapias: Heritability Estimates and Correlated Growth Responses at Suboptimal Temperatures

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BEHRENDS, L.L., J.B. KINGSLEY and M.J. BULLS. 1996. Cold tolerance in maternal mouthbrooding tilapias: heritability estimates and correlated growth responses at suboptimal temperatures, p. 257-265. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Based on data derived from bidirectional selection for cold tolerance in tilapias, realized heritability estimates were calculated according to the ratio response:selection differential. After one generation of selection, estimates for up-selected populations (most cold tolerant) were: *Oreochromis aureus*, 0.33; *O. niloticus*, -0.05; and F_3 hybrid, 0.31. Estimates for two down-selected populations (least cold tolerant) were: *O. aureus*, 0.04; and *O. niloticus*, 0.42. Based on these initial estimates, it should be possible to increase cold tolerance in *O. aureus* and related hybrids via directed selection.

Following two generations of bidirectional selection, companion studies were undertaken to evaluate growth at low suboptimal temperatures. Fingerlings from up-selected populations grew significantly faster ($P<0.05$) than fingerlings from down-selected populations at temperatures ranging from 17.2 to 21.2°C. Under low temperature regimes, growth rate of *O. aureus* was greater than *O. niloticus*, irrespective of selection criteria ($P<0.05$).

Introduction

Rapid expansion of tilapia (*Oreochromis* spp.) culture into the warmwater aquaculture industry of the United States is hampered by their sensitivity to winter temperatures, and in certain instances, low market acceptability. Growth and reproduction of commercial strains are negligible at 20 and 22°C, respectively (Behrends and Smitherman 1983). Species specific mortality ensues after prolonged exposure to temperatures of 10 to 15°C (Shafland and Pestrak 1982; Wohlfarth and Hulata 1983; Behrends et al. 1990a).

Red-colored tilapias are often perceived as being more attractive than "normal-colored" tilapias and can command a premium price. However, many commercial strains of red tilapias grow slowly, suffer high mortalities (El Gamal et al. 1988), and are more sensitive to low temperatures than other commercial strains of normal-colored tilapias (Behrends et al. 1990a).

It may be possible to develop fast-growing, cold-tolerant hybrid strains of red tilapias via interspecific hybridization and introgression coupled with directed selection. This requires use of two breeding schemes: introgression of the red hereditary material into commercial strains of wild-type colored tilapias, and mass selection of improved cold tolerance within specific populations, or in segregating F_{2+} generations. Introgression of desirable red coloration

from otherwise unproductive strains into valuable commercial strains has been accomplished in the genus *Oreochromis* of which many species hybridize readily (Behrends and Smitherman 1984; Behrends et al. 1990b). However, improving cold tolerance in warmwater fishes via directed selection has not been previously investigated (Gjedrem 1983). It requires establishing selection criteria and adequate selection techniques.

Objectives of this research included: (1) documenting first-order estimates of realized heritability for cold tolerance in several distinct populations of tilapia and (2) evaluating growth of bidirectionally selected strains over a range of low suboptimal temperatures.

Materials and Methods

Fish Stocks

Populations tested included *Oreochromis aureus* (Israeli strain), *O. niloticus* (Egypt strain) and a red and normal-colored F_{2+} hybrid strain (red *aureus* females x red *niloticus* males). Fingerlings used in tests of cold tolerance, realized heritability and growth at suboptimal temperatures were derived from populations encompassing 10 to 40 spawns per population.

Red and normally pigmented hybrid strains of *O. aureus* and *O. niloticus* were produced over several generations via introgressive hybridization (Behrends et al. 1990a). This entailed, for example, producing F_1 hybrids by crossing *O. aureus* females with a red hybrid (predominantly *O. mossambicus*) and subsequently backcrossing red hybrid males to *O. aureus* females for multiple generations. The two red hybrid strains were subsequently crossed to combine the cold tolerance of *O. aureus* with the superior growth of *O. niloticus*.

Realized Heritability Estimates

Three control populations of tilapia (*O. aureus, O. niloticus* and a red F_2 hybrid strain), consisting of 600 fingerlings per population, were subjected to standardized long-term, cold-tolerance tests. Procedural details and phenotypic responses are summarized in a companion paper (Behrends et al. 1990a). Individual weights of fingerlings used in cold tolerance and selection trials ranged from 2 to 90 g. Fingerlings were challenged by slowly lowering water temperature from 16°C (acclimation temperature) to 5°C at a mean rate of $1°C \cdot day^{-1}$. Selection criteria were based on time (to the nearest hour) and temperature (to the nearest 0.1°C) at loss of response to stimuli due to cold narcosis. Mean cumulative degree hours (MCDH) to an unresponsive state were calculated for each population. MCDH is the sum total of degree hours below a set threshold (acclimation) temperature, e.g., 16°C. For example, a fish held for one hour at 14°C, would accumulate two degree hours if the threshold temperature was 16°C; if held an additional hour at 13°C, the sum would be (16-14)+(16-13), or five degree hours, etc.

Within populations, bidirectional selection was accomplished by selecting the most cold tolerant, i.e. those last to lose responsiveness to stimuli (upper 10%) and the least cold tolerant, i.e., those first to lose responsiveness (lower 10%). Fifty to sixty broodstock from each of the selected populations (up- and down-selected) were assortatively mated in replicate earthen ponds. Control populations were recreated in each generation by respawning the original parental lines. Resulting offspring, consisting of several thousands of each population, were cultured for approximately three months, harvested

in the fall and overwintered at 16°C. Two hundred randomly selected fingerlings from each population were subsequently cold-branded (Myers and Iwamoto 1986), stocked communally into replicate aquaria and subjected to a standardized cold-tolerance test.

A second generation of bidirectional selection was also completed during the spring of 1990. Selection was accomplished by retaining the upper 50% (100 most cold tolerant) and the lower 50% (100 least cold tolerant) of the selected fingerlings from the first generation selection trial (see previous paragraph). Second generation selects and their respective control populations were assortatively mated, fingerlings produced, overwintered, freeze-branded and subsequently subjected to a standardized long-term, cold-tolerance test.

Realized heritability estimates for the first and second generations of selection were calculated based on the ratio response: selection differential (Falconer 1981). Standard errors of the estimates were calculated for only the first generation of selection, due to the limiting assumptions of the formula (Prout 1962).

Correlated Responses: Growth at Suboptimal Temperatures

O. aureus and *O. niloticus* fingerlings used in this study were offspring from second generation select broodstock and appropriate controls. Fingerlings were mechanically graded by population to a mean weight of approximately 2 g to eliminate, as much as possible, differences in initial weight. Fingerlings were randomly allocated by group to aquaria and cultured for three contiguous 30-day trials at mean suboptimum temperatures of 17.2, 19.2 and 21.2°C, respectively. Each temperature-group treatment was replicated three times with 20 fingerlings per replicate.

All treatments received a salmon starter diet (50% crude protein) at 3% of body weight in equal morning and afternoon feedings. Feed rates were adjusted biweekly based on the average biomass of the fastest growing group. Water management was passthrough with a retention time of 5 minutes. Thus, with the exception of water temperature, water quality was near optimum for realized growth potential. Fingerlings were weighed individually to the nearest 0.1 g at two-week intervals.

Two-way analysis of variance and means separation tests were used to detect differences in growth rates and mean final weights (Barr et al. 1979). Main effects were species (*O. aureus* vs. *O. niloticus*) and selection criteria (up-selected vs. down-selected vs. control). Where warranted, covariance was used to adjust final mean weights by initial weights.

Results

Realized Heritability Estimates

Table 1 summarizes cold-tolerance means, standard deviations, coefficients of variation and selection differentials for three base populations and their respective bidirectionally selected subpopulations. Tables 2 and 3 summarize analogous data for progeny from select and control populations for generations I and II, respectively.

Based on a single generation of bidirectional selection, realized heritability estimates were calculated according to the ratio response: selection differential. The ratio can range from -1.0 to 1.0. Theoretically, $0 \leq h^2 \leq 1$, but there is no absolute limit for R/S: $-\infty < R/S < +\infty$ depending on experimental errors.

The estimates and respective standard errors, based on cumulative degree

Table 1. Cold-tolerance parameters for three populations of tilapia (*Oreochromis* spp.) and selected subpopulations subjected to temperature declines of 1°C·day^{-1}. Figures in parentheses indicate percentates.

Population	n	Mean lower[a] critical temperature (°C)			Cumulative degree hours (base 15°C)			Selection differential
		$\bar{x}$	SD	CV	$\bar{x}$	SD	CV	
O. aureus								
Base	594	6.8	0.44	6.5	739	72.2	9.8	-
Up-selected (10)	60	6.5	0.14	2.1	844	13.8	1.6	+105
Down-selected (10)	56	7.6	0.52	6.8	593	72.3	12.2	-146
O. niloticus								
Base	584	7.9	0.56	7.0	540	69.0	12.8	-
Up-selected (10)	60	7.4	0.12	1.6	644	19.6	3.0	+104
Down-selected (10)	60	8.8	0.40	4.5	415	43.1	10.3	-125
Red hybrid[b]								
Base	603	7.8	0.71	9.1	552	97.3	17.6	-
Up-selected (10)	60	7.0	0.46	6.6	698	19.6	2.8	+146
Down-selected (10)	60	9.0	0.69	7.7	366	68.9	18.8	-186

[a]Temperature at which fish lose responsiveness to stimuli due to cold narcosis.
[b]F_2 hybrid derived from cross (red *O. aureus* x red *O. niloticus*).

Table 2. Cold-tolerance parameters for progeny from selected and control populations of tilapia (*Oreochromis* spp.).

Population	n	Mean lower[a] critical temperature (°C)			Cumulative degree hours (base 15°C)			Selection response
		$\bar{x}$	SD	CV	$\bar{x}$	SD	CV	
O. aureus								
Control	204	6.4	0.49	7.6	853.5	75.22	8.8	-
Up-selected	189	6.2	0.59	9.3	888.7	85.48	9.6	+35.2
Down-selected	208	6.4	0.72	11.3	847.6	114.99	13.6	-5.9
O. niloticus								
Control	198	7.1	0.64	9.1	715.1	91.39	12.8	-
Up-selected	209	7.1	0.52	7.2	709.5	74.31	10.5	-5.6
Down-selected	196	7.3	0.60	8.1	662.2	93.22	14.1	-52.9
F_3 hybrid[b]								
Control	148	7.2	0.56	7.8	699.5	77.99	11.1	-
Up-selected	172	7.0	0.67	9.6	745.1	82.45	11.1	+45.6
Down-selected[c]	-	-	-	-	-	-	-	-

[a]Temperature at which fish lose responsiveness to stimuli due to cold narcosis.
[b](*O. aureus* x *O. niloticus*).
[c]Down-selects lost to oxygen depletion in nursery pond.

Table 3. Cold-tolerance parameters (means±SD) for progeny of three populations of tilapia (*Oreochromis* spp.) after two generations of selection.[a] Means within columns followed by different letters are significantly different (P<0.05).

Population	n	Mean lower[b] critical temperature (°C)			Cumulative degree hours (base 15°C)		
		$\bar{x}$	SD	CV	$\bar{x}$	SD	CV
O. aureus							
Up-selected	53	6.6a	0.63	9.5	1,043a	122.7	11.8
Control	44	6.4a	0.63	9.8	1,089a	118.2	10.9
Down-selected	42	6.4a	0.57	8.9	1,073a	113.9	10.6
O. niloticus							
Up-selected	46	7.2a	0.40	5.5	920a	74.6	8.1
Control	44	7.2a	0.45	6.2	906a	79.0	8.7
Down-selected	50	7.4b	0.61	8.2	853b	98.1	11.5
Red hybrid (F-4)[c]							
Up-selected	51	7.2a	0.96	13.4	931a	148.0	15.9
Control	50	7.1a	0.57	8.0	927a	102.8	11.1
Down-selected[d]	49	7.3a	0.51	7.0	870b	85.3	9.8

[a]Intensity of selection in Generation I is equal to 60/600; Generation II 100/200.
[b]Temperature decreased 1°C·day^{-1} from 16°C to 5°C.
[c]F_4 hybrid derived from cross (red *O. aureus* x red *O. niloticus*).
[d]Down-selects derived from lower 50% of the previous up-selected generation.

hours, are summarized in Table 4. Estimates for up-selected populations (most cold tolerant) were: *O. aureus*, 0.33; *O. niloticus*, -0.05; and F_3 red hybrid, 0.31. Estimates for two down-selected populations were: *O. aureus*, 0.04; and *O. niloticus*, 0.42.

Second generation selection results (Table 3) revealed that up-selection did not lead to further improvements in cold tolerance in any of the selected populations (P>0.05). However, significant responses were still evident in down-selected lines of *O. niloticus* and the interspecific hybrid.

These first-ever heritability estimates for cold tolerance in tilapias indicate that at relatively high intensities of individual selection, statistically significant responses can be obtained. However, the magnitude and direction of the responses appear to be species- or strain-specific. For instance, results indicate that selection may be useful for improving cold tolerance in *O. aureus* and its hybrids, but not in *O. niloticus*. Conversely, relatively cold-intolerant strains of *O. niloticus* and the interspecific hybrid may be developed via down-selection, but not cold-intolerant strains of *O. aureus*.

Correlated Responses: Growth at Suboptimal Temperatures

At rearing temperatures of 17.2°C, absolute growth rates (g·individual^{-1}·month^{-1}) of selected subpopulations of *O. aureus* and *O. niloticus* were minimal, ranging from -0.04 to 0.29 g·individual^{-1}·month^{-1} (Table 5). Although there was little divergence of growth rate among selected subpopulations at this temperature, it was apparent that growth of *O. aureus*, irrespective of selection criteria, was superior to *O.*

Table 4. Response to bidirectional selection for cold tolerance in tilapias (*Oreochromis* spp.). Parameter measured was cumulative degree hours below acclimation temperatures (15-16°C). Standard errors calculated according to Prout (1962).[a]

	Generation I				Generation II[d]		
Population	Selection differential	Response	Realized heritability (R/S)	Standard error (population mean)	Selection differential	Response	Realized heritability (R/S)
O. aureus							
Up-selected	+105	+35.2	0.33[c]	0.004	+46	-46.0	-1.00
Down-selected	-146	-5.9	0.04	0.007	-59	-16.0	0.27
O. niloticus							
Up-selected	+104	-5.6	-0.05[c]	0.016	+37	13.0	0.35
Down-selected	-125	-52.9	0.42	0.004	-72	-53.0	0.74
$F_{3,4}$ hybrid							
Up-selected	+146	+45.6	0.31[c]	0.002	+54	4.0	0.08
Down-selected	-186	-[b]	-	-	-54.3	-56.8	1.05

[a]Standard error formula only applicable for first generation of selection.
[b]100% mortality in fingerling production phase.
[c]Significant response to selection.
[d]Down-selects in Generation II derived from lower 50% of the previous up-selected population.

Table 5. Growth rate data for selected[a] populations of tilapia (*Oreochromis* spp.) cultured at suboptimal temperatures. Each mean based on three replicates of 20 fish each. Growth trials at each temperature regime were conducted for 30 days.

	17.2°C			19.2°C			21.2°C		
Population	Initial weight (g)	Final weight (g)	Difference[b] (g)	Initial weight (g)	Final weight (g)	Difference (g)	Initial weight (g)	Final weight (g)	Difference (g)
O. aureus									
Up-selected	3.11	3.30	0.19a	3.30	3.65	0.35a	3.65	4.71	1.06a
Control	2.62	2.91	0.29a	2.91	3.17	0.26a	3.17	3.94	0.77b
Down-selected	3.04	3.22	0.18a	3.22	3.40	0.18a	3.40	4.15	0.75b
O. niloticus									
Up-selected	2.99	2.95	-0.04a	2.95	3.12	0.17a	3.12	4.03	0.91a
Control	3.08	3.17	0.09b	3.09	3.21	0.12a	3.23	3.72	0.49b
Down-selected	2.99	3.09	0.10b	3.09	3.15	0.06a	3.20	3.77	0.57b

[a]Bidirectional selection for cold tolerance applied for two generations (see text for details).
[b]Means followed by a different letter are significantly different ($P<0.05$); vertical comparisons within species only.

niloticus. Similarly, *O. aureus* outperformed *O. niloticus* at 19.2 and 21.2°C. This is in contrast to growth performance at higher temperatures (26 to 32°C), where *O. niloticus* generally grows faster than *O. aureus*.

Divergence of growth rates among selected populations within species was weakly manifested at 19.2°C ($P>0.05$), but readily apparent ($P<0.05$) at 21.2°C (Table 5). Results indicate that directional selection for cold tolerance in two species of tilapia may have significantly influenced absolute growth rates at suboptimal temperatures ($P<0.05$). However, in several instances the results were unexpected. While there was no significant response to up-selection after two generations in *O. niloticus* (Table 4), there was a noticeable improvement in growth rate of the up-selected population at suboptimal growth temperatures (Table 5). Conversely, two generations of downward selection for cold tolerance resulted

in a subpopulation of *O. niloticus* that was significantly less cold-tolerant than its respective control (Table 4). Despite this fact, growth rate at suboptimal temperatures was not significantly diminished by downward selection (Table 5).

Discussion

Intraspecific differences in cold tolerance among geographically distinct strains have been reported for *O. niloticus* (Khater 1985). The most northerly distributed strain (Egypt 31°N) was significantly more cold-tolerant than strains from Côte d'Ivoire (10°N) or Ghana (6°N). Intraspecific hybridization and backcrossing among the Egypt and Côte d'Ivoire strains revealed no significant heterosis (Tave et al. 1990), indicating that cold tolerance in those populations is not controlled by dominant genetic variance. Furthermore, clinal variation among strains within a species (Khater 1985) indicates: (1) an intraspecific component of additive genetic variance and (2) that natural selection has probably operated to extend the range of certain *Oreochromis* species. Statistically significant heritability estimates for cold tolerance in tilapias (Table 4) provide further experimental evidence that cold tolerance is controlled by additive genes. Therefore, progress in either direction, upward or downward, should be possible at prescribed intensities of selection. The erratic and asymmetrical responses observed within and among species and hybrids during two generations of selection (Table 4) have many potential causes including: random drift, experimental error, selection differential, inbreeding depression, maternal effects, genetic asymmetry, genes with large effects, scalar asymmetry and indirect selection (see Falconer 1981).

Responses to selection in the first generation were attained at relatively high levels of selection intensity, while responses in the second generation were achieved at much reduced levels of selection intensity. Unfortunately, positive gains made in cold tolerance in up-selected populations of *O. aureus* and its hybrid during the first generation of selection were lost during the second generation of selection. In contrast, rapid gains in down-selected populations were achieved in *O. niloticus*, irrespective of selection intensity. Such results may indicate that cold tolerance is near a selection limit in these populations. Lack of response to up-selection in the second generation may have resulted from a combination of factors including: sampling error, selection plateau, misidentification of freeze brands and/or a relaxing of the intensity of selection. It should be noted that the selection differential in the first generation was based on the upper and lower 10% of a base population consisting of 600 individuals. However, selection differential in the second generation of selection was based on the upper and lower 50% of 200 individuals/population. Thus the intensity of selection in the second generation was significantly less than that practiced in the first generation.

It should be noted that many of the causes for lack of response and asymmetry of response are exacerbated by small founding populations. In the US, certain tilapia populations were founded as early as 1957 with as few as five broodstock (R.O. Smitherman, Auburn University, pers. comm.). Also many of the US populations have experienced multiple and severe bottlenecks, further reducing genetic variance. Despite limited phenotypic variance among several tested populations (Behrends et al. 1990a), it

was still possible to elicit selection responses.

The positive correlated response between cold tolerance and growth at suboptimal temperatures needs to be confirmed by further studies. Although strains up-selected for cold tolerance grew faster than control and down-selected populations at suboptimal temperatures, the results in certain instances were confounded by differences in initial starting weights. Controlling differences in initial weight among test strains either by statistical blocking or mechanical grading is important since even small differences in initial weight can mask genetically mediated differences in growth rate.

Correlated responses to selection revealed that it may be possible to improve growth at suboptimal temperatures by selecting for cold tolerance. From a practical standpoint, this may provide a marginal increase in the growing season for temperate climates and allow overwintering at lower temperatures. From a technical standpoint, such a response indicates that indirect selection for cold tolerance may be possible by selecting for growth at suboptimal temperatures. From a logistical and technical standpoint, this has important implications. Growth at suboptimal temperatures is an indirect measure of cold tolerance and is much easier to measure than standardized cold-tolerance tests. Measurements of growth can also be monitored over longer periods of time, with greater objectivity and precision. A combination of direct and indirect selection could conceivably result in rapid gains.

In conclusion, these studies have revealed several important points that should be understood by others undertaking similar studies: (1) cold tolerance is a malleable physiological trait apparently controlled by additive genetic variance; (2) phenotypic expression is greatly influenced by acclimation history; and (3) within populations, genotypic and phenotypic variances are relatively small. Cold-intolerant strains of tilapia may be desirable for culture in states or regions where there are restrictions against importation of relatively cold-tolerant strains (Kingsley 1987).

Acknowledgements

This research was supported, in part, by a grant from the US-Israel Binational Agricultural Development Fund (BARD US-1062-86). We are grateful to Ms. Linda Tackett and Mr. Mike Montgomery (TVA Agricultural Institute) for their assistance in typing and editing this manuscript.

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Mitochondrial DNA Restriction Endonuclease and Isozyme Analyses of Three Strains of *Oreochromis niloticus*

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CAPILI, J.B. and D.O.F. SKIBINSKI. 1996. Mitochondrial DNA restriction endonuclease and isozyme analyses of three strains of *Oreochromis niloticus*, p. 266-272. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Restriction endonuclease analysis of mitochondrial DNA (mtDNA) and isozyme analysis were used to characterize samples of three populations of *Oreochromis niloticus*: *O.n. niloticus* (Lake Manzala, Egypt), *O.n. baringoensis* (Lake Baringo, Kenya) and a "selected" strain of *O. niloticus* derived from hybridization of different strains of *O. niloticus* originating from Lakes Turkana, Victoria, Baringo and Manzala. Samples of *O. aureus* and *O. mossambicus* were included for comparison. mtDNA was examined with 14 six-base restriction endonucleases using ethidium bromide-staining of 1.0% agarose gel to study fragment patterns. Of the 10 restriction endonucleases that cleaved mtDNA, only one (*Dra*I) could discriminate among the three *O. niloticus* populations. Species-specificity of restriction endonucleases was shown by *Bgl*I, *Eco*RV and *Pvu*II.

Isozyme analysis was carried out at 10 enzyme loci that were known to be polymorphic in some *O. niloticus* populations. Results showed introgression of *O. aureus* genes in the "selected" strain of *O. niloticus* and in *O.n. niloticus*. Limitations of the two molecular techniques used in this study are discussed.

Introduction

Tilapias are of great importance in the tropics and subtropics as a cheap source of animal protein. There has been wide distribution of tilapia species beyond their native range, especially for *Oreochromis niloticus* (Welcomme 1981). In the Philippines, there are four strains of *O. niloticus* that are presently used for aquaculture. The consequences and implications of introductions of tilapia to Asia were extensively discussed by Pullin and Capili (1988).

The possibility of marking these strains and other stocks of *O. niloticus* from hatcheries, genetically and biologically, has been explored by means of electrophoresis (Macaranas et al. 1986 and unpubl. data). They revealed the introgression of *O. mossambicus* into different stocks of *O. niloticus*. Strain-specific markers, however, have not yet been identified. In parallel with this effort, the use of morphometric and meristic characters was tried by Pante et al. (1988) using canonical discriminant analysis to find indices that could be used by culturists and field biologists. This

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technique can only discriminate species such as *O. niloticus* and *O. mossambicus* and not strains or closely related hybrids.

Although allelic data can sometimes discriminate among stocks, there are many instances in which electrophoresis fails to identify genetically discrete stocks. Such failures could be partially due to the insensitivity of the technique which reflects differences at the DNA level (Ferris and Berg 1987).

It has been recognized that restriction endonuclease analysis of mtDNA is an attractive method for quantifying genetic differences among fish populations. The use of this method with tilapias from East Africa has been reported by Seyoum and Kornfield (1992). Their success in identifying subspecies of *O. niloticus* prompted the present study, which aims to identify specific diagnostic loci and mtDNA markers in three populations of *O. niloticus* which are thought to be highly inbred, but are widely used in genetic and aquaculture research on tilapia. The identification of such markers would be of value in monitoring contamination and hybridization among these and other strains and species of tilapia. It was also the aim of this study to evaluate the use of rapid and simple technique of phenol extraction of mtDNA and visualization of fragments on ethidium bromide-stained agarose gels for use under laboratory conditions in the Philippines.

Materials and Methods

The three populations of *O. niloticus* used in this study were: *O.n. niloticus*, origin Lake Manzala, Egypt; *O.n. baringoensis*, origin Lake Baringo, Kenya; and a "selected" strain of *O. niloticus* derived from hybridization of different strains of *O. niloticus* from Lakes Turkana, Victoria, Baringo and Manzala. Fingerlings from two families of *O.n. baringoensis* and three families each of *O.n. niloticus* and the "selected" strain of *O. niloticus* were reared for four months in a recirculating water tank system. Stocks of *O. aureus* and *O. mossambicus* were originally obtained from Kenya and Zimbabwe, respectively.

Liver and muscle tissues were used for isozyme analysis. The enzyme loci *Adh*, *Ck-3*, *Gpi-1*, *Ga-3-pdh*, *Sdh*, *Sod*, *Mdh-1*, *Mp-3* and *Mp-7* that had been observed to be polymorphic by Taniguchi et al. (1985) and Macaranas et al. (1986) in *O. niloticus* were electrophoretically analyzed in this study. *Ada* which was found to be polymorphic by McAndrew and Majumdar (1983) in *Sarotherodon* and *Oreochromis* species was also included in the analysis. Isozyme methods were based on the work of Taniguchi et al. (1985) and Macaranas et al. (1986).

Mature ovarian tissue or liver was isolated from four to six individuals from each family for mtDNA extraction. The methods of mtDNA purification are based closely on those described by Fisher and Skibinski (1990). Mitochondria were prepared by differential centrifugation from tissue homogenized with a polytron mechanical homogenizer. A phenol/chloroform extraction procedure incorporating a CTAB step was used to purify mtDNA.

mtDNA digestion was carried out using the conditions recommended by the supplier (Bethesda Research Laboratories, Harthersburg, Maryland).

Restriction endonucleases used in this study were: *Apa*I, *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, *Nde*I, *Pst*I, *Pvu*II and *Xba*I.

Restriction fragments were separated by horizontal electrophoresis in a 1.0% agarose gel which was then stained with ethidium bromide. Tris acetate buffer

(0.4 M Tris Base; 0.2 M Na acetate; 0.02 M EDTA; 0.18 M NaCl; and pH 8.05) was used for gel and electrode buffers. *Hind*III-digest of Lambda DNA was used as the molecular weight standard on all gels. Electrophoresis was carried out for four to five hours with current of 75 mA. Gels were illuminated with short wavelength UV light (260 nm) and photographed through a Polaroid MP4 camera.

Results

Isozyme Analysis

In Table 1, alleles are designated with capital letters and the fastest migrating allele is designated as A. Allelic expressions for *Adh*, *Mdh-1* and *Ada* loci that were found polymorphic in the three populations of *O. niloticus* are shown in Fig. 1. The banding pattern for the reference *O. niloticus* strain was obtained from Macaranas et al. (1986). Tissue samples from *O. aureus* and *O. mossambicus* were run side by side with the samples of *O. niloticus* to see clearly the allelic expression differences among species of *Oreochromis*. Although the number of samples from *O. aureus* and *O. mossambicus* were very small, the gene frequencies obtained from this study and the work of Macaranas et al. (unpubl. data) were similar. Gene frequencies for the seven protein loci observed in the three populations of *O. niloticus*, *O. aureus* and *O. mossambicus*, and the results from Macaranas et al. (1986) are shown in Table 2.

Restriction Endonuclease Analysis of mtDNA

Of the 14 restriction endonucleases used, four did not cleave the mtDNA genome (*Apa*I, *Bste*I, *BamH*I and *Bgl*II). In the work of Seyoum and Kornfield (1992), *Apa*I-digested mtDNA yielded fragments which discriminated subspecies of *O. niloticus*. It is possible that in this study, impurities remaining in mtDNA preparations inhibited this enzyme.

mtDNA fragment patterns yielded by digestion with *Dra*I, *Bgl*I, *EcoRV* and *Pvu*II are shown in Fig. 2. Variation among the three families of the "selected" strain of *O. niloticus* was observed in the fragment patterns of mtDNA digested with *Dra*I. Families 1 and 2 of the "selected" strain share the same three restriction fragments with all the three families of *O.n. niloticus*, whereas Family 3 shares the same fragment patterns with the two families of *O.n. baringoensis*.

Table 1. List of enzymes and proteins investigated in strains of *Oreochromis niloticus*, *O. aureus* and *O. mossambicus*.

Enzymes and proteins	EC no.	Structure	Locus	Allele	Tissue
Alcohol dehydrogenase (ADH)	1.1.1.1	dimer	*Adh*	A, B	liver
Creatine kinase (CK)	2.7.3.2	monomer	*Ck-3*	A, B	muscle
Glucose phosphase isomerase (GPI)	5.3.1.9	dimer	*Gpi-1*	A, B	muscle
Glyceraldehyde-3 phosphate dehydrogenase (GA-3PDH)	1.2.1.12	dimer	*Ga-3-pdh*	A, B	muscle
Sorbitol dehydrogenase (SDH)	1.1.1.14	dimer	*Sdh*	A, B	liver
Superoxide dismutase (SOD)	1.15.1.1	dimer	*Sod*	A, B	liver
Malate dehydrogenase (MDH)	1.1.1.37	dimer	*Mdh-1*	A, B	muscle
Adenosine deaminase (ADA)	3.5.4.4	monomer	*Ada*	A-F	muscle
Muscle protein (MP)		monomer	*Mp-3*	A, B	muscle
			Mp-7	A-C	muscle

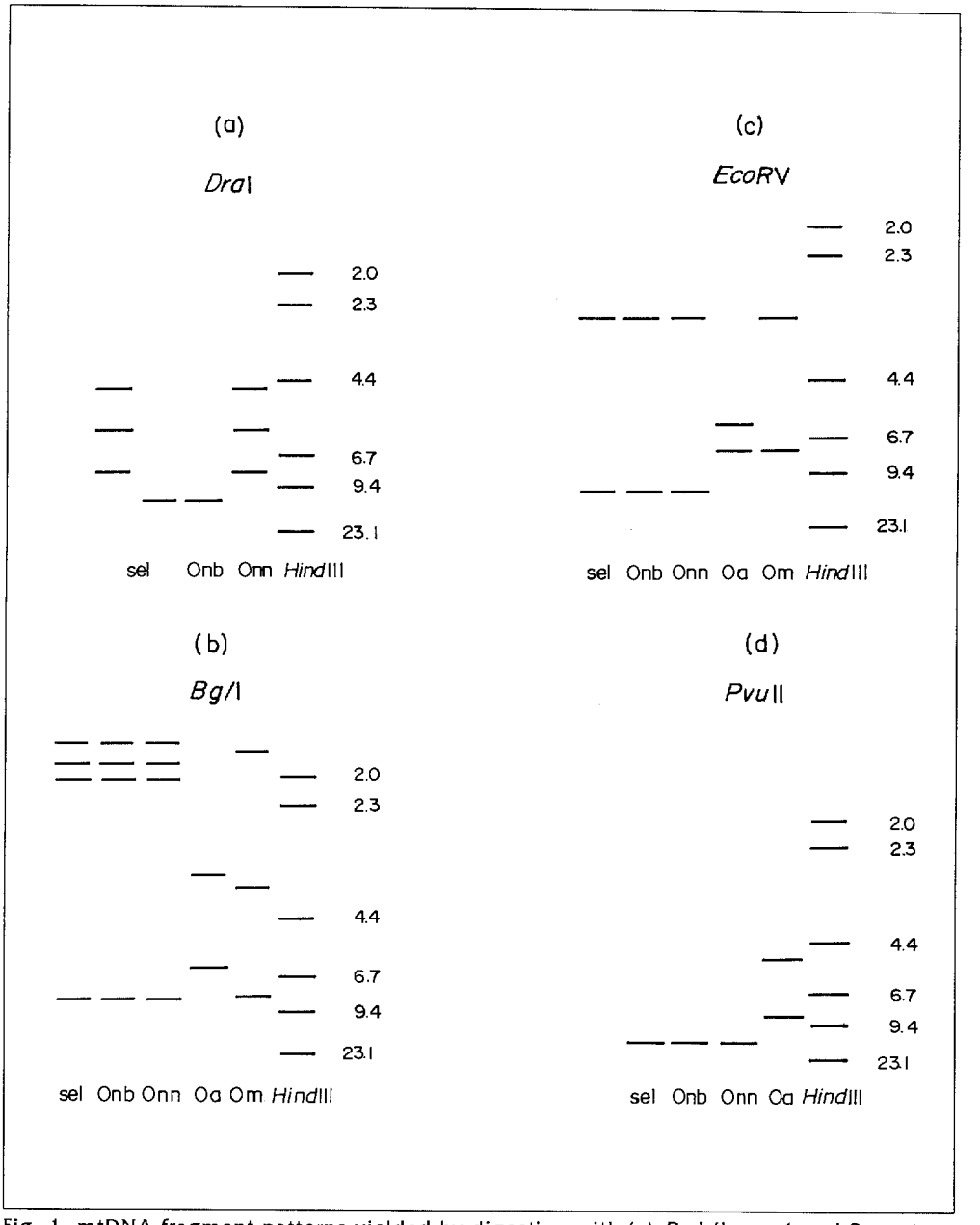

Fig. 1. mtDNA fragment patterns yielded by digestion with (a) *Dra*I (lanes 1 and 2 are two fragment patterns from three families of the "selected" strain [sel]; lane 3 is the fragment pattern of *O.n. baringoensis* [Onb]; lane 4 is the fragment pattern of *O.n. niloticus* [Onn]; and lane 5 is the standard which is a *Hind*III-digest of Lambda DNA); (b) *Bgl*I and (c) *EcoRV* (lanes 1 to 3 are the fragment patterns for all families from "selected" strain [sel], *O.n. baringoensis* [Onb] and *O.n. niloticus* [Onn]; lanes 4 and 5 are fragment patterns of *O. aureus* [Oa] and *O. mossambicus* [Om], respectively; and lane 6 is a standard *Hind*III-digest of Lambda DNA); and (d) *Pvu*II (lanes 1 to 3 are the fragment patterns for all families from "selected" strain [sel], *O.n. baringoensis* [Onb] and *O.n. niloticus* [Onn]; lane 4 is the fragment pattern of *O. aureus* [Oa]; and lane 5 is the standard which is a *Hind*III-digest of Lambda DNA).

Table 2. Gene frequencies at seven protein loci in *Oreochromis* spp.

Locus	Allele	*O. niloticus*				*O. aureus*	*O. mossambicus*
		Reference of Macaranas et al. (1986)	"Selected" strain (27)	*O.n. baringoensis* (12)	*O.n. niloticus* (24)	(3)	(1)
Adh	A	0	0.042	0	0.426	1.000	0
	B	1.000	0.958	1.000	0.574	0	1.000
Ck-3	A	0	1.000	1.000	1.000	1.000	0
	B	1.000	0	0	0	0	1.00
Ga-3-pdh	A	1.000	1.000	1.000	1.000	1.000	0
	B	0	0	0	0	0	1.000
Gpi-1	A	0	0	0	0	0	1.000
	B	1.000	1.000	1.000	1.000	1.000	0
Sod	A	1.000	1.000	1.000	1.000	1.000	0
	B	0	0	0	0	0	1.000
Mp-3	A	1.000	1.000	1.000	1.000	1.000	0
	B	0	0	0	0	0	1.000
Ada	A	-	0.020	0	-	-	-
	B	-	0.140	0.265	-	-	-
	C	-	0.240	0.647	-	-	-
	D	-	0.300	0	-	-	-
	E	-	0.300	0.044	-	-	-
	F	-	0	0.044	-	-	-

Fragment patterns of mtDNA digested with *Bgl*I show no variation among families and among the three populations of *O. niloticus*. There were four restriction fragments observed for this species, whereas in the other reference species, two restriction fragments were observed in *O. aureus* and three in *O. mossambicus*. The total summed approximate sizes of restriction fragments for *O. niloticus* populations, *O. aureus* and *O. mossambicus* were 13.8 kb, 9.4 kb and 13.0 kb, respectively. The total fragment size of *O. aureus* was very small as compared to the fragment sizes of *O. niloticus* and *O. mossambicus*. Kornfield and Bogdanowicz (1987) have the same observation when they used ethidium bromide-staining in their attempt to differentiate mtDNA in Atlantic herring *(Clupea harengus)*. This could likely be due to the presence of small fragments which could not be visualized on the agarose gel stained with ethidium bromide. Another possible explanation is that some bands reflected doublets, that is, two co-migrating fragments of identical sizes.

Restriction fragments yielded by *Eco*RV and *Pvu*II digests show no variation among families and populations of *O. niloticus*. However, variation was observed among the different species of *Oreochromis*.

Other restriction endonucleases *Bcl*I, *Eco*RV, *Hind*III, *Nde*I, *Pst*I and *Xba*I yielded invariant patterns across all species.

Discussion

In this study, protein electrophoresis could not easily discriminate among populations of *O. niloticus*. However, differences among three species of *Oreochromis* were observed from the gene frequencies obtained from six enzyme loci (*Adh*, *Ck-3*, *Ga-3-pdh*, *Gpi-1*, *Sod* and *Mp-3*). Most of the enzyme banding patterns were identical for *O. niloticus* and *O. aureus*, but different between these two species and *O. mossambicus*,

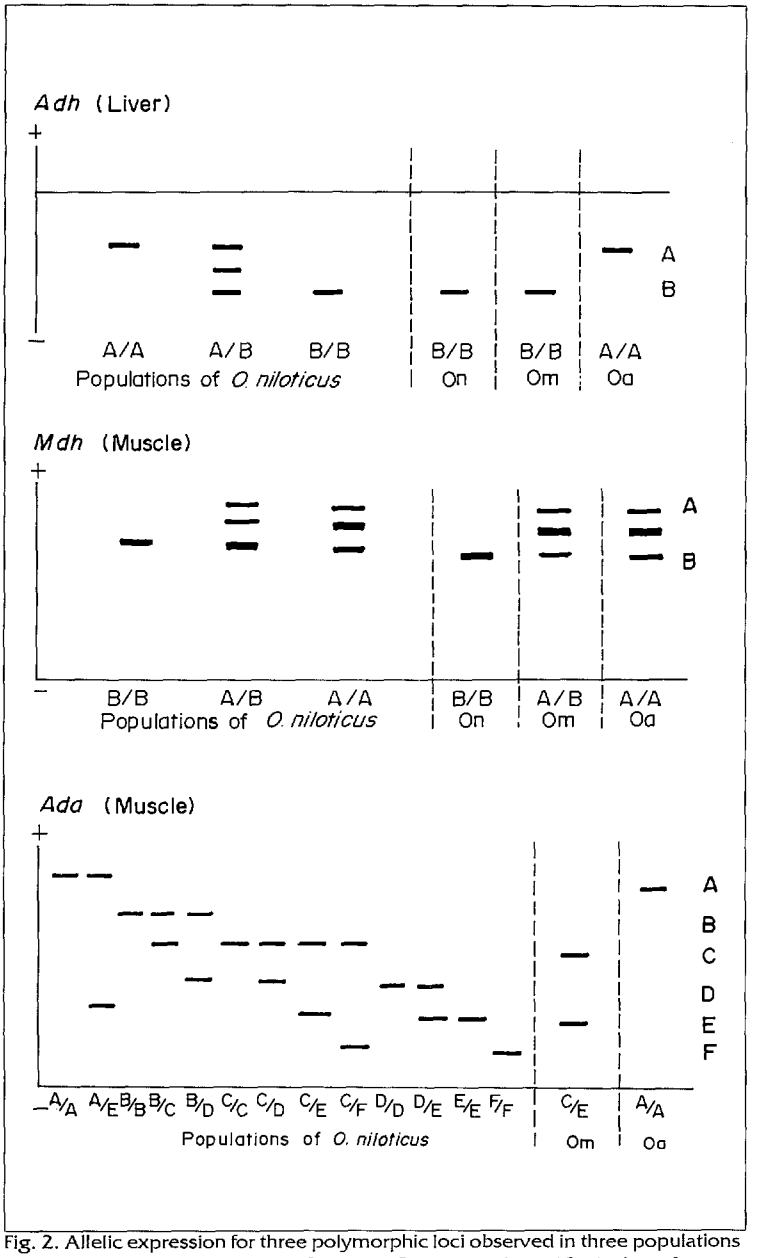

Fig. 2. Allelic expression for three polymorphic loci observed in three populations of *O. niloticus* (On); *O. aureus* (Oa); and *O. mossambicus* (Om); the reference *O. niloticus* (On) strain obtained from the work of Macaranas et al. (1986) is included in the diagram. Note that for the *Ada* locus, allelic expression for the reference *O. niloticus* strain of Macaranas et al. (1986) is not available.

except for *Adh*, where *O. niloticus* and *O. mossambicus* share the same pattern (allele A) and *O. aureus* has allele B (Table 2). Thus, the *O.n. niloticus* and the "selected" strain of *O. niloticus* studied here are polymorphic at the *Adh* locus. This suggests the possibility of introgression of *O. aureus* genes into these two populations of *O. niloticus*.

Restriction fragment patterns of mtDNA cleaved with *BglI* and *EcoRV* were observed to be different among species of *Oreochromis*. There was no evidence of introgression of mtDNA which contrasts with the results for *Adh*. This points to a potential limitation of mtDNA analysis in stock identification and monitoring. The technique cannot, by its nature, be informative about nuclear contamination. The results for *DraI* provide evidence of mtDNA variation within *O. niloticus*. The families of the selected strain share restriction patterns possessed by the two other populations, *O.n. baringoensis* and *O.n. niloticus*. With data on only a limited number of families, it is difficult to draw conclusions about the ancestry or phylogenetic relationships between these populations; however, it is of interest that the selected strain, which may have a heterogeneous ancestry, possesses two different *DraI* genotypes.

This study shows that mtDNA genotypes can be found for genetically

marking different populations within a species or even different strains within a family, as with the selected strain in this study. Thus, mtDNA techniques may have some uses in aquaculture. Because of the mode of inheritance of mtDNA, it is likely that families will be fixed for specific mtDNA genotypes in circumstances in which isozyme variation is still segregating. Thus, it may be easier to develop mtDNA than isozyme markers within strains.

In summary, results obtained in this study showed that phenol extraction of mtDNA and visualization of restriction fragments on an ethidium bromide-stained agarose gel is feasible in these tilapia species. Although only a few restriction endonucleases cleaved the mtDNA, this gave informative results and diagnostic mtDNA markers were identified among species of *Oreochromis* and among populations of *O. niloticus*. Further investigations using similar techniques and more restriction endonucleases should be tried.

Acknowledgements

We thank C. Fisher, M. O'Connell and C. Hurst for their valuable technical advice; E. Roderick, S. McConnell, C. Gilling, C. Pongsri and T. Abella for their assistance in the collection of fish samples; and the Overseas Development Administration for the research grant to Prof. J. Beardmore and Drs. D.O.F. Skibinski and G.C. Mair. The British Council is gratefully acknowledged for a scholarship grant to J.B. Capili.

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Triploidy Induced by Heat Shock in *Oreochromis aureus*

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Abstract

Optimum conditions were sought for induction of triploidy by heat shock in tilapia, *Oreochromis aureus*. Triploids were identified using karyological techniques that detected metaphase chromosomes in the tail epithelial cells of larvae. Heat shock treatment at 41°C gave 100% triploidy when started at the zygotic age of 3 minutes and applied for 4 minutes. Higher percentages of triploidy were produced at higher water temperatures at short duration times in a range of heat shock treatments (40-43°C) applied for 2-8 minutes at the zygotic age of 3 minutes. No triploidy was induced when heat shock treatment was conducted at the zygotic age of 7 minutes or at a temperature of 39°C within a duration of 8 minutes. To produce 100% triploidy in *O. aureus*, heat shock treatment should be conducted at the zygotic age of 3 minutes, temperatures of 40 or 41°C, and with durations of 8 or 4 minutes, respectively.

Introduction

Triploidy in fish leads to functional sterility (Gervai et al. 1980; Wolters et al. 1982; Don and Avtalion 1986). Fish sterility is potentially profitable in aquaculture especially in precocious species with high reproductive capacities. A high reproductive capacity can lead to overstocking in fishponds, which may have negative impacts on growth and survival. The higher heterozygosity of triploid fish may enhance growth (Stanley et al. 1984; Thorgaard 1986; Scheerer and Thorgaard 1987).

Triploidy in tilapias has been reported by Valenti (1975), Chourrout and Itskovich (1983), Don and Avtalion (1986, 1988a), Penman et al. (1987a, 1987b), Pandian and Varadaraj (1988) and Mair et al. (1991a, 1991b). Although induction of 100% triploidy had been reported, results have not been consistent. The question remains as to the suitable zygotic age for induction. In the present study, the relationships between heat shock intensity, zygotic age and duration of treatment for induction of triploidy in *Oreochromis aureus* were investigated.

Materials and Methods

The study was conducted at the Tungkang Marine Laboratory, Taiwan Fisheries Research Institute. Four fiber-glass aquaria with a water capacity of 0.35 t (1.2x0.6x0.6 m) were used as breeding tanks. Each aquarium contained three females (395±113 g mean body weight) and one male (500-700 g body weight). The premaxillae of the males were removed to prevent injury to the female during courtship as suggested by Lee (1979). The water was kept clean by pumping it

through a filter. Observations were made through the glass of the aquaria. Eel feed containing 44% crude protein was given two to three times per day. The experiment was conducted under natural photoperiod (12L:12D).

Artificial fertilization was conducted using the methods of Rothbard and Pruginin (1975). The eggs obtained from one female was mixed with 0.6-1.0 ml of sperm. After shaking the mixture of eggs and sperm several times manually, about 50 ml of underground freshwater at 30°C was added to activate the sperm. This was considered to be the start of fertilization. The eggs were immediately divided among 10 plastic bowls, and the contents of each poured into a plastic tube (6 cm length and 4 cm diameter) sealed at one end with a 0.8 mm mesh net. The tubes were put into an incubation chamber at a constant water temperature of 30°C until the time of the heat shock treatment.

According to the experimental design (Table 1), the suitable zygotic ages for induction of triploidy were determined first in Trial 1. Then, durations of 2, 4, 6, and 8 minutes of various heat shock temperatures were used in Trials 2 to 6. The plastic tube containing the eggs was put directly into another incubation chamber for heat shock treatment. After the treatment, the tube was put back directly into the original incubation chamber with a water temperature of 30°C. Every treatment was duplicated.

After counting, the eggs were incubated in a plastic soda bottle using recirculated underground water. The survival rate of the yolksac larvae (fifth day) was calculated. No disinfection treatments were used.

Identification of metaphase chromosomes was modified from the methods of Kligerman and Bloom (1977), Chourrout and Itskovich (1983), and Baksi and Means (1988). Three- to eight-day old larvae were put into a 50- or 100-ml beaker containing 10 ml of 0.03-0.05% colchicine for 2-4 hours. The tail part of the larva was cut off and put in distilled water for 2 hours. After removing the distilled water, the tissue was fixed for more than 30 minutes in a mixture of three parts methyl alcohol and one part acetic acid. It was then placed in 50% acetic acid and stroked with the flat side of a forceps to form a cell suspension. The cell suspension was extracted with a pipette and dropped on to a prewarmed slide. A cell ring was formed on the slide after the solution (50% acetic acid) was removed. The slide was then air dried for one day after which the slide was stained in 6% Giemsa for 1 hour and air dried again. For every treatment, 20-30 larvae were killed for preparations to identify metaphase chromosomes. Two cell rings per larva were made on each slide.

Table 1. Summary of the experimental conditions and treatments used to investigate heat shocking of *Oreochromis aureus* eggs for production of triploid larvae.

	Heat shock		
Trial	Temperature (°C)	Zygotic age (minute)[a]	Duration (minute)
1	41	1,3,5,7	4
2	39	3	2,4,6,8
3	40	3	2,4,6,8
4	41	3	2,4,6,8
5	42	3	2,4,6,8
6	43	3	2,4,6,8

[a]After artificial fertilization.

Results

Survival of Yolksac Larvae and Induction of Triploidy by Heat Shock of 41°C at Different Zygotic Ages

Trial 1. The survivals of the yolksac larvae induced at 41°C lasting for 4 minutes at the zygotic ages of 1, 3, 5, and 7 minutes were 54, 74, 55 and 93%, respectively, in comparison with the control group (Table 2). There

were no significant differences (P>0.05) between the survivals after a heat shock treatment beginning at the zygotic age of 7 minutes (60%) and the control group (65%). Although the survival rate of yolksac larvae from eggs that had a heat shock treatment beginning at the zygotic age of 7 minutes was highest among these treatment groups, no triploidy was induced. Triploidies of 93, 100 and 80% were obtained by heat shock treatment after fertilization of 1, 3, and 5 minutes, respectively. There was no difference (χ^2=0.7, P>0.05) between heat shock treatment at the zygotic ages of 1 and 3 minutes, but a significant difference (χ^2=3.91, P<0.05) was found between heat shock treatments at the zygotic ages of 3 and 5 minutes.

Because induction of triploidy was highest at 3 minutes after fertilization in Trial 1 and because this time was sufficient to put the eggs in the bowls and tubes after fertilization, the zygotic age of 3 minutes was chosen for subsequent trials with different heat shock temperatures and durations.

Survival of Yolksac Larvae and Induction of Triploidy by Different Heat Shock Temperatures and Durations

Trial 2. The survivals of the yolksac larvae from eggs that had a 39°C heat shock treatment beginning at a zygotic age of 3 minutes for 2, 4, 6, and 8 minutes duration were 58, 47, 31 and 70%, respectively, in comparison with controls (Table 3). No triploidy was induced in this trial. A heat shock treatment of 39°C for 8 minutes could not restrain the release of the second polar body in meiosis II.

Trial 3. The survivals of the yolksac larvae from eggs that had a 40°C heat shock treatment at a zygotic age of 3 minutes for 2, 4, 6, and 8 minutes duration were 91, 66, 59 and 91% (Table 4), respectively, in comparison with controls. Triploidies of 4, 50, 94 and 100% were obtained at durations of 2, 4, 6 and 8 minutes, respectively. There was no significant difference among the percentages of induced triploidy at a duration of 2 minutes and the control group (χ^2=1.4, P>0.05), 6 minutes and 8 minutes (χ^2=0.04, P>0.05).

Trial 4. The survivals of the yolksac larvae from eggs that had a 41°C heat shock treatment at a zygotic age of 3 minutes at durations of 2, 4, 6, and 8 minutes were 90, 84, 8 and 1%, respectively (Table 5), in comparison with controls. No triploidy was induced at a duration of 2 minutes; 100% triploidy was obtained at a duration of 4 minutes. No yolksac larvae survived at durations of 6 and 8 minutes for examination of chromosomes.

Table 2. Survival and percent triploids of *Oreochromis aureus* yolksac larvae from eggs subjected to heat shocks (of 4 minutes duration at 41°C), applied at various time intervals after fertilization (Trial 1).

		Yolksac larvae							
		Survival				Diploidy		Triploidy	
Duration of heat shock (minute)[a]	Initial no.	No.	%	% of control	No. of fish sampled	No.	%	No.	%
Control	152	99	65	100	20	20	100	0	0
1	161	57	35	54	30	2	7	28	93
3	261	125	48	74	26	0	0	26	100
5	228	81	36	55	30	6	20	24	80
7	199	120	60	93	29	29	100	0	0

[a]After artificial fertilization.

Table 3. Survival and percent triploids among *Oreochromis aureus* yolksac larvae from eggs subjected to heat shocks (39°C at 3 minutes after fertilization) of various duration times (Trial 2).

		Yolksac larvae							
		Survival				Diploidy		Triploidy	
Duration of heat shock (minute)[a]	Initial no.	No.	%	% of control	No. of fish sampled	No.	%	No.	%
Control	329	108	33	100	20	20	100	0	0
2	327	62	19	58	30	30	100	0	0
4	337	52	15	47	30	30	100	0	0
6	334	34	10	31	29	29	100	0	0
8	304	70	23	70	30	30	100	0	0

[a]After artificial fertilization.

Table 4. Survival and percent triploids of *Oreochromis aureus* yolksac larvae from eggs subjected to heat shock (40°C at 3 minutes after fertilization) of various duration times (Trial 3).

		Yolksac larvae							
		Survival				Diploidy		Triploidy	
Duration of heat shock (minute)[a]	Initial no.	No.	%	% of control	No. of fish sampled	No.	%	No.	%
Control	263	17	7	100	19	19	100	0	0
2	372	22	6	91	28	27	96	1	4
4	345	15	4	66	24	12	50	12	50
6	399	15	4	59	18	1	6	17	94
8	388	23	6	91	25	0	0	25	100

[a]After artificial fertilization.

Table 5. Survival and percent triploids of *Oreochromis aureus* yolksac larvae from eggs subjected to heat shock (41°C at 3 minutes after fertilization) of various duration times (Trial 4).

		Yolksac larvae							
		Survival				Diploidy		Triploidy	
Duration of heat shock (minute)[a]	Initial no.	No.	%	% of control	No. of fish sampled	No.	%	No.	%
Control	178	157	88	100	20	20	100	0	0
2	214	170	79	90	29	29	100	0	0
4	202	150	74	84	30	0	0	30	100
6	217	15	7	8	0	-	-	-	-
8	195	1	1	1	0	-	-	-	-

[a]After artificial fertilization.

Trial 5. The survivals of the yolksac larvae from eggs that had a 42°C heat shock treatment at a zygotic age of 3 minutes at durations of 2 and 4 minutes were 76 and 49% (Table 6), respectively, in comparison with controls. Triploidies of 18 and 97% occurred at durations of 2 and 4 minutes, respectively. No yolksac larvae survived at durations of 6 and 8 minutes for examination of chromosomes.

Trial 6. A survival of 71% observed for yolksac larvae (Table 7) was obtained in comparison with controls and there was 100% triploidy after a heat shock treatment of 43°C at a zygotic age of 3 minutes, with a duration of 2 minutes. No yolksac larvae survived in the other heat shock treatment groups for examination of chromosomes.

Discussion and Conclusions

Triploidy of 100% was obtained by heat shock treatment lasting for 2 minutes at 43°C at a zygotic age of 3 minutes, but there was a lower percentage of triploidy by heat shock treatment at 40-42°C at the same duration. At 40°C, a longer duration of 8 minutes was needed to obtain 100% triploidy. These results suggest that the formation of microtubules was interrupted by the shorter time with the higher temperature or by the longer time with lower

Table 6. Survival and percent triploids of *Oreochromis aureus* yolksac larvae from eggs subjected to heat shock (42°C at 3 minutes after fertilization) of various duration times (Trial 5).

		Yolksac larvae							
		Survival				Diploidy		Triploidy	
Duration of heat shock (minute)[a]	Initial no.	No.	%	% of control	No. of fish sampled	No.	%	No.	%
Control	152	58	38	100	18	18	100	0	0
2	141	41	29	76	28	23	82	5	18
4	177	33	19	49	29	1	3	28	97
6	139	0	0	0	0	-	-	-	-
8	154	0	0	0	0	-	-	-	-

[a]After artificial fertilization.

Table 7. Survival and percent triploids *of Oreochromis aureus* yolksac larvae from eggs subjected to heat shock (43°C at 3 minutes after fertilization) of various duration times (Trial 6).

		Yolksac larvae							
		Survival				Diploidy		Triploidy	
Duration of heat shock (minute)[a]	Initial no.	No.	%	% of control	No. of fish sampled	No.	%	No.	%
Control	135	34	25	100	20	20	100	0	0
2	167	30	18	71	25	0	0	25	100
4	139	0	0	0	0	-	-	-	-
6	162	0	0	0	0	-	-	-	-
8	169	0	0	0	0	-	-	-	-

[a]After artificial fertilization.

temperature, thus in each case causing the metaphase chromosomes that stayed on the equator plate of the cell to fail to separate. Diploidy was only obtained completely when heat shock treatment was started at the zygotic age of 7 minutes. On the other hand, the second polar body of the egg was released completely after 7 minutes of fertilization. These observations do not match those of Valenti (1975) who also produced triploidy in *O. aureus* after 15 minutes of fertilization when the eggs were incubated at 32°C. Don and Avtalion (1988a) found that triploidy using cold shock treatment (11°C for 60 minutes) could be induced within a wide range of zygotic ages (0-15 minutes), but the induction of triploidy using heat shock was possible only within a narrow range of zygotic ages: 2.5-3.5 and 3.5-4.5 minutes for *O. aureus* and *O. niloticus*, respectively. These inconsistent results are hard to explain.

In the present study, survival of yolksac larvae was higher in the 100 and 0% triploid groups for which heat shock treatment was started at the zygotic age of 3 and 7 minutes (Table 2). This suggests that the embryos were more tolerant to temperature shock when the second polar body was retained or released completely. Except in the 2-minute duration group, the same result was obtained in Trial 3 (Table 4).

The identification of ploidy in tilapias using karyological techniques has been widely used (Chourrout and Itskovich 1983; Myers 1985; Don and Avtalion 1986, 1988a; Pandian and Varadaraj 1988). In diploidy, there are two distinct marker chromosomes much larger than the others. On the other hand, it is quite easy to identify triploidy from metaphase chromosomes—it has three larger chromosomes in comparison with the others. The tissues commonly used for the examination of chromosomes are the embryo, larval tail, gill and kidney. Although pigmented tilapia embryos should have abundant metaphase chromosomes due to a lot of cell cleavage, it is difficult to separate the tail bud and yolk. Furthermore, yolk particles can easily contaminate the slide. A longer time would be needed to rear fry if the gills and kidneys were to be used for examination of chromosome. Undoubtedly, using the tail part of three- to eight-day larvae is the most convenient and effective method for identification of ploidy.

Mass production of triploids by retention of the second polar body in tilapias would not be profitable because only a few eggs could be squeezed out per spawning female. Moreover, the spawning time is difficult to control. The production of sterile triploidy by establishing tetraploid broodstock with suppressed first cleavage and crossing with diploids may be a potential way to overcome this problem as suggested also by Chourrout et al. (1986) and Don and Avtalion (1988b).

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Effects of Substrate and Water Quality on Seasonal Fry Production by *Tilapia rendalli* in Tanks[a]

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COSTA-PIERCE, B.A. 1996. Effects of substrate and water quality on seasonal fry production by *Tilapia rendalli* in tanks, p. 280-289. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Effects of presence/absence of a spawning substrate, and of temperature and water quality changes on seasonal fry production by *Tilapia rendalli* broodstock, were investigated from 29 December 1989 to 19 February 1991 (416 days) in tanks at the National Aquaculture Center, Domasi, Malaŵi. For each of the two treatments (with vs. without substrate), four 5-m^3 tanks were stocked with *T. rendalli* broodstock of similar mean size (35.8-44.2 g). Fish produced batches of sticky eggs which adhered strongly to side and bottom walls of cement tanks. Fry production was not significantly different between treatments ($P>0.05$, one-way ANOVA; $n=81$). Fry production was highly seasonal, with little or no fry production from 3 July to 25 September 1990 when minimum water temperatures dropped below 20°C. A multiple regression on fry production as the dependent variable was significant ($P<0.05$; $n=136$) with broodstock weight, temperature, dissolved oxygen and Secchi disk visibility (all positive regression coefficients) and substrate (negative coefficient) (as a dummy variable). Temperature was a highly significant positive predictor of fry production ($P<0.01$; $n=136$).

Introduction

Tilapia rendalli is one of the most important cultured indigenous fishes in rural areas of central and southern Africa and has good potential for smallholder aquaculture where vegetation is available for feeding (Pullin 1982, 1988; Chikafumbwa and Costa-Pierce 1992). In Malaŵi, its firm flesh, harvestability with traditional gears and ability to grow when fed on the types of vegetation available on the majority of smallholder farms make it a desirable fish for low-cost aquaculture in rural areas. However, little experimental work has been done to develop hatchery systems for *T. rendalli*. This paper reports on seasonal variabilities of fry production by *T. rendalli* broodstock in tanks with and without a spawning substrate.

Materials and Methods

The study was conducted in eight 5-m^3 concrete tanks at the National Aquaculture Center, Domasi, Malaŵi to test the effect of providing a spawning substrate (a 10-cm layer of washed river sand/small gravel from the Domasi River [approx. 50:50% by weight] at the tank bottom) and the resulting seasonal water quality changes on *T. rendalli* fry production.

Two treatments, with and without substrate, were laid out in two randomized

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blocks of four tanks. All substrates were washed at least three times with new water inside the tanks, or were washed repeatedly until the washing water was clear. Four tanks were left with bare concrete walls and bases without substrate.

T. rendalli broodstock (mean individual body weights, 35.8-44.2 g) were stocked at a 2:1, female:male sex ratio; six females:three males per 5-m^3 tank. Fish were fed daily with maize bran at 5% body weight·day^{-1}, calculated for a five-day week. The amount of feed given was adjusted to the new mean body weight every three weeks when free-swimming fry were harvested from each tank by draining half of its volume and using a small mesh scoop net to remove all fry and adults. Fry were counted and each adult fish weighed and measured individually for standard and total length. The experiment ran from 29 December 1989 to 19 February 1991 (416 days).

Fry production in the two treatments was compared by one-way ANOVA. Water quality was monitored every three weeks on the day before fry harvests according to the "checking" mode of Costa-Pierce (1990). Dissolved oxygen (DO) was measured at 0600-0700 hours. On eight occasions when pH exceeded 8.0, total ammonia concentrations were checked using a Hach kit. Water quality differences between treatments were analyzed by paired t-tests. A multiple regression analysis was performed with fry number/tank/harvest as the dependent variable and broodstock weight, temperature, dissolved oxygen and substrate (added as a dummy variable) as independent variables. Analysis was performed using methods defined previously (Costa-Pierce et al. 1993).

A length-based growth index, ϕ' (Pauly et al. 1988), was calculated using a growth spreadsheet solution developed by Vakily (1988). Standard lengths were used in the calculations.

Results

Over 416 days (19 fry harvesting periods), broodstock in ponds with substrate yielded a total of 18,217 fry, while the broodstock with no substrate produced 22,220 (Table 1). Fry production was strongly seasonal and very variable, being concentrated from 16 October to 12 June when minimum water temperatures were above 20°C (Fig. 1). During the 76 sampling periods, 40 periods (53%) yielded no fry in the tanks with substrate and 29 periods (38%) with no fry were experienced in the no substrate tanks.

Mean (+SE) fry production ranged from 13.71 to 28.14 (6.68-37.35) fry·100 g broodstock day^{-1} (substrate) and from 5.72 to 32.44 (2.63-40.54) (no substrate). Mean (+SE) fry production·m^{-2}·day^{-1} ranged from 1.39 to 3.83 (0.66-5.15) (substrate) and from 0.77 to 4.30 (0.35-5.35) (no substrate) (Fig. 2). One-way ANOVA showed no significant differences in fry production with or without substrate ($P>0.05$; $n=81$ paired comparisons).

Broodstock reached a mean (+SE) size at harvest of 85.3 g (82.6-88.0 g) (substrate) and 93.8 g (90.2-97.3 g) (no substrate) from 41.8 g (41.0-42.6 g) and 39.0 (37.6-40.4 g) stocking weights. Calculations using a length-based growth index (ϕ') showed mean (+SE) indices for *T. rendalli* in tanks with and without substrate of 2.31+0.06 and 2.34 +0.08, respectively (Table 2). No correlation was found between growth indices and fry·100 g broodstock weight·day^{-1} ($P>0.05$; $n=8$).

Weight-based estimates of broodstock growth were very variable. Of the 72 growth intervals measured (18/tank x 4 tanks), 11 (15%) showed weight losses where substrate was present compared to 19 (26%) with no substrate. Overall, of the 144 total weight growth intervals, 30 (21%) showed weight losses of broodstock. Eighteen (60%) of these weight loss periods occurred during the cool season from 3 July to 16 October 1990.

Table 1. *Tilapia rendalli* fry production in 5-m³ tanks with and without a spawning substrate over 416 days.

Tank	Fry number	Fry/female	Number of spawnings
Substrate 1	3,568	595	8
Substrate 2	2,953	492	6
Substrate 3	3,935	656	10
Substrate 4	7,761	1,294	12
Range	2,953-7,761	492-1,294	
Mean	4,554	759	
SD	1,884	314	
No substrate 1	5,240	873	12
No substrate 2	6,285	1,048	15
No substrate 3	1,972	329	6
No substrate 4	8,723	1,454	14
Range	1,972-8,723	329-1,454	-
Mean	5,555	926	-
SD	2,424	404	-

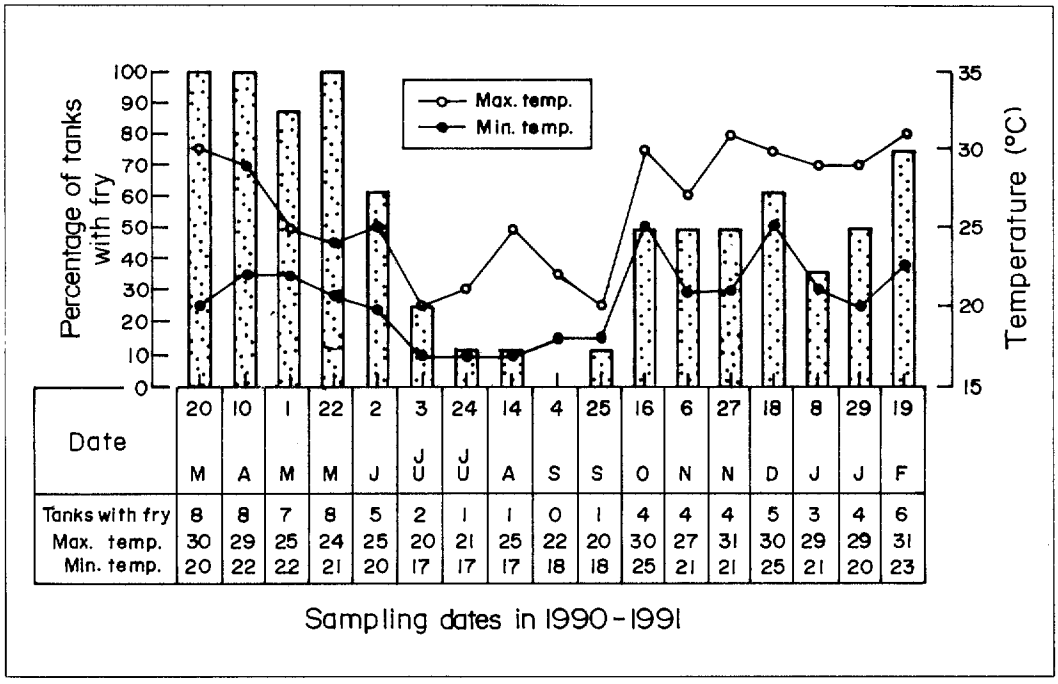

Fig. 1. Seasonal pattern of free-swimming fry production by *Tilapia rendalli* in eight 5-m³ concrete tanks, with details of water temperature.

Table 2. Growth parameters for *Tilapia rendalli* broodstock in tanks with and without a spawning substrate, compared to results of a previous study.

Treatment	Growth parameters L_∞ (SL, cm)	K (year^{-1})	ϕ' (= log K + 2 log L_∞)
Substrate 1	13.4	0.841	2.18
Substrate 2	13.3	1.372	2.39
Substrate 1	15.5	0.627	2.18
Substrate 1	13.2	1.677	2.47
No substrate 1	13.9	1.630	2.50
No substrate 2	14.3	0.860	2.25
No substrate 1	13.2	1.677	2.47
No substrate 1	20.7	0.305	2.12

	n	ϕ' range	Mean ϕ' (SE)
Pauly et al. (1988), (Aquaculture)	4	2.34-2.81	2.60 (0.12)
Pauly et al. (1988), (Nature)	16	2.24-2.80	2.45 (0.03)
This study (Aquaculture)			
Substrate	4	2.18-2.47	2.31 (0.06)
No substrate	4	2.12-2.50	2.34 (0.08)

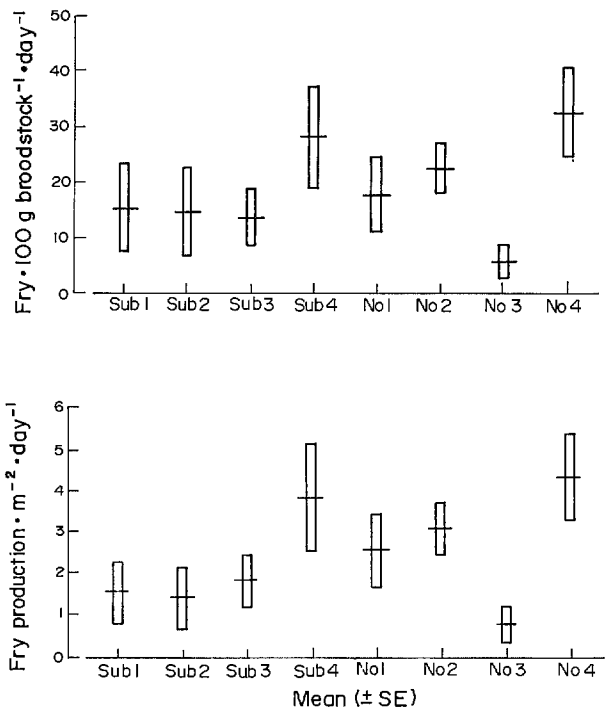

Fig. 2. *Tilapia rendalli* fry production per day in concrete tanks with (sub) and without (no) substrate. Fry production is plotted per 100 g of broodstock biomass (top) and per m^2 of tank area (bottom). Lines and rectangles are means + standard error (SE).

Mean temperatures ranged from 23.1 to 23.9°C in the eight tanks (Table 3), with three distinct seasons (cool, 2 July-24 September; hot, dry, 15 October-25 November; and hot, wet, 17 December-30 April) separated by a transitional cooling period (21 May-11 June) (Fig. 3). The seasonal pattern of conductivity changes was similar to water temperature. Correlation of temperature with conductivity was highly significant ($P<0.001$; $n=136$).

Mean Secchi disk visibilities (SDVs) showed a similar trend in substrate/no substrate treatments as phytoplankton populations developed (Fig. 3); however, SDVs were significantly lower (paired t-test, $P<0.001$, $n=68$) in tanks with substrate (Table 3). A multiple regression of SDVs as the dependent variable with temperature and substrate as independent variables was highly significant ($P<0.001$; $n=136$) (Table 4).

Dissolved oxygen concentrations (DOs) showed a seasonal trend similar to temperature and conductivity (Fig. 3). With one exception, the date of the lowest DO was 17 December in all tanks. Tanks with substrate had 12, and those with no substrate, seven dates with DOs below 1.0 $mg \cdot l^{-1}$. DOs were significantly lower (paired t-test, $P<0.001$, $n=68$) in tanks with substrate (Table 3). The mean pH was significantly higher (paired t-test, $P<0.001$, $n=68$) in tanks with no substrate (Table 3).

In a multiple regression analysis of fry production with broodstock weight, temperature, DOs and SDVs as independent variables, temperature was the only significant positive predictor ($P<0.01$) of fry production (Table 5).

Discussion

In natural waters, *T. rendalli* spawn in grassy, shallow margins of lakes and rivers, usually at a depth of 120-130 cm, but sometimes spawn in waters as shallow as 50 cm (Caulton 1978). In this study, *T. rendalli* did not need a substrate to spawn in 1-m deep concrete tanks. Nesting behavior, fertilization and egg development were readily accomplished on bare concrete tank walls. When tanks were drained to collect fry, egg masses were commonly seen sticking strongly to tank walls in both treatments. Jubb (1967) reported that *T. rendalli* produced sticky eggs which are "moved from pit to pit, guarded and oxygenated by tail fanning by both parents."

Fry production per female in this study was, however, lower than reported previously from wild and captive fish. The 24 females in the two treatments produced a total of 18,217 (substrate) and 22,220 (no substrate) free-swimming fry. During the 416 days of the experiment, 2,953-7,761 fry (492-1,294/female) were produced in six to 12 spawnings (substrate) and 1,972-8,723 fry (329-1,454/female) in six to 12 spawnings (no substrate) (Table 2). Kenmuir (1973) observed a pair of *T. rendalli* in a tank could breed eight times a year. A 21.1-cm (190-g) female produced 48,000 $fry \cdot year^{-1}$ (8 broods x 6,000 fry/brood), whereas a 31.5 cm female produced 70,000 $fry \cdot year^{-1}$ (Kenmuir 1973). De Bont (1950) obtained 14,380 *T. rendalli* larvae from five pairs breeding in captivity (2,876/female).

The lower fry production found in the present study at Domasi may be attributed to cooler temperatures and a shorter reproductive season. Temperature was found to be a strong predictor of fry production, thus, a strong seasonality in fry production was found. Reproductive activity nearly ceased from July to September when minimum water temperatures fell below 20°C. Chervinski (1982) reported that *T. rendalli* is capable of living at temperatures as low as 11°C, but does not breed below 21°C. Balarin (1988) reported spawning temperatures of 20-28°C and de Pienaar (1978) reported

Table 3. Summary of water quality data in *Tilapia rendalli* tanks. Temperature in degrees C; conductivity in $\mu mho \cdot cm^{-1}$; dissolved oxygen (DO) in $mg \cdot l^{-1}$; Secchi disk visibility (SDV) in cm; R = ranges; M = arithmetic means; SD = standard deviations; and CV = coefficients of variation. Values are all ranges of four tanks of two treatments.

Parameter	Statistics	Substrate	No substrate	Significance levels (n = 68)
Temperature	R	18.0 - 28.4	18.0 - 28.5	
	M	23.2 - 23.8	23.1 - 23.9	P>0.05
	SD	3.2 - 3.4	2.8 - 3.0	
	CV	14 - 15	12 - 13	
Conductivity	R	83.4 - 200.0	91.5 - 197.7	
	M	83.4 - 99.7	91.5 - 101.0	P>0.05
	SD	21.0 - 27.5	18.2 - 24.9	
	CV	17 - 22	15 - 19	
DO	R	0.5 - 5.2	0.4 - 8.9	
	M	1.5 - 1.9	2.1 - 3.5	P<0.001
	SD	0.8 - 1.2	2.0 - 2.3	
	CV	48 - 66	60 - 93	
pH	R	6.56 - 7.98	6.93 - 9.50	
	M	7.31 - 7.34	7.78 - 8.14	P<0.001
	SD	0.30 - 0.38	0.29 - 0.65	
	CV	4 - 5	4 - 8	
SDV	R	0.25 - 1.00	0.33 - 1.00	
	M	0.69 - 0.73	0.77 - 0.91	P<0.001
	SD	0.21 - 0.28	0.10 - 0.23	
	CV	28 - 40	11 - 30	

Table 4. Multiple regression on Secchi disk visibility (SDV) in *Tilapia rendalli* broodstock tanks, as the dependent variable, with temperature and presence/absence of substrate as independent variables. Significance levels are indicated with stars (*=0.05; **=0.01; ***=0.001); b = regression coefficient; and SE = standard error of the regression coefficient.

Independent variables	b	SE
Temperature	-0.030	0.006***
Substrate	-0.104	0.035**
Constant (a)	1.518	
r^2	0.209	
F value	17.614	
Probability	< 0.001	
n	136	

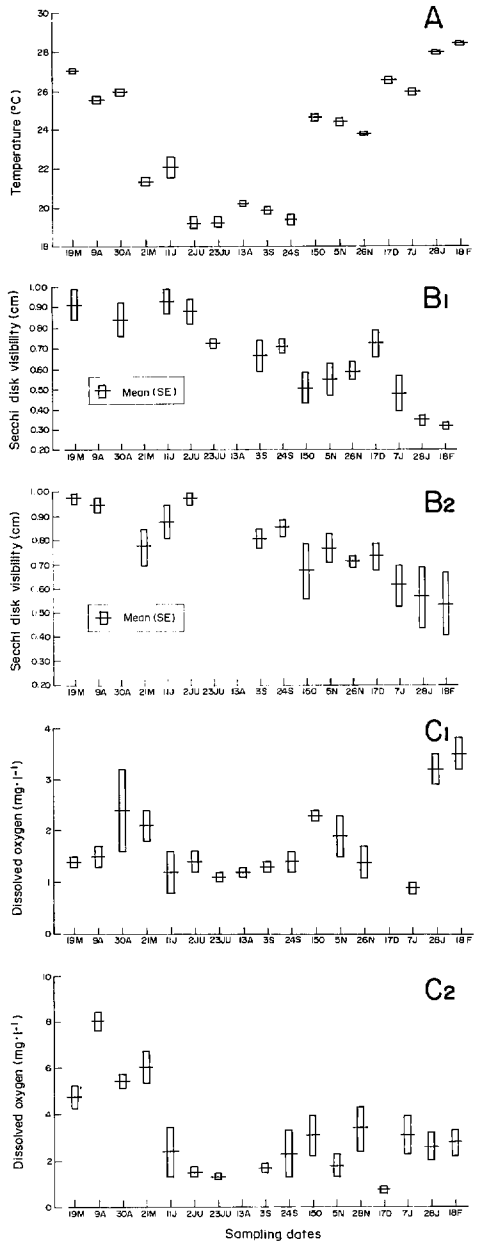

Fig. 3. Seasonal variations in temperature, Secchi disk visibilities and dissolved oxygen at 0600-0700 hours measured in eight 5-m^3 concrete tanks containing *Tilapia rendalli* broodstock: four tanks had a spawning substrate, four had none. A - temperature for all eight tanks. B - Secchi disk visibilities in 1. tanks with substrate; and 2. tanks without substrate. C - dissolved oxygen in 1. tanks with substrate; and 2. tanks without substrate. The lines are mean values with rectangles representing standard errors.

Table 5. Multiple regression on fry production by *Tilapia rendalli* with broodstock weight, temperature, dissolved oxygen, Secchi disk visibility and presence/absence of substrate as independent variables. Dependent variable is the number of free-swimming fry/tank/harvest period. Significance levels and symbols are as in Table 3.

Independent variables	b	SE
Broodstock weight	2.515	3.77
Temperature	35.396	11.864**
Dissolved oxygen	17.787	21.596
Secchi disk visibility	160.599	216.062
Substrate	-14.296	90.636
Constant (a)	-940.874	
r^2	0.099	
F value	2.856	
Probability	< 0.05	
n	136	

maturity as well as breeding are slowed or cease below 21°C.

Up to 15 spawnings were noted in one tank during this experiment (Table 1), with spawnings concentrated during the rainy season (November to March). In nature, *T. rendalli* spawns as long as water temperatures remain above 21°C; and, in southern Africa, spawning reaches a peak during the hot, rainy season (October to December) (de Pienaar 1978; Kenmuir 1983). The number of spawnings is determined by the duration of the rainy season (De Bont 1950) and suitable water temperatures (Kenmuir 1983). Spermatogenesis is continuous but oogenesis is highly influenced by temperature and photoperiod (season) (Moreau 1982). During the hot, rainy season, reproduction can take place every four weeks (de Pienaar 1978).

Growth rates of broodstock using a length-based index (ϕ') were similar to those reported from ponds in Zambia and Uganda (Pauly et al. 1988). Growth of *T. rendalli* was good compared to previous trials using vegetation as a feed input to earthen ponds at Domasi (Chikafumbwa and Costa-Pierce 1992). Fish reached mean weights over 80 g in 417 days in both treatments with maize bran as the only feed. Growth rates and fish size at harvest in this experiment were superior to those reported previously in Malaŵi. Noble and Costa-Pierce (1992) reported that the mean weight of fish sold from 16 harvests of smallholder fishponds in the Zomba District was only 26.6 g. *T. rendalli* is not strictly herbivorous but feeds actively on particles of maize bran.

Fry production in tanks could have been affected by at least two factors: water quality and the choice of a 2:1, female:male broodstock ratio. Water quality conditions differed between the two treatments. Tanks with substrate had lower SDVs, likely due to more dense natural food concentrations. Therefore, early morning DOs and pHs were lower in tanks with substrate. However, all water quality conditions in the two treatments were within ranges acceptable for *T. rendalli* (Philippart and Ruwet 1982). When water quality was suspect (e.g., pH>8.0 when ammonia concentrations were checked), toxic concentrations were not found.

T. rendalli are known to be monogamous; one spawning female pairs with a single male (De Bont 1950). In this study, suboptimal sex ratios with some females being "surplus" may have constrained maximum fry production. As pointed out by Philippart and Ruwet (1982), substrate spawners form stable territorial pairs Surplus fish do not participate in breeding but can disturb breeders.

Acknowledgements

This study was conducted through funding provided by the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) to ICLARM. I thank GTZ and the Malaŵi Department of Fisheries for funding, access to facilities and approval to conduct this study. My appreciation goes to Dr. Martin Bilio, Boniface Mkoko, Brian Rashidi and Jaston Mutambo. Technical assistance in the design, coordination and implementation of this study by Fredson Chikafumbwa, Foster Makuwa and John Balarin is also gratefully acknowledged.

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A Practical Quantitative Method to Estimate Relative Reproductive Activity in *Oreochromis niloticus*[a]

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Abstract

Exact quantitative measurements of reproductive traits such as variation in age and size at maturity, frequency of spawning and their relationship with growth performance require regular draining of ponds and sampling of all individuals, which is impossible to carry out during routine production cycles. The method proposed here attempts to utilize the information on morphological and behavioral changes associated with reproduction that can be routinely recorded during regular random sampling of individuals, to construct a "reproduction index." A total of 7,652 individually tagged fingerlings of seven strains of *Oreochromis niloticus* were communally reared in diverse farming systems including ponds, cages and rice-fish systems. Regular random sampling (about 30% of the population) was done every three weeks during a 90-day production cycle. Individual females were scored from 0 to 5 based on condition of the genital papilla and belly, and the presence of eggs/fry in the mouth. A reproduction index value of 0 indicates that the population is reproductively inactive, whereas a value of 5 indicates that all individuals have completed reproduction.

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[a]ICLARM Contribution No. 973.

The reproduction index was low in cages (<1) and rice-fish systems (2), and was high in ponds (4 to 5). Reproduction indexes were highly variable among the different strains, but relatively consistent across different farming systems. Reproductive activity starts much earlier and at lower mean body weights in slow growing strains than in the faster growing strains.

Introduction

Tilapias are widely recognized as one of the most important species for farming in a wide range of aquaculture systems from simple small-scale waste-fed fishponds to intensive culture systems (Maclean 1984; Pullin 1985). They form the mainstay of many resource-poor fish farmers. One of the major problems associated with tilapia culture, however, is its ability to reproduce at small sizes and consequent overcrowding of culture environments. Controlled reproduction has been an area of considerable research (Mair and Little 1991).

The present study forms part of a major collaborative research effort of the Genetic Improvement of Farmed Tilapias (GIFT) project. A sequential approach has been followed in the GIFT project, from systematic documentation of the poor status of the Asian farmed stocks and identification of wild tilapia genetic resources in Africa; evaluation of promising strains of *Oreochromis niloticus*; and then the establishment of base populations and plans to develop a more productive tilapia (Pullin et al. 1991). The primary focus of the GIFT project is selection for fast growth. Inclusion in a selection index of important reproductive traits, such as age and size at maturity, is also being envisaged for possible selection for late maturity.

The results presented here are based on a preliminary attempt to quantify reproductive activity of individual females of different strains of *O. niloticus* under communal stocking. The approach is to score individual females based on their morphological and behavioral characteristics associated with reproduction. The objective of this study was to compare the relative reproductive activity of different strains of *O. niloticus* in different farm environments.

Materials and Methods

This study was a part of an experiment designed to evaluate the growth performance of seven strains of *O. niloticus* reared in 11 different test environments (Eknath et al. 1993). Altogether, 7,652 individually tagged fingerlings of three newly imported African strains (Egypt [E1], Ghana [Gh] and Senegal [Se]) and four established Philippine farmed stocks (locally known as "Israel" [Is], "Singapore" [Si], "Taiwan" [Tw] and "Thailand" [Th]) were communally reared in a wide range of Philippine low input tilapia farming systems (test environments): fertilized ponds (with and without supplementary feeding), ponds fertilized with on-farm agricultural residues (ipil-ipil leaves[e], and leaves and vines of sweet potato), rice-fish systems, cages (different stocking densities; with and without feeding) and three hatcheries (BFAR satellite stations) located in different regions of the island of Luzon (Table 1). The origin of strains and the experimental set-up were described in detail by Eknath et al. (1993).

Single-pair mating (25 breeding pairs of each strain) was done in 175 hapas (1 m^3) installed in breeding ponds. The progeny of each strain were reared separately in hapas until they reached a mean body weight of 3-5 g. The fish were individually tagged and reared in different test environments for 90 days.

[e]Leguminous tree.

Table 1. Description of test environments for genetic research on different strains of *Oreochromis niloticus*, from which data were gathered to assess reproductive activity.

Environment code	Description
S1	Philippine Bureau of Fisheries and Aquatic Resources (BFAR) satellite station located in the lowlands near Laguna Lake, southern Luzon. *Pond culture*. Standard[a] management and fertilization. Stocking density: $0.6 \cdot m^{-2}$.
S2	BFAR statellite station located in the coastal region of northwest Luzon. *Pond culture*. Standard[a] management and fertilization. Stocking density: $0.6 \cdot m^{-2}$.
S3	BFAR satellite station located in the highlands of central Luzon (temperature range 18-20°C). *Pond culture*. Stocking density: $0.6 \cdot m^{-2}$.
P1	On-station[b]. *Pond culture*. Standard[a] management and fertilization. Stocking density: $0.6 \cdot m^{-2}$.
C1	BFAR statellite station located in the lowlands near Laguna Lake, southern Luzon. *Cage culture* in a farm reservoir without fertilization or feeding. Stocking density: $50 \cdot m^{-2}$.
C2	On-station[b]. *Cage culture* in reservoir without fertilization. Feeding at 20% body weight once daily (70% rice bran and 30% fish meal). Stocking density: $30 \cdot m^{-2}$.
C3	On-station[b]. *Cage culture* in ponds (Standard[a] management and fertilization) with supplementary feeding at 10% body weight twice daily (70% rice bran and 30% fish meal). Stocking density: $22 \cdot m^{-2}$.
W1	On-station[b]. *Pond culture*. Fertilized with chicken manure (1,000 $kg \cdot ha^{-1}$) every second week. Stocking density: $0.6 \cdot m^{-2}$.
W2	On-station[b]. *Pond culture*. Fertilized with untreated ipil-ipil leaves (*Leucaena* sp.) at 50 kg dry matter$\cdot ha^{-1}$ daily. Stocking density: $0.6 \cdot m^{-2}$.
W3	On-station[b]. *Pond culture*. Fertilized with untreated leaves and vines of sweet potato (*Ipomea batata*) at 50 kg dry matter$\cdot ha^{-1}$ daily. Stocking density: $0.6 \cdot m^{-2}$.
RF	On-station[b]. *Rice-fish culture*. Trench refuge system (0.75 m wide x 0.5 m deep). Plots planted to IR-70 variety of rice. Stocking density: $0.5 \cdot m^{-2}$.

[a]Inorganic fertilizer (16-20-0) at 50 $kg \cdot ha^{-1}$ and chicken manure at 1,000 $kg \cdot ha^{-1}$ every second week.
[b]The BFAR national broodstock center and the Freshwater Aquaculture Center of Central Luzon State University facilities, Muñoz, lowlands of central Luzon.

The mean age and mean initial body weights of tagged fingerlings at stocking were 108 days (range 98 to 121) and 5.34 g (range from 1.6 to 14.6), respectively. Regular sampling (about 30% of the population) to record individual body weights and reproductive activity was done every 21 days, except in the rice-fish systems.

Individual females were scored from 0 to 5 based on the condition of the genital papilla and belly, and the presence of eggs/fry in the mouth as follows:

score = 1 - genital papilla reddish and swollen, genital pore slightly open;

score = 2 - genital papilla swollen, genital pore fully open, abdomen fully swollen, ready to spawn;

score = 3 - mouthbrooding, eggs in the mouth;

score = 4 - mouthbrooding, fry in the mouth;

score = 5 - spent females with depressed abdomen, swollen jaw, emaciated appearance; and

score = 0 - none of the above, females not reproductively active.

The frequency of individuals by strain and test environment in a given stage

was used to construct the reproduction index. A reproduction index of 0 indicates that the population is immature or reproductively inactive, whereas a value of 5 indicates that all individuals are sexually mature and have spawned.

Results

Mortality and Sex Ratio

The total numbers of individually tagged fingerlings, estimated percentage total mortality and sex ratio at harvest across strains within each test environment are presented in Table 2. Mortality was variable across different test environments, but was not strain-specific (Eknath et al. 1993). Sex ratios across test environments were not significantly different ($P<0.05$) from 1:1.

Growth

The mean body weights of females of the different strains in different test environments were highly variable (Table 3). Among strains, strain E1 was consistently the fastest growing and Gh the slowest. Growth performance of other strains was intermediate. The mean body weights of females of all strains across test environments (environment means; Table 3) were also highly variable. They range from 107 g in S1 to 9.1 g in C1.

Reproduction Indexes in Different Test Environments

The reproduction indexes of all strains in each of the 11 different test environments at successive samplings are presented in Fig. 1. They were highly variable, ranging from almost 0 in environments C1, C2, W1, W3 to 4.6 in S1, at harvest. Interestingly, the reproduction indexes were highly variable in some of the test environments (C2, S3, W1, W2 and W3) where the final mean body weights were similar (Fig. 1 and Table 3). Test environment RF registered a relatively higher reproduction index when compared to its environmental mean value.

Reproduction Indexes of Strains Across Environments

The reproduction indexes of the different strains across test environments at successive samplings are presented in Fig. 2. Onset of reproductive activity occurred 42 days after stocking. The reproduction index was highly variable among strains. The Philippine strains exhibited relatively higher reproduction index values on day 63 (poststocking) than the African strains. At harvest, however, strains Gh and Se showed higher reproduction index values than the other strains.

The reproduction indexes of strains in four representative environments (farming systems) presented in Table 4 indicate the following:

Ponds (S1). The reproduction indexes were highly variable on day 63, with Philippine strains showing relatively higher values than the African strains. However, at harvest, all the strains were reproductively active and showed similar reproduction indexes.

Low-temperature Ponds (S3). For the few surviving females, the reproduction index at harvest was highly variable. The reproduction indexes of strains Gh and Si were considerably lower than the other strains.

Cages (C3). Although the mean body weight for these environments was relatively high (Table 3), the reproduction indexes of the different strains were low. At harvest, strain Gh showed the highest reproduction index and E1 the lowest.

Rice-fish (RF). Reproduction indexes in this environment were considerably

Table 2. Numbers of tagged *Oreochromis niloticus* fingerlings stocked; estimated total mortality (%) and sex ratio at harvest in different test environments.

Test environment (see Table 1)	No. of fish stocked	Estimated mortality[a] (%)	Sex ratio (M:F)[b]
S1	829	10	1.20:1
S2	702	12	0.95:1
S3	680	59	1.20:1
P1	2,714	25	0.96:1
C1	693	5	0.90:1
C2	104	4	0.79:1
C3	596	16	0.79:1
W1	212	49	1.10:1
W2	214	32	0.96:1
W3	218	38	0.97:1
RF	690	51	0.95:1

[a]From Eknath et al. (1993).
[b]Not significantly different from 1:1 sex ratio ($P<0.05$).

Table 3. Mean body weights (g) of females of seven strains of *Oreochromis niloticus* after 90 days of rearing in different test environments.

Test environment (see Table 1)	Strains (see text)							Environment mean
	E1	Gh	Se	Is	Si	Tw	Th	
S1	117.0	76.9	99.8	92.0	90.3	101.2	106.8	99.4
S2	55.3	33.5	39.7	45.9	48.5	49.0	49.8	46.5
S3	32.9	27.2	28.9	27.4	27.5	30.4	26.2	28.7
P1	75.4	42.1	57.1	57.8	55.9	65.6	60.7	59.0
C1	8.3	8.3	9.7	8.1	7.4	8.9	9.1	8.4
C2	26.4	22.2	28.3	21.5	18.1	23.6	30.2	22.7
C3	70.1	47.0	67.0	63.5	-	63.1	63.8	62.1
W1	26.8	23.4	23.2	23.9	19.9	22.0	19.3	22.7
W2	37.5	19.3	23.3	23.5	25.7	27.9	25.3	25.1
W3	26.2	18.5	25.2	27.7	24.9	30.5	26.1	25.3
RF	20.0	12.0	17.2	14.2	13.7	17.5	14.8	15.5
Mean across strains	66.7	38.2	49.1	46.2	41.3	52.2	51.8	

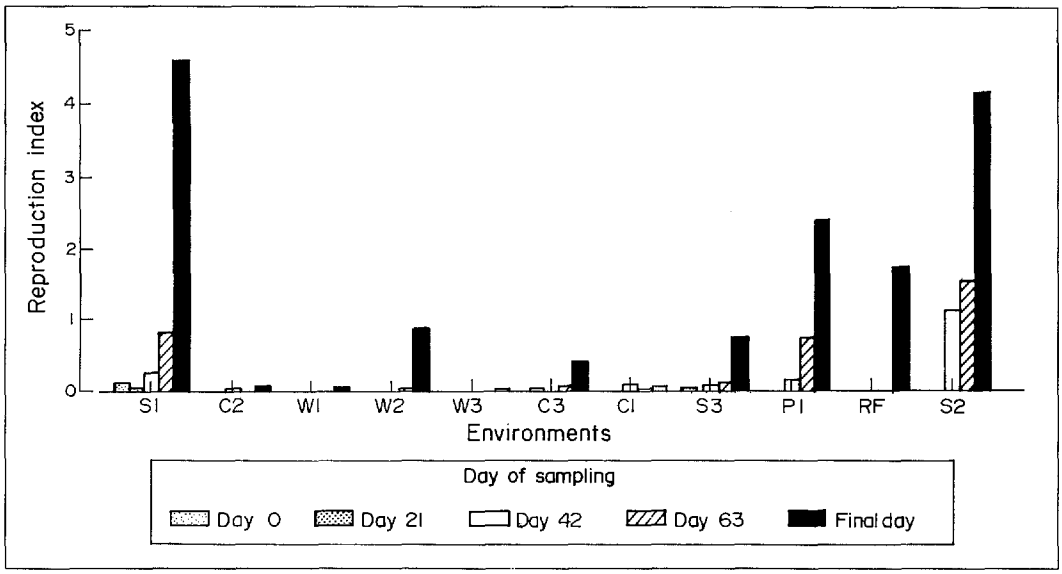

Fig. 1. Reproduction indexes for *Oreochromis niloticus* populations in 11 different test environments: for explanation of environments, see Table 1 and text.

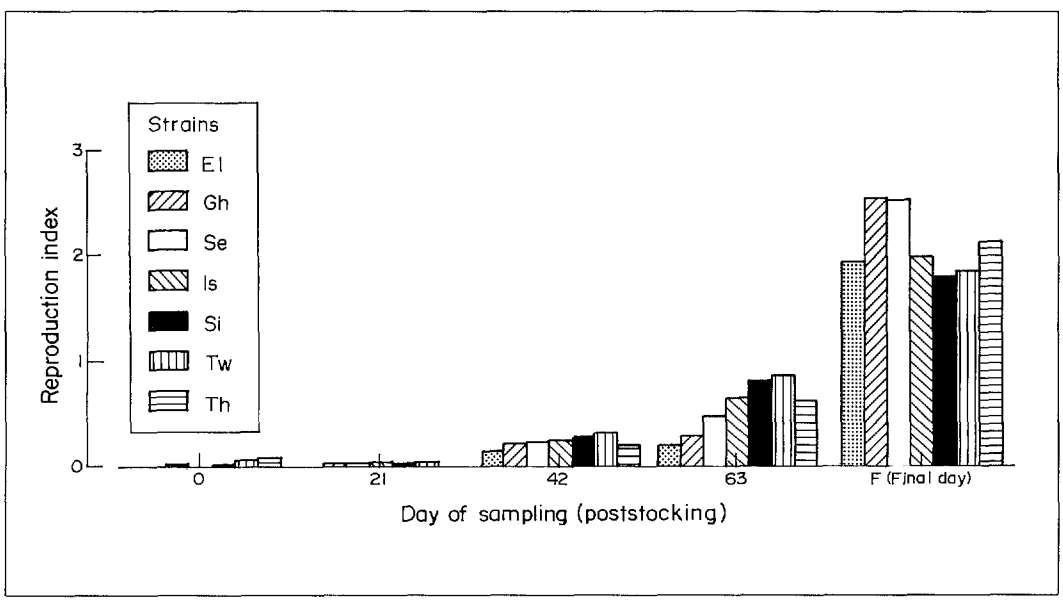

Fig. 2. Reproduction indexes of seven strains of *Oreochromis niloticus* in all the test environments (day 0 = at stocking; day F = at harvest): for explanation of strains, see text.

Table 4. Reproduction indexes of seven strains of *Oreochromis niloticus* in four representative test environments.

Test environment (See Table 1)	Strains (See Text)	Sampling Days (poststocking) 0	21	42	63	90
S1	E1	0	0	0.07	0.45	4.67
	Gh	0.1	0	0.23	0.34	4.55
	Se	0.13	0.04	0.29	0.63	4.65
	Is	0	0.12	0.13	1.10	4.69
	Si	0.15	0.10	0.41	1.27	4.51
	Tw	0.30	0.13	0.43	1.57	4.57
	Th	0.23	0	0.40	0.57	4.63
S3	E1	0	0	0	0	0.89
	Gh	0	0	0	0	0.21
	Se	0	0	0	0	0.86
	IS	0	0	0	0	0.78
	Si	0.17	0	0.33	0	0.33
	Tw	0	0	0.35	0.75	1.20
	Th	0.22	0	0	0	0.94
C3	E1	0	0	0.04	0	0.13
	Gh	0	1.10	0	0.03	0.90
	Se	0	0.06	0	0.06	0.17
	Is	0	0.10	0	0.19	0.56
	Si	*	*	*	*	*
	Tw	0	0.06	0	0.06	0.50
	Th	0	0	0.07	0.11	0.30
RF	E1	0	-	-	-	0.58
	Gh	0	-	-	-	3.30
	Se	0	-	-	-	2.03
	Is	0	-	-	-	2.20
	Si	0	-	-	-	1.19
	Tw	0	-	-	-	1.22
	Th	-	-	-	-	1.61

* = strain Si not stocked in C3.
- = no intermediate sampling.

higher than for other test environments relative to the environmental mean value. The reproduction indexes among strains were highly variable. Interestingly, the slowest growing strain (Gh) showed the highest reproduction index and the fastest growing strain (E1) the lowest.

Reproduction Indexes and Growth

The relationship between reproduction index and the mean body weights of the different strains across test environments is presented in Fig. 3. Onset of reproductive activity and the corresponding mean body weights were highly variable. The slowest growing strain (Gh) was at its highest reproductive activity when the mean body weight at harvest was less than 40 g, whereas in the fastest growing strain (E1), the onset of reproduction occurred at a higher mean body weight (52 g). E1 reached its highest reproduction index value when mean body weight was about 67 g (about 68% higher mean body weight compared to strain Gh).

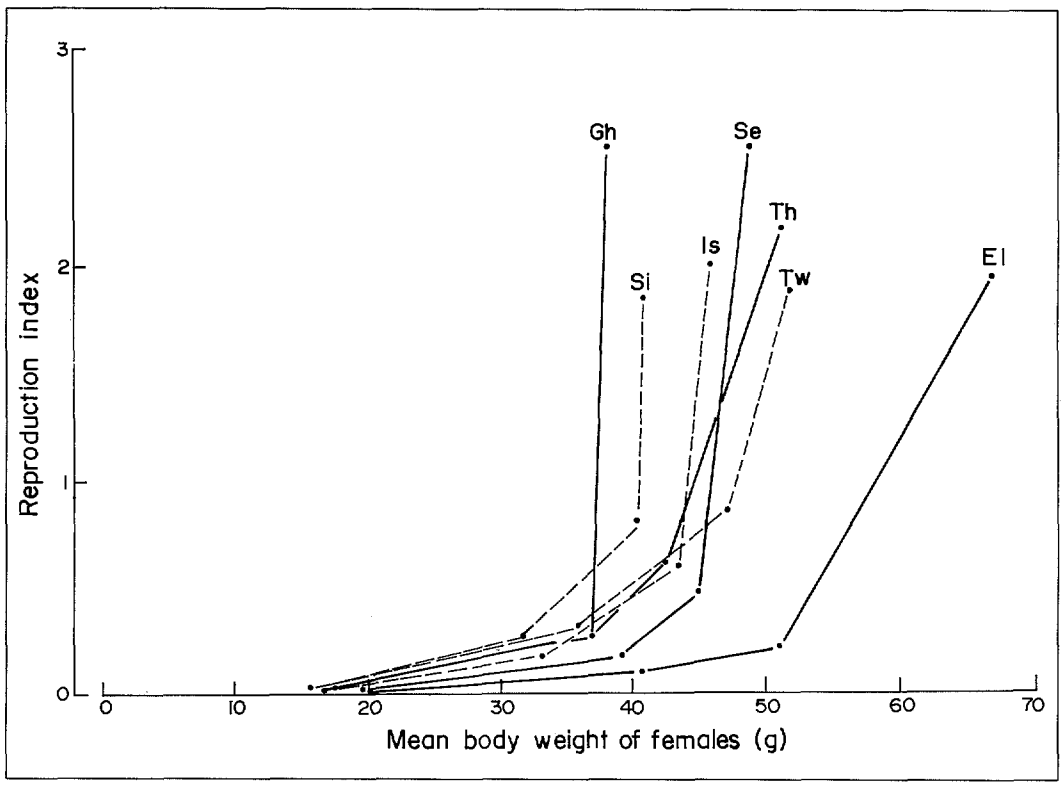

Fig. 3. Reproduction index as a function of the mean body weight (g) of seven strains of female *Oreochromis niloticus* across test environments.

Discussion

The reproduction index developed here provides valuable insights into the reproductive activity of the different tilapia strains across diverse test environments. As can be expected, the reproduction index of females of comparable mean body weights was relatively lower in cages than in other environments. In fertilized earthen ponds, where an adequate substrate for nesting is available, the reproduction index was relatively high. Across test environments, it appeared that reproduction commenced when females reached a mean body weight of about 25 g. The apparent exceptions were RF and one of the pond environments fertilized with leaves and vines of sweet potato (W3). In the RF environment, reproductive activity commenced at lower mean body weights, while in W3 there was no sign of reproductive activity. It is possible that the exudates from the leaves and vines may deter reproduction. This is being investigated in the GIFT project.

There was considerable variation in reproduction indexes among the strains tested (Fig. 3). In general, the slower growing strains commenced reproductive activity earlier and at relatively smaller body sizes than the faster growing strains. In a related study, it has been shown that the divergent growth performance of sexes is strain-specific. Divergence of growth rates occurred at later ages and at higher mean body weights in the fastest growing strain (E1) than in the slowest growing strain (Gh) (Palada-de Vera and Eknath 1993).

Overall, the results here suggest that the reproductive activity of the strains

is relatively consistent across these test environments (Fig. 2). The relative growth performance of the same strains was also shown to be consistent across a range of test environments (Eknath et al. 1993). The GIFT project has now built a base population composed of best performing strains and their crosses. Selection for fast growth is in progress. The future strategy is to incorporate the procedure outlined here to estimate the reproductive activity of full- and half-sib families, and to include this trait in a selection index. Families in representative test environments will be screened for reproductive activity once during the production cycle. The approach is to assign lower weighting to families with relatively higher reproduction index values when estimating breeding values. Meanwhile, research will continue to develop more objective criteria for assessing reproductive performance of both males and females.

This study focused on estimating the reproductive activity of female tilapias. An analogous reproduction index for males, based on their nuptial coloration, stripping of milt by applying gentle pressure on the abdomen and counting of nests following draining of ponds, was also attempted. This was found impractical because nuptial coloration was highly variable among strains, expression of milt was difficult to quantify, and nests were almost invariably destroyed during seining and draining operations. Furthermore, counting of nests was almost impossible in the RF environment.

Acknowledgements

This work forms part of the collaborative research project on the Genetic Improvement of Farmed Tilapias (GIFT), co-financed by the Asian Development Bank (RETA 5279) and the United Nations Development Program/Division for Global and Interregional Programs (INT/88/019). We are grateful to Drs. Hans Bentsen, David Penman, Daniel Pauly and Roger S.V. Pullin for their valuable comments and suggestions.

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Searching for Behavioral Isolating Mechanisms in Tilapias

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FALTER, U. 1996. Searching for behavioral isolating mechanisms in tilapia, p. 299-307. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

In tilapia, especially in species belonging to the genus *Oreochromis*, hybridization occurs spontaneously at a high rate. It therefore does not seem to happen by accident. Given its consequences which differ according to the environment (disastrous in the wild, desirable in a controlled culture system), there is a need to determine the factors which are involved in this process. The present analysis focuses on behavior. Using mainly two species, *O. niloticus* and *O. mossambicus* (two strains), but including in some experiments *O. macrochir*, *O. aureus* or *O. salinicolus*, several experiments were run in which intra- and interspecific communication during courting and mating was studied. Although visual, chemical and/or acoustic signals contributed to the identification of the homospecific mate, their importance seems to be limited, the major mating barrier being the species-specific aggression level, which characterizes not only the sexes but also the species.

Introduction

In tilapias, hybridization is a common phenomenon in natural and artificial environments such as lakes, ponds or tanks. The consequences may be considered either as disastrous (natural environment) or as beneficial (human-controlled environment). During the last decade, many studies and experiments have been performed to evaluate the biological and economic impacts of tilapia hybrids. However, little effort has been spent on the study of the mechanisms which actually enable hybridization, i.e., spawning between heterospecific mates.

There are three major reasons for studying these mechanisms. First, hybrids challenge the concept of species (Templeton 1989). In tilapias, this is reflected by the different criteria used in several classification systems, the genus being characterized either by criteria on which the natural populations diverge or, in contrast, by those on which they converge. Second, once two species meet in open waters subsequent to a voluntary or accidental introduction of at least one of the species, the possible spontaneous apparition of the hybrids generally has a negative effect on the natural production of the waters (Daget and Moreau 1981). Improved knowledge of the mechanisms involved in hybridization would lead to more accurate predictions on the consequences of such introductions. Third, since the culture of hybrids in human-controlled rearing systems can be attractive for economic reasons, insight into the underlying mechanisms could not only improve the techniques of hybrid production, but also might encourage the breeding of new hybrid crosses with good potential for culture in extreme habitats like those with high salinity.

Since interspecific mating in tilapias primarily involves behavioral factors, the present analysis used ethological methods to investigate possible behavioral isolating mechanisms. It was limited to maternal mouthbrooding species, a subgroup in which spontaneous hybridization occurs most frequently and is of most practical and economic interest.

Isolating Mechanisms in *Oreochromis* spp.

Applying the categories of isolating barriers described by Dobzhansky (1970) to the genus *Oreochromis*, Table 1 formulates them in behavioral terms whenever behavioral mechanisms are involved. The development control includes parental care, such as mouthbrooding in this genus. It can be seen from this table that even when the topic is limited to behavioral mechanisms, a multitude of factors, including those concerning the nest site or the time of spawning, must be studied in the natural environment, those related to communication between mates being more easily tested in the laboratory.

The present paper focuses on interspecific mate communication by testing the effect of visual, chemical or acoustic signals on the behavior of the heterospecific mate and by analyzing the sequence of behavioral interactions during interspecific courtship.

Visual Signals

Vision plays a dominant role in cichlid behavior, including spawning (Baerends and Baerends-Van Roon 1950). Mates are visually informed of the sexual status of a potential partner either by its morphological characteristics, which may be permanent (secondary sexual characteristics) or temporary (protrusion of specific organs, i.e., genital papilla, exhibition or specific color pattern), or by the form and the sequence of its behavioral display.

In order to evaluate the ease with which cichlids recognize conspecifics on the basis of visual cues, an experiment was designed in which the behavior of *O. niloticus*, *O. mossambicus* (albino strain) or F_1-hybrid females that had ovulated towards males of the two pure species was recorded. Males of similar weight were confined behind a sealed glass pane and presented first separately, then simultaneously, to the females as described in Falter and Charlier (1989). Once the experiment was completed, females were given access to the males they had chosen.

Females generally chose the males of their own species, although some females were not only attracted to, but also accepted spawning with a heterospecific male (Tables 2 and 3). Also, according to the species to which the male and the female belonged, the probability of mating differed. Crosses between *O. mossambicus* females and *O. niloticus* males regularly resulted in oviposition, whereas the reverse cross only reached this point with difficulty due to the excessive aggression of the *O. niloticus* female. Mate choice in the hybrid females was less restricted than in the pure females, with hybrid females accepting the males of both parental species equally.

From this experiment, it can be concluded that in most of the encounters, visual cues alone ensure mate recognition. Nevertheless, these stimuli are not sufficient to prevent interspecific matings in all cases, since a few females were significantly more attracted by the heterospecific male despite its differences in coloration, and in the form and frequency of its courting behavior.

Table 1. Isolating mechanisms between species of *Oreochromis*.

Barrier for males	Male of one species	Process and results	Female of another species	Barrier for females
Pre-mating	Mature and having specific requirements concerning the place and the time of spawning	Encounter Same place, same time	Mature and having specific requirements concerning the place and the time of spawning	Etholo-gical
	Innate mate scheme, and possible individual experience with previous mates	Mutual acceptance as potential mate	Innate mate scheme, and possible individual experience with previous mates	
	Balance between sexual, fight and flight motivation	Reciprocal adjustment of the behavior during courtship to reach synchronization of mating (90 seconds)	Balance between sexual, fight and flight motivation	
None (mating)	Fertilization	Gamete fusion	Oviposition	None
Post-mating	Development controlled in some cases by the male	Egg stage Wriggler stage Fry stage	Development controlled in most of the cases by the female	Multiple
		Viability and flexibility of the F_1, F_2 and F_n-hybrids		

Table 2. Time (in seconds) the three categories of female spent near the *Oreochromis niloticus*, *O. mossambicus* male or in the neutral zone (means and standard error of means) (Wilcoxon test).

Type of females	*O. mossambicus* male	*O. niloticus* male	Neutral zone	Probability
Hybrid	426 (317)	498 (372)	876 (312)	N.S.
O. mossambicus	766 (196)	259 (180)	775 (276)	<0.0001
O. niloticus	227 (148)	881 (358)	692 (249)	<0.0001

Chemical Signals

Since sexual pheromones are released by female cichlids subsequent to ovulation, the role of these chemical signals was determined in a second series of tests (Falter and Dolisy 1989). This time, the aim of the experiments was to ascertain the existence of sexual pheromones in prespawning *O. niloticus* and *O. mossambicus* females, to determine the specificity of these substances and to evaluate the preference of hybrid males (parents: ♀ = *O. niloticus*, ♂ = *O. mossambicus* albino strain) for the chemical signals released by the females of both parental species.

Samples of water, presumed to contain the pheromones, were collected from small tanks in which ovulated females of either species had been isolated for four to six hours. The samples were transferred into Baxter bottles and released drop-by-drop in the two opposite corners of the test tank containing the males. A mirror was placed in each of these corners to provide a neutral cue to which the test male could direct its sexual and/or aggressive behavior.

Four series of experiments were carried out in which *O. niloticus* or F_1-hybrid males (parents: ♀ = *O. niloticus*, ♂ = *O. mossambicus*) were provided with pairs of test substances:

Series 1:
O. niloticus ♂ : clean water
vs.
ovulated *O. niloticus* ♀

Series 2:
O. niloticus ♂ : ovulated *O. niloticus* ♀
vs.
non-ovulated *O. niloticus* ♀

Series 3:
O. niloticus ♂ : ovulated *O. mossambicus* ♀
vs.
ovulated *O. niloticus* ♀

Series 4:
F_1-hybrid ♂ : ovulated *O. mossambicus* ♀
vs.
ovulated *O. niloticus* ♀

In series 1, *O. niloticus* males directed nearly all their behavior towards the side containing the sample taken from the tank of the ovulated female (Table 4).

Table 3. Occurrence of oviposition during and after mate selection tests.

Type of Females	Frequency of oviposition	During the test near: *O. mossambicus* male	During the test near: *O. niloticus* male	During the 15 hours following the test near: *O. mossambicus* male	During the 15 hours following the test near: *O. niloticus* male	Alone
Hybrid	14/17	0	1	5/6	6/6	2/5
O. mossambicus	3/13	1	0	0/4	2/4[a]	0/5
O. niloticus	0/12	0	0	0/4	0/4	0/4

[a]One *O. mossambicus* female was placed in the compartment of the *O. niloticus* male after the test.

Table 4. Differences in biting and digging frequencies (and time in seconds) between *Oreochromis niloticus* (M) or F_1 hybrid (H) males (both types with n=12) exposed to either an ovulated *O. niloticus* female or another treatment.

Series	Behavior	Ovulated ♀	Other treatment	Prob. of difference
1(M)			Clear water	
	Biting	42.9	7.7	<0.01
	Digging	21.5	0.6	<0.02
	Time	631	260	<0.001
2(M)			Non-ovulated ♀ *O. niloticus*	
	Biting	14.6	4.3	<0.001
	Digging	14.5	3.4	<0.001
	Time	600	299	<0.001
3(M)			Ovulated ♀ *O. mossambicus*	
	Biting	38.1	10.4	<0.01
	Digging	11.4	2.8	<0.01
	Time	587	313	<0.001
4(H)			Ovulated ♀ *O. mossambicus*	
	Biting	7.2	4.4	<0.05
	Digging	11.7	3.7	<0.001
	Time	486	414	<0.05

In series 2, *O. niloticus* males displayed more sexual activity to the side which contained the sample taken from the ovulated female as opposed to a nonovulated female. In series 3, *O. niloticus* males preferentially displayed near the sample from their own females, but also displayed near the heterospecific one. In series 4, F_1-hybrid males were more attracted by the sample taken from the tank of *O. niloticus* females, the species to which their mother had belonged.

The conclusions concerning the chemical signals are therefore similar to those mentioned above for visual cues. Females, once they have ovulated, release chemical attractants which are perceived by the males. In addition, males are able to discriminate between pheromones released by different species, although the substances released by heterospecific females remain attractive to them.

Acoustic Signals

Cichlids also communicate by acoustic signals, especially during territorial defense and courtship, hence some preliminary studies were also made of this aspect. In this experiment, males of several *Oreochromis* spp. were placed in large noise-attenuated tanks in which they defended a breeding territory. For each species, a sonogram reflecting the frequencies of the emitted sounds was established confirming species-specific differences. Using a hydrophone, these differences were discernible by the human ear, with territorial *O. niloticus* emitting single bursts of clearly distinct emissions (four to five knocks) and territorial *O. mossambicus* males emitting several bursts consecutively with less distinct emissions (drumroll). Females also emitted sounds, but only when they were aggressive, never when they were

spawning. Cross-species communication was not examined.

Taken together, all these results concerning sensory communication confirm that each species has developed its distinct visual, olfactory and acoustic signals which enable it to distinguish between its own and a closely related species. However, these distinctions do not appear to be of the all-or-none type but rather reflect some kind of specific preferences, which may lead, in some cases, to a preference by an individual for the other species.

Courtship Sequences In Intra- and Interspecific Encounters

It is apparent from the above that sensory cues are not sufficient to prevent hybridization. Behavioral premating barriers, if they exist, should therefore be likely to occur during the courtship phase and to operate by disrupting the behavioral interactions which are necessary for the mutual synchronization of the mates. Since pairbonding is absent in the *Oreochromis* species, the courtship phase is generally short, varying, under laboratory conditions, between a few minutes and several hours. As a consequence, isolating mechanisms have to be effective within this short period.

The behavioral dynamics during the courtship phase were examined in an experiment in which males and females of *O. niloticus* and *O. macrochir* met each other in intra- and interspecific encounters (Falter and Dufayt 1991). Records were taken of the sequence of the different male and female behavioral patterns, with observations lasting either until the female laid her first clutch of eggs or until one fish definitively moved away.

Spawning success depends upon the type of cross, with a strong barrier preventing hybridization between *O. niloticus* females and *O. macrochir* males (Table 5). A possible explanation of the mechanism by which hybridization is prevented in this cross was provided by the comparison of the behavioral interaction sequences shown by the four crosses. This analysis revealed that the behavioral sequences were composed of two separate classes of behavior, the first comprising the acts performed outside the nest (mostly aggression and courtship), and the second comprising the behavioral patterns performed inside the nest (mostly related to courtship and spawning). Species-specific differences mainly appeared in the first

Table 5. Spawning success in two intra- and interspecific encounters of *Oreochromis* spp. (Falter and Dufayt 1991).

Females	Males	
	O. niloticus	*O. macrochir*
O. niloticus	6 spawnings from 11 attempts	0 spawning[a] from 7 attempts
O. macrochir	6 spawnings from 10 attempts	6 spawnings from 7 attempts

[a]Six of these seven *O. niloticus* females spawned immediately when confronted afterwards to a male of their own species.

class; *O. niloticus* males and females were more aggressive than *O. macrochir* males and females. Differences in aggression also characterized the two interspecific encounters: aggression was almost absent in the cross culminating in oviposition, whereas aggression was the dominant feature in the other cross in which interactions between mates broke off before spawning had occurred.

The results suggested that the interactions between *O. niloticus* females and *O. macrochir* males had been disrupted by the excessive aggression level of the female. Two explanations could account for this: either the *O. macrochir* males were not aggressive enough to withstand the aggression of the *O. niloticus* females or, since territorial *O. niloticus* males are brightly colored, the *O. niloticus* female did not accept the black-colored *O. macrochir* male as a potential mate and therefore reacted aggressively.

To investigate these two explanations, an additional experiment was designed in which *O. niloticus* females were confronted with F_1-hybrid males which combined the "wrong" color pattern (nearly as black as the *O. macrochir* males) with the "right" aggression level (nearly as aggressive as the *O. niloticus* males). In six out of nine attempts, *O. niloticus* females readily accepted spawning with the F_1-hybrid males, revealing that an appropriate aggression level was essential for the mating success, sexual coloration playing only a secondary role, if any.

These results clearly show that *Oreochromis* species have developed species-specific signals and ways to interact. Within this maternal mouthbrooding group, the species-specific characteristics appear to have evolved in a quantitative rather than qualitative manner, the animals expressing stronger preferences for the traits of their own species but still remaining receptive to signals emitted by other species.

This quantitative rule applies to aggression, the only efficient barrier which was found to operate at the behavioral level. Aggression is a major component for success in breeding. Males generally have to eject a territory owner from its nest site by aggression to get access to the territory. Once they have succeeded, they have to fight either to attain a better territory or just to defend it against other competitors. When a female starts visiting a male's nest, she is often initially treated like a male and chased away. Females, whose own aggression level has drastically increased during the period preceding ovulation, generally withstand these attacks and return to the male. In most cases, males adapt their behavior after a while, switching from aggression to courtship. In some cases, however, males, especially those who have to defend a coveted area, fail to change their strategy and continue to attack the females. In this case, females switch to another male who courts them more readily but who is still able to keep away other intruders. The role of aggression in spawning success was investigated in an experiment in which behavioral interactions between *O. mossambicus* males and females were followed over several months in small breeding colonies (Falter and Foucart 1991). Thus, although aggression can sometimes endanger spawning even within a homospecific population, the aggression level between the two sexes is generally equilibrated, i.e., high enough in the females to resist the attacks of the males of their own species.

One of the consequences of these quantitative barriers is that differences among individuals are of major importance for hybridization. In fact, it is possible to rank the species on an aggression gradient with overlapping zones

between species. Moreover, by adapting the environment to the behavioral requirements of the most demanding species, it should be possible to enlarge the extent to which individuals of the two species would accept to interact with each other.

This analysis leads to two major practical applications. Concerning the question whether to introduce a tilapia species into a natural environment, the decision should depend on the inventory of the species already existing in this habitat. In the case where two species of the same genus would meet, the risk of spontaneous hybridization would be high due to the large behavioral plasticity of the individuals. In contrast, this risk is limited in the case of intergeneric encounters, voluntary hybridization being absent between *Sarotherodon* and *Oreochromis* species (Fishelson 1988) as well as between *Tilapia* and *Oreochromis* species (Lovshin 1982).

These findings could contribute to a more systematic exploitation of the spontaneous tendency that tilapias have to hybridize. New crosses could be tested. In this respect, the cross between *O. niloticus* and *O. salinicolus* seems to be particularly promising because it could produce a hybrid which resists high salinity and extreme temperature fluctuations. *O. salinicolus* is a maternal mouthbrooding species which lives in the saline springs of Mwashia (Shaba, Zaire) and resists the extreme environmental conditions of high temperature and high salinity. In the wild, their growth seems to be stunted, the total length ranging between 1.5 and 9.0 cm (Thys van den Audenaerde 1964). Under laboratory conditions, progressively adapted to freshwater, their growth rate is much higher: one individual reached 22.5 cm TL and weighed 217 g after three years (unpubl. data). Individuals caught in the wild are extremely aggressive under laboratory conditions requiring therefore to be housed in very large aquaria, but their spawning behavior is similar to other *Oreochromis* species and hybridization should therefore be possible.

Conclusion

Hybridization occurs frequently in tilapias, with barriers acting as spacing rather than isolating mechanisms. Moreover, among the tilapiine cichlids, the speciation rate has been considered low, the fish considered generalists and hybridization thought of as accidental. An alternative hypothesis could, however, be suggested. Given the special features (e.g., broad ecological tolerance, good resistance to diseases, efficient breeding systems, high behavioral plasticity, etc.) which characterize this group (Fryer and Iles 1972), hybridization could also be considered an active process by which these species counteract their tendency to speciate and which helps them to remain generalists. This view would fit the cohesion concept of species proposed by Templeton (1989) who defines a species as the most inclusive population of individuals having potential for phenotypic cohesion through genetic and/or demographic exchangeability, a definition which goes beyond the solely reproductive criteria.

Acknowledgements

I would like to thank Prof. G. Thinès, Prof. J.C. Micha and Prof. J.P. Gosse for their advice and encouragement.

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Plasticity in the Parental Cycle of *Oreochromis niloticus*

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GAUTIER, J.Y, M.A. RICHARD-YRIS, B. LE FAUCHEUX and M. FORASTE. 1996. Plasticity in the parental cycle of *Oreochromis niloticus*, p. 308-313. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Applied to mouthbrooding females of *Oreochromis niloticus*, the cross-adoption method (adoption of young fry after removal of the eggs or adoption of eggs after removal of the fry) shows plasticity in the maternal behavior of this species. Brood rejuvenation (adoption of eggs) is accompanied by a significant increase in the total duration of the parental cycle and of each of its phases (incubation before and after release, and aggressive behavior). The aging of the brood (adoption of fry) produces the opposite effects of which the reduction of the incubation period after release of the fry is the clearest.

This shows that the females tend to adjust their parental behavior to the spawn age. However, this adjustment is not optimal in relation to the age of the fry at the time of first release. This suggests a double control in maternal behavior: an external control involving buccal stimulation by the brood and an internal control the nature of which is not yet fully understood.

Introduction

The complex nature of their parental behavior is one of the major characteristics of the reproductive cycle in species of the genus *Sarotherodon* and *Oreochromis* (Cichlidae) (Ruwet et al. 1976; Perrone and Zaret 1979). The physiology of reproduction in these species has been the subject of many studies which have significantly contributed to the development of their culture (Baroiller and Jalabert 1989).

The objective of our study on *Oreochromis niloticus* was to analyze the regulation of maternal behavior. After spawning and fertilization of the eggs, the female takes them in its mouth and incubates them, and then the fry, until hatching which also takes place in the mouth.

This first period of normally uninterrupted mouthbrooding ends with the mass release of all fry. Then the second period begins, characterized by incubations of variable duration of all or part of the fry with a total reabsorption at night, and by the aggressive behavior of the female to protect the fry. This behavior persists even after all diurnal incubations have stopped. Later, the female loses interest in the fry and cannibalism is frequent if the fry and the female are kept in the same aquarium.

The aim of the present study was to establish the role of the buccal stimulations by the eggs and the fry in

[a]Research conducted under the auspices of the Groupement de concertation scientifique sur les bases biologiques de l'aquaculture, "Groupe aquaculture tropicale."

the sequential organization, and the duration of the different periods of the maternal cycle. To do this, we used the cross-adoption method leading either to the rejuvenation or to the aging of the incubated brood (eggs or fry).

Materials and Methods

Animals and Maintenance

The fish (*O. niloticus*) used in this study came from the Pisci-Meuse fish culture station in Tihange, Belgium. The female and the male mean weights were.304.6 g (±7.8) and 450-500 g, respectively. Females taken from a mass production pond where males and females live separately were weighed, identified individually using Alcian blue, then regrouped at the rate of three to six individuals per 300-l aquaria with gravel at the bottom. Recirculating warm water (26±1.5°C) was used to supply the aquaria, which were located in a room with a 12/12 photoperiod. Animals were fed a supplementary feed twice a day (2 g per 100 g of body weight).

When a female showed signs of sexual receptivity, particularly the enlargement of the genital papilla, it was transferred to a 300-l aquarium where a male breeder was established. After spawning and fertilization, the female was transferred to a 120-l aquarium. Mouthbrooding females were isolated, keeping only visual contacts with their conspecifics. They were no longer fed until the release of the fry.

Experimental Batches

Three batches of females were used:

- one "control" batch, n=21; after fertilization of the brood, females incubated their eggs then the fry without being subjected to any particular experimental procedure;
- one batch labeled "adoption of eggs," n=10; after incubation of their eggs then of their young fry, the females were subjected to an egg adoption procedure (Table 1); and
- one batch labeled "adoption of fry," n=12; females incubating their eggs were subjected to a procedure of adoption of young fry (Table 1).

Cross-adoption Technique

The female incubating the eggs and the female incubating the fry were again removed from the aquarium. Then, each

Table 1. Age of the adopted spawn (eggs or fry of *Oreochromis niloticus*) and intervals between the dates of spawning and the dates of adoption.

	Age of the adopted spawn: eggs or fry	Intervals spawn-adoption
Adoption of eggs	1-3 days	7-12 days
Adoption of fry	7-12 days	1-3 days

of the females was captured by hand to induce the regurgitation of the eggs or of the fry into a bucket. The eggs and the fry were then deposited at the bottom of the aquarium in an area where the gravel had been removed, and the adoptive female was introduced into the aquarium immediately afterwards.

The first adoption tests were conducted without anesthesia. Later, phenoxyethanol was used to anesthetize the females in the bucket to reduce the effects of stress produced during the procedure. Since light anesthesia did not affect the behavior of the females, they were anesthetized routinely. The age of the adopted eggs as well as the intervals between spawn and adoption are shown in Table 1.

Measurements

We used four parameters expressed in days: the total incubation period from spawning up to the "first release" of the fry (100% of the fry released); the real age of the fry at first release; the incubation period after release of the fry corresponding to the number of days after the first release during which the female takes back all or part of the fry into its mouth, particularly at night; and the duration of the aggressive behavior of the female after first release. The aggressive behavior displayed in response to the introduction of the hand along the aquarium was recorded every day.

Statistical Analysis

An analysis of variance was done to compare the three experimental batches. When F values were significant, the mean results of the different treatments were compared at the probability level of 5% (ANOVA and Scheffé's test).

Results

Behavior of Females during Adoption

ADOPTION OF THE FRY

The experiment was conducted on 14 females, but due to technical problems (capture of one female and imprecise date of release of fry for another female), results were analyzed for 12 animals only. The process of uptake of the fry into the mouth of the female was rapid, usually starting when the fry and the female met. In our experiment, the uptake was total or near-total in less than 30 minutes for nine of the females and in 40-90 minutes for three others. However, it took over six hours for the uptake of the fry to be completed in two females.

The number of adopted fry varied from 100 to 500, and on the day of release their number decreased only slightly or not at all, except in two instances where fry decreased from 200 to 100 and from 500 to 50. In these instances, the fry were not very vigorous.

ADOPTION OF EGGS

The experiment was conducted on 14 females, but one female did not incubate the eggs; two females stopped mouthbrooding two and three days after the adoption and ate the eggs; and one female only incubated a few fry after their release. Therefore, results were analyzed for 10 animals only. Although the incubation periods were variable from female to female, the adoption of eggs was slower than for the fry. While seven females incubated more than half of the eggs in 30 minutes, the adoption was slower for the others, reaching up to six hours and over in some instances.

Only a small number of females adopted all eggs present in the aquarium.

The number of adopted eggs varied from 100 to 400 and the number of fry released decreased very little or not at all, except in two females (50-60 fry at the moment of release). The same phenomenon was observed in the control animals.

Comparison between Batches

An analysis of variance showed significant differences among batches with $P<0.05$ for each of the four parameters established (see results summarized in Fig. 1).

The adoption of eggs (corresponding to a brood rejuvenation) caused the incubation period before release to lengthen compared to the controls ($P<0.02$), whereas the adoption of fry (corresponding to the aging of the brood) caused the incubation period before release to shorten ($P<0.05$).

The examination of the age of the fry at the time of release supplemented the analysis. When eggs were adopted, they were on the average three days younger than the controls ($P<0.02$). Therefore, despite the lengthened incubation period, fry were released too early. On the other hand, when fry were adopted, they were five to six days older than

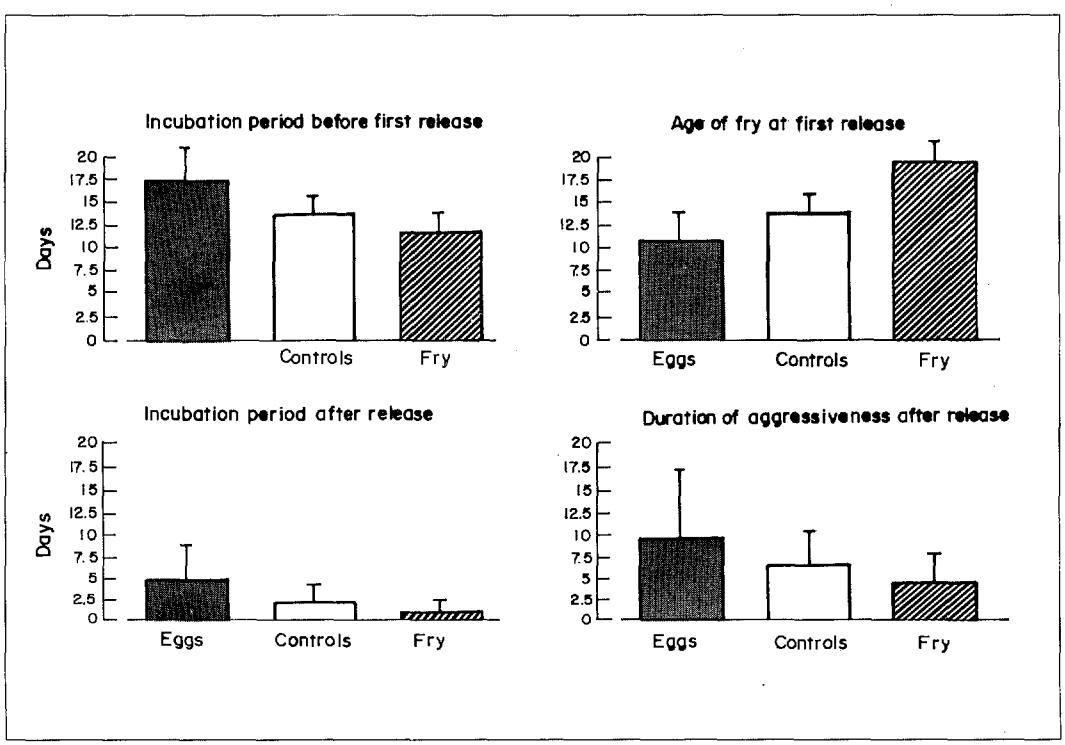

Fig. 1. Results of experiments on adoption of eggs or fry by females of *Oreochromis niloticus* (mean values and standard deviations of the four parameters: incubation period before first release of the fry; real age of the fry at first release; incubation period after release of the fry; and duration of the aggressive behavior of the female after first release).
Eggs: adoption of eggs by the females (n=10).
Controls: control females (n=21).
Fry: adoption of fry by the females (n=12).

the controls ($P<0.02$) at the time of first release. The adopted fry were therefore "abnormally" aged at the time of release despite the shortened period of incubation by the adoptive females.

Concerning the last two parameters, the incubation period after release was longer in females adopting eggs than in controls ($P<0.05$) and in females adopting fry ($P<0.02$) where it was actually nonexistent in eight females. However, the difference between controls and adoptions of fry did not reach the 0.05 level of significance.

The mean values for the duration of the aggressive behavior after release of the fry followed the same tendency as with the previous criterion, but the difference was significant only between adoption of eggs (9.7 days) and adoption of fry (4.4 days).

Discussion

Females of *O. niloticus* can adopt either eggs or fry after the removal of their own brood. Females adopt fry easily, probably because they see them better than the eggs since the fry are capable of small movements (seven-day-old fry) or start swimming (10-12-day-old fry). Moreover, older fry (12 days) facilitate their rapid adoption as they move towards the female. This stimulation of the females by the fry may explain the rapidity with which all fry are adopted.

Females encounter more difficulties with immobile eggs lying at the bottom. Eggs are detected and adopted by searching the bottom of the aquarium. On the other hand, females that are distressed by the removal of their own brood tend to swim in midwater. This may explain the failures observed and the longer periods of time needed to reabsorb the eggs into the mouth compared to the time needed for the fry. Finally, the females return several times above the brood to take up again the eggs into their mouth; these movements disperse the brood and eggs that are too scattered are left on the bottom.

This cross-adoption technique shows the plasticity of the parental cycle in the females of *O. niloticus*. Adoption of eggs causes a significant increase in the total duration of the parental cycle and in that of each of its phases: the incubation period before and after first release of the fry and the period of aggressive behavior both lengthen. However, the cycle plasticity is not optimal because fry are released prematurely, despite the extension of the incubation period before release. However, because the post-release incubation is longer than in controls, this compensates for the lack of incubation before release. Fry from adopted eggs and controls enjoy the same incubation period when the incubation periods, before and after release, are added. After the adoption of fry, females shorten the incubation period before, and especially after, release and shorten the duration of the aggressive behavior period as well. But here again, the incubation period before release is not optimal. These females continue to incubate abnormally-aged fry.

To conclude, females try to adjust their behavior to the age of the brood: the younger the fry, the more maternal they become (incubation and defense of the fry); and they quickly lose interest in the older fry after their first release. Although the incubation period before the first release of the fry is modified by the adoptions, it seems to be the least elastic phase of the parental cycle.

These results suggest a double control of the maternal behavior: on the one hand, an external control shown by the above adjustments, and involving stimulation of the mouth of the females by the eggs and the fry; and on the other hand, an internal

control, probably related to hormonal balance, which could oppose the optimal adjustment of the female's behavior to the age of the fry. This hormonal component remains to be identified. Smith and Haley (1987) compared non-incubating females of *Oreochromis mossambicus* (eggs were eaten by the females) with incubating females and observed that in the incubating females, the next oocyte development was slower and the post-ovulatory follicles did not degenerate as fast. These authors suggest that follicles have an inhibiting effect, at least partially, on oocyte development. This hypothesis is supported by the study on the hormonal profiles of incubating and nonincubating females (eggs removed or eaten within 24 hours after spawning). Comparable levels of 17-ß oestradiol and testosterone were observed later in incubating females (Smith and Haley 1988). Follicles could also be involved in maintaining the parental behavior.

On the other hand, the influence of prolactin on behavior is not excluded. In fact, prolactin is known to stimulate ventilation in cichlids (Blum and Fiedler 1965; Blum 1966). Although Wendelaar Bonga et al. (1984) did not show any increase in prolactin production during the parental cycle in *O. mossambicus*, Tacon (1991) had opposite results (tendency of the prolactin to increase during maternal care) in *O. niloticus*.

These data are not incompatible with the hypothesis of the buccal stimulation by the eggs and then by the fry, which, through a neuroendocrine reflex, would allow the post-ovulatory follicles to remain with their secretions and their inhibiting effects on oogenesis.

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Sex Reversal of Tilapia Fry by Immersion in Water Containing Estrogens

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GILLING, C.J., D.O.F. SKIBINSKI and J.A. BEARDMORE. 1996. Sex reversal of tilapia fry by immersion in water containing estrogens, p. 314-319. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Oreochromis niloticus (L.) fry were subjected to immersion treatments in three different estrogens at concentrations of between 4 and 500 $\mu g \cdot l^{-1}$ for durations of up to 30 days. Ethynylestradiol (EE) proved the most successful in inducing feminization, although 17ß-estradiol and diethylstilbestrol achieved significant conversion to female in some treatment groups. The optimal time of starting treatment was found to be on or before the first feeding stage and the optimal duration of treatment was 18 days at a concentration of 170-200 $\mu g \cdot l^{-1}$ EE. Implications for the production of all-male fry for aquaculture are discussed.

Introduction

Culture of all-male tilapia populations has been proposed as the best solution to the problem of precocious sexual maturity, unwanted reproduction and overpopulation in tilapia ponds. Although alternative methods exist whereby this can be achieved, recent research has concentrated on the production of genotypic males through the manipulation of sex determining mechanisms in broodstock (Shelton 1987; Scott et al. 1989). The production of the novel genotypes, heterogametic XY females in *Oreochromis niloticus* and homogametic ZZ females in *O. aureus*, provide models for all-male fry production in these species. Two fundamental problems in the use of this approach are the unreliability of treatments to induce sex reversal of males to females (feminization) and the conflicting evidence on the precise mechanisms of sex determination in *Oreochromis* species.

Large-scale production of YY male broodstock in *O. niloticus* is dependent upon the efficient feminization of sexually undifferentiated fry. Masculinization of fry for the production of male fish with female genotypes needed for breeding programs and direct stocking is readily achieved by adding androgens to fry food (Guerrero 1975). Tayamen and Shelton (1978) demonstrated that *O. niloticus* could be feminized by feeding of diethylstilbestrol (DES) at 100 $mg \cdot kg^{-1}$ of food and Varadaraj (1989) reported 100% feminization of *O. mossambicus* by feeding DES at concentrations of 100 $mg \cdot kg^{-1}$ or more to young fry. However, routine use of this treatment on *O. niloticus* fry in our laboratories has produced highly variable rates of sex reversal and there is some evidence of strain-specific susceptibility to sex reversal in this species (G.C. Mair, pers. comm.). In this paper, we present the results of attempts to feminize *O. niloticus* fry by immersing them in water

containing three estrogens at a range of concentrations.

Materials and Methods

The species initially used was a strain of *O. niloticus* which had been selected at Baobab Farm (Kenya) for fast growth, light body color and high depth to length ratio. Individual sexually mature males and females were kept in 200-l glass aquaria with undergravel filtration at 27°C with a 12-hour light photoperiod and fed a commercial trout feed at approximately 5% body weight per day. Males and females were separated by a perspex sheet but when a female showed signs of imminent spawning (extended genital papilla and intense nest-building activity), the male was introduced and spawning was allowed to occur.

After three days, the eggs were removed from the female and placed in incubators until the start of the hormone treatment. Larvae were placed in vigorously aerated 20-l aquaria at a stocking density of approximately 3 per liter and were fed on Artemia nauplii for the first few days, prior to a powdered commercial trout diet fed ad libitum. Water was changed and the tanks thoroughly cleaned every other day.

All hormone treatments were conducted in a constant temperature room at 28°C, until the fry had reached a mean standard length (SL) of 20 mm (regardless of the duration of hormone treatment). They were then transferred to plastic bins in a closed recirculating system where they were grown-on to 60 mm SL. Fish were sexed by removing the gonads, staining squashes with acetocarmine and examining them under 40x magnification (Guerrero and Shelton 1974).

Data from each replicated experiment were subjected to heterogeneity Chi-squared analysis and, where samples were homogeneous, data were pooled. Treatment data were then tested against their control group for goodness of fit using a Chi-squared contingency test.

The hormones used were 17ß-estradiol (BE), DES and ethynylestradiol (EE), all obtained from Sigma Chemicals, UK. They were dissolved in 95% ethanol at a concentration of 2 $g \cdot l^{-1}$. This solution was added to the water after each tank water change to make up the required concentration. The initial number of fry in each treatment was the same as that of the control group. High mortality has been reported in salmonid fry immersed in estrogen solutions at high concentrations (Nakamura 1981). So, initially, a range of concentrations of between 4 and 500 $\mu g \cdot l^{-1}$ was used and mortality was monitored over a 30-day period.

Results

Hormone Concentration and Mortality

Table 1 shows the results of the initial trials. DES proved to be the most toxic and BE the least. Concentrations equal to or greater than 250 $\mu g \cdot l^{-1}$ EE produced an unacceptable level of mortality (>50%), as did 500 $\mu g \cdot l^{-1}$ DES. Few fry survived for more than 18 days in 200 $\mu g \cdot l^{-1}$ DES. In all cases, mortality was correlated with increasing hormone concentration. Treatment mortality is given as a percentage of the number remaining in its control group at the time of removal from the tanks to bins in the recirculating systems (30 days after first feeding). Mean survival in control groups was 96.4%.

Concentrations of 200 $\mu g \cdot l^{-1}$ BE and greater, 100 $\mu g \cdot l^{-1}$ EE, and 100 $\mu g \cdot l^{-1}$ DES gave a significant bias towards females in treated groups. In addition to causing high mortality, very high concentrations of hormone (500 $\mu g \cdot l^{-1}$ BE, and 300 $\mu g \cdot l^{-1}$ EE and DES) did not feminize all of the few fish that survived.

Table 1. The effect of three estrogens in inducing feminization of *Oreochromis niloticus* (Baobab strain) fry. Data are from crosses where control sex ratios did not differ significantly from 1:1. (NS = not significant; ***P<0.001; **P<0.025; NA = Chi-squared test not applicable due to low survival; BE = 17ß-estradiol; DES = diethylstilbestrol; and EE = ethynylestradiol.)

Hormone concentration ($\mu g \cdot l^{-1}$)		Duration (days)	No. of replicates	% Mortality	♀	♂	⚥	Proportion of females	Chi-squared
BE	4	30	2	0	68	62	0	0.52	0.139 NS
	20	30	2	2	70	63	0	0.53	0.184 NS
	100	30	4	5	99	77	3	0.55	1.008 NS
	200	30	5	18	159	50	21	0.69	17.474***
	250	30	2	68	19	4	3	0.73	2.925 NS
	250	14	3	18	96	20	7	0.78	38.704***
	500	30	2	94	3	2	0	0.60	NA
	500	14	2	42	62	14	2	0.80	10.268**
EE	4	30	2	0	77	69	0	0.53	0.219 NS
	20	30	2	0	56	64	0	0.47	0.267 NS
	100	30	8	7	384	41	43	0.82	107.215***
	200	30	2	79	23	3	2	0.82	6.452**
	300	30	1	93	1	2	1	0.25	NA
	400	30	1	100	-	-	-	-	NA
DES	100	30	1	17	32	2	1	0.91	14.380***
	200	30	1	70	19	2	1	0.86	6.482**
	300	30	1	95	3	1	1	0.60	NA
	400	30	1	99	-	-	-	-	NA

Timing of Start of Treatment

There would be an advantage for fry survival if treatment could be started as late as possible, when fry had first fed and would therefore be fitter, with corresponding lower rates of mortality. This would also permit treatment of fry of differing ages collected from various waterbodies. A series of treatments was set up using 250 $\mu g \cdot l^{-1}$ BE for 14 days because this concentration had been successful in feminizing fry with acceptably low mortality. Treatments were started at first feeding and six, 12 and 18 days after first feeding. Only the first treatment had a significant effect on the sex ratio (Table 2).

Similar experiments were undertaken, starting treatment with 100 $\mu g \cdot l^{-1}$ EE for 14 days starting two days or one day before the swim-up stage, when fry (in incubators) are free swimming but still absorbing the yolksac; at swim-up, and three and six days after swim-up. The results (Table 3) indicate that there is no significant advantage in starting immersion treatments earlier than swim-up when fry are able to survive without an incubator. These data also confirmed that hormone treatments are not effective if initiated more than three days after swim-up.

Duration of Treatment

Treatments with 100 $\mu g \cdot l^{-1}$ EE were started at first feeding and continued for six, 12, 18, 24 and 28 days. The results (Table 4) indicate that sex reversal started after 12 days, but an optimum is reached between 12 and 24 days. Mortality appeared to be correlated with duration of treatment.

Discussion

In our laboratory, 95-100% female progeny is now routinely achieved by treating fry from crosses where control groups approximate to 1:1 sex ratios. EE is used in preference to BE since it is more effective at

Table 2. The number of females, males and intersexes produced from immersion of *Oreochromis niloticus* (Baobab strain) fry in 250 $\mu g \cdot l^{-1}$ 17ß-estradiol (BE) for 14 days starting at first feeding, six, 12 and 18 days later. Data are pooled from three homogeneous replicates. (Data bearing different suffix letters are significantly different; ***P<0.001; NS = not significant; and P>0.05.)

Start of treatment (days after first feeding)	% Mortality	No. of females	No. of males	No. of intersexes	Proportion of females	Chi-squared
Control	3.4	64	54	0	0.54[a]	-
0	10.3	99	19	3	0.82[b]	24.292***
6	8.0	75	56	0	0.56[a]	0.228 NS
12	5.2	70	53	0	0.57[a]	0.173 NS
18	3.1	62	60	1	0.51[a]	0.281 NS

Table 3. The number of females, males and intersexes produced from immersion of *Oreochromis niloticus* (Baobab strain) fry immersed in 100 $\mu g \cdot l^{-1}$ ethynylestradiol (EE) for 20 days, starting two days before to six days after the swim-up stage. Data are pooled from two homogeneous replicates. (Data bearing different suffix letters are significantly different; **P<0.025; NS = not significant; and P>0.05.)

Start of treatment (days after first feeding)	% Mortality	No. of females	No. of males	No. of intersexes	Proportion of females	Chi-squared
Control	4.1	40	40	0	0.50[a]	-
-2	6.1	50	8	14	0.69[b]	5.932**
-1	5.4	40	6	11	0.70[b]	9.568**
0	3.9	50	10	6	0.76[b]	10.146**
+3	4.9	39	30	12	0.48[a]	0.005 NS
+6	6.8	37	42	1	0.46[a]	0.225 NS

Table 4. The number of females, males and intersexes produced from immersion of *Oreochromis niloticus* (Baobab strain) fry in 100 $\mu g \cdot l^{-1}$ ethynylestradiol (EE) for six, 12, 18, 24 and 28 days starting at the swim-up stage. Data are pooled from three homogeneous replicates. (Data bearing different suffix letters are significantly different; ***P<0.001; and NS = not significant. Data with the same suffix letter were not significantly different [P>0.05]).

Start of treatment (days from swim-up)	% Mortality	No. of females	No. of males	No. of intersexes	Proportion of females	Chi-squared
Control	2.0	62	60	0	0.51[a]	-
6	1.5	63	58	0	0.52[a]	0.037 NS
12	2.9	139	38	5	0.76[b]	21.290***
18	8.4	93	12	3	0.86[b]	32.468***
24	6.3	57	3	5	0.88[b]	24.915***
28	7.2	68	13	7	0.77[b]	15.170***

lower concentrations. The two molecules are very similar but EE is slightly more soluble in water and this may be the reason for its relatively greater success. DES is usually the preferred artificial estrogen for oral administration. Initial trials with DES were promising but fears about its carcinogenic properties (IARC Monographs 1974) have inhibited its use in our laboratory.

Administration of EE at a concentration of 100 $\mu g \cdot l^{-1}$ for 12+ days is the optimal treatment. Fry attained a standard length of 13-15 mm at this age under the growing conditions used, but it is probable that a lower protein diet or lower temperatures would necessitate a longer treatment period because of reduced growth. This treatment has no significant deleterious effects on growth and survival in comparison with controls. Eckstein and Spira (1965) induced sterility in *O. aureus* when administering estrogens for five to six weeks, commencing at four to five weeks post-hatching. With the longer treatment periods there appeared to be a lessening of oogonia development (Gilling et al. 1992) at 60-70 mm standard length, but most adults matured normally.

Srisakultiew and Rana (1991) reported that sexual differentiation can be seen in histological preparations of tilapia gonads at 14 days after fertilization. Our results indicate that germ cells are developing prior to this and that, for environmental influences to overcome any genetic influences, they must be initiated at first feeding or soon after.

It is hoped that, through progeny testing, it will be possible to isolate YY males amongst the progeny from sex reversed females and that, using the method described, females can be produced with a YY genotype. These could then be backcrossed to YY males giving all YY progeny which would form the basis of a broodstock producing all-male fry for ongrowing under commercial conditions. However, deviations from the sex ratios predicted by a theory of monofactorial sex determination as described by Mair et al. (1991) may prove to be a limitation to the success of this approach. In addition, the viability of the YY genotype, especially in a female phenotype, needs investigation.

Acknowledgements

We acknowledge the help of Eric Roderick and Richard Morgan for their technical assistance, Dr. Graham Mair and Stuart McConnell for helpful discussions, and the British Overseas Development Agency for providing funds for the project.

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Effects of Triploidy on Sexual Maturation and Reproduction in Nile Tilapia, *Oreochromis niloticus* L.

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HUSSAIN, M.G., D.J. PENMAN and B.J. McANDREW. 1996. Effects of triploidy on sexual maturation and reproduction in Nile tilapia, *Oreochromis niloticus* L., p. 320-325. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

The effects of triploidy on sexual development and reproduction were examined in female and male Nile tilapia (*Oreochromis niloticus* L.). Triploidy was induced by exposing newly fertilized eggs to either a hydrostatic pressure shock (8,000 psi for 2 minutes) at 9 minutes after fertilization (a.f.) or a heat shock (41°C for 3.5 minutes) at 5 minutes a.f. Highly significant differences in ovary weight and GSI were observed between triploid and control diploid females from 5 months of age to the end of the growing period (10 months). The string-like ovaries of triploid contained mainly oogonia and very few small oocytes; in contrast, diploid ovaries were packed with developing or well-developed secondary and vitellogenic oocytes. Triploid testes contained watery milt with a very few motile spermatozoa, while diploid testes were full of motile spermatozoa. The results of 10 different crosses between triploid males and diploid females revealed that triploid spermatozoa were mostly unable to fertilize normal eggs. In a few crosses, where fertilization occurred, the hatched larvae were deformed and died before yolksac resorption. Karyotypic analysis revealed that hatched embryos from such crosses were all aneuploid. The impact of such reproductive sterility of triploid individuals on the mixed-sex farming of *Oreochromis* spp. is discussed.

Introduction

Tilapias (*Oreochromis, Sarotherodon* and *Tilapia* spp.) are fishes of major economic importance in tropical and subtropical countries, but their uncontrolled and prolific breeding at a small size in mixed-sex culture constitutes a serious constraint on their efficient production. Production of all-triploid progeny of *Oreochromis* spp., by genome manipulation techniques or by crossing diploid and tetraploid individuals, has great potential for commercial application to replace hybridization or the use of hormones as means of producing all-male or other nonbreeding tilapia production stocks. Triploid individuals are expected to be functionally sterile because of the failure of homologous chromosomes to synapse correctly during the first meiotic division. Triploidy in tilapias has been achieved in a number of studies (Valenti 1975; Chourrout and Itskovich 1983; Penman et al. 1987; Don and Avtalion 1988; Varadaraj and Pandian 1988; Hussain et al. 1991). In other teleost-fish species, the ovaries of female triploids contain few oogonia and primary oocytes fail to mature with the result that the ovaries remain smaller at all stages of development compared to those of the diploids (see, e.g., Thorgaard and Gall 1979;

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Chrisman et al. 1983; Lincoln and Scott 1984; Richter et al. 1987). In male triploids, the size of the testes do not differ significantly from diploids. During spermatogenesis, when the primary spermatocytes start to develop into secondary spermatocytes there is mass atresia and degeneration of these germ cells. If active division of spermatocytes does occur, and they are transformed to spermatids, a few aneuploid spermatozoa are produced. This ultimately leads to functional sterility of the testes (see, e.g., Wolters et al. 1982; Richter et al. 1987).

In tilapia, there are a few reports on sexual maturation and fertility of triploids (Penman et al. 1987; Pandian and Varadaraj 1988; Varadaraj and Pandian 1990), but the nature of sterility in these fishes is still unclear due to lack of follow-up trials, particularly on the histological and endocrinological aspects of triploid and diploid gonads.

In this paper, we aim to summarize the results of our experiments on the condition of functionally but reproductively sterile gonads (having nonviable gametes) in successive age groups of triploid Nile tilapia, *Oreochromis niloticus* L. and the implications for the culture of this species.

Materials and Methods

The *O. niloticus* L. broodstock used in this study came from the Tilapia Reference Collection maintained at the Institute of Aquaculture, University of Stirling, Scotland and were descended from a pure stock originally obtained from a wild population in Lake Manzala, Egypt in 1979 (McAndrew and Majumdar 1983). The husbandry of the stock, stripping, egg fertilization and incubation protocols were as described by Hussain et al. (1991).

Eggs stripped from a female fish were fertilized with fresh spermatozoa and were then divided into three equal batches. The first batch of eggs was exposed to a heat shock (41°C for 3.5 minutes) at 5 minutes after fertilization (a.f.) and the second batch to a hydrostatic pressure shock (8,000 psi for 2 minutes) at 9 minutes a.f. (Hussain et al. 1991). The third batch was used as a control.

The ploidy status of all treated and control fish was assessed by chromosome preparations from newly hatched or one-day-old larvae, using the technique of Hussain et al. (1991). The ploidy of individual large fish in the ongrowing trial was checked by estimating the mean erythrocyte nuclear major axis. Blood smears from individual fish were made on glass slides and stained with Wright's blood stain (10 red blood cell [RBC] nuclei were measured per fish with an eye piece graticule at x1,000 magnification using an Olympus compound microscope [Penman et al. 1987]).

The first feeding triploid and diploid fry (10-12 days a.f.) were transferred from incubating jars into 20-l tanks, provided with a recirculated, aerated and controlled temperature (28±1°C) water supply. Triploids and diploids were kept separately at a stocking density of 100 fry·tank^{-1} for five weeks. No differences in triploid treatment groups were noted so, at the end of this period, triploid sib fry from the two shock treatments were stocked together for ongrowing.

The early fry were fed commercial trout feeds following the methods of McAndrew and Majumdar (1989). At the end of the initial rearing period, the triploid (mean weight 0.35±0.13 g) fry were stocked separately into 500-l tanks at a density of 70 fish·tank^{-1} and kept on a 12-hour photoperiod in a recirculated water system maintained at 28°C. Ongrowing fish were fed twice daily at 3-5% body weight with trout pellets (Ewos Baker, Nos. 3, 4, and 5 pellets; 35-50% protein).

Monthly estimates of standard length, body weight and gonad weight for male and female triploid and diploid fish (5 fish/sex/treatment) were made on random subsamples of total fish in each category

from four months of age to the end of the growing period (10 months). The values for gonadosomatic index (GSI) were calculated as individual gonad weight x 100/ individual body weight.

The ovaries and testes collected were transferred to Bouin's fluid overnight, fixed in 70% ethanol, and examined by routine histological methods (paraffin wax embedding; haematoxylin/eosin staining).

Ten crosses were made between triploid males and diploid females. Some males and females were used more than once, but in different crosses. All the milt from sexually ripe triploid and diploid males was checked under a microscope to assess the motility and density of spermatozoa. Eggs collected from a normal female were divided into two batches, one being fertilized with triploid and the other with diploid sperm. The survival rate of embryos from all the crosses was estimated at four development stages: morula (MOR), pigmentation (PIG), hatching (HAT) and yolk-sac resorption (YSR). The numbers of normal and deformed fry at HAT and YSR stages were also recorded.

Data on standard length, body weight, gonad weight and GSI were collected from all groups. Differences between groups for these variables were analyzed using a one-way analysis of variance (ANOVA). The relationship between gonad weight and body weight was analyzed by simple regression analysis, using Statgraphics (Version 3.0).

Results and Discussion

Mean standard length, body weight, gonad weight and GSI for both sexes of triploid and diploid fish belonging to successive age groups are given in Table 1. In this preliminary growth study, no significant length differences were found between the treatments within a sex. Diploid females were significantly heavier ($P<0.05$) at four months than their triploid sibs, but this disappeared in succeeding samples (5-10 months). Diploid males always showed increased body weight over triploid males throughout the growing period but this was not significant except in month 6. These measurements were mostly incidental for calculation of GSI and a more indepth investigation on comparative growth of triploid and diploid individuals is in progress.

Highly significant differences in ovary weight and GSI were found between triploid and diploid females from five to 10 months of age (Table 1). Diploid ovaries were 20-50 times heavier than ovaries of their triploid sibs. The former were packed with numerous developing oocytes from four months of age to the end of the growing period (10 months). In contrast, triploid females showed very poor development of the urogenital papilla and the ovaries were very thin, string-like and sometimes short and plump. The mean ovary weight and body weight for females of both ploidy levels were positively correlated during the course of the study period and the values of correlation coefficients (R) for linear regression equation were mostly significant.

Diploid testes were soft, elongate, milky and full of motile spermatozoa. Triploid testes were more or less of similar size, sometimes thin and flat: all had watery milt. No significant differences in testes weight and GSI were found between triploid and diploid males. A positive correlation was also found between mean testes weight and body weight for diploid and triploid males. Triploid males, like diploids, developed secondary sexual characters such as a prominent urogenital papilla and the shiny body color.

Diploid ovaries from fish of six to eight months of age contained oogonia and many maturing previtellogenic and vitellogenic oocytes with irregular nuclei and vacuolated cytoplasm associated with endogenous and exogenous yolk formation (Plate 1A). Triploid ovaries contained mainly oogonia and a few small primary or previtellogenic oocytes (Plate 1B). In the earlier stages (four to seven

Table 1. Mean standard length, body weight, gonad weight and gonadosomatic index (GSI) for female and male triploid and diploid *Oreochromis niloticus* in successive age groups (4, 5, 6, 7, 8, and 10 months). All values are mean ± SE based on five fish in each category. Significance levels of ANOVA for comparisons between 2n and 3n fish of each sex, at every age, are given against the 3n sex.

	Ploidy/Sex	Age (months)					
		4	5	6	7	8	10
Length (cm)	2n Female	11.10 ± 0.20	13.10 ± 0.50	13.20 ± 0.90	15.90 ± 0.50	17.50 ± 0.40	18.00 ± 0.04
	3n Female	10.10 ± 0.20	11.70 ± 0.80	13.60 ± 0.30	14.50 ± 0.40	17.70 ± 0.40	19.00 ± 0.30
	2n Male	12.20 ± 0.40	14.50 ± 0.10	15.60 ± 0.80	17.80 ± 0.40	18.20 ± 0.40	18.80 ± 0.30
	3n Male	11.60 ± 0.40	13.40 ± 0.30	14.20 ± 0.60	16.40 ± 0.60	17.60 ± 0.50	18.60 ± 0.20
Weight (g)	2n Female	56.80 ± 3.00	88.20 ± 7.90	95.90 ± 19.10	164.20 ± 18.60	212.90 ± 14.70	227.00 ± 9.50
	3n Female	44.20 ± 2.80*	73.90 ± 0.30	123.90 ± 6.00	147.90 ± 14.10	238.60 ± 10.50	257.50 ± 5.30
	2n Male	71.60 ± 7.70	119.70 ± 5.00	193.40 ± 10.70	231.40 ± 17.30	252.70 ± 11.30	305.90 ± 20.80
	3n Male	70.80 ± 4.70	100.00 ± 6.60	118.70 ± 9.90**	210.30 ± 22.90	219.30 ± 9.90	280.70 ± 17.90
Gonad weight (g)	2n Female	0.50 ± 1.60	1.60 ± 0.20	1.70 ± 0.20	3.30 ± 0.40	4.60 ± 0.50	5.50 ± 0.90
	3n Female	0.01 ± 0.00	0.03 ± 0.01***	0.08 ± 0.01***	0.10 ± 0.01***	0.20 ± 0.02***	0.30 ± 0.02**
	2n Male	0.40 ± 0.10	1.70 ± 0.60	2.30 ± 0.30	2.70 ± 0.30	4.50 ± 1.10	5.50 ± 0.60
	3n Male	0.40 ± 0.10	1.40 ± 0.20	1.90 ± 0.60	3.30 ± 0.30	3.80 ± 0.40	4.80 ± 0.40
GSI	2n Female	0.82 ± 0.44	1.80 ± 0.30	1.80 ± 0.20	2.00 ± 0.30	2.20 ± 0.30	2.40 ± 0.40
	3n Female	0.02 ± 0.00	0.04 ± 0.10***	0.06 ± 0.10***	0.07 ± 0.01***	0.08 ± 0.01***	0.10 ± 0.01**
	2n Male	0.55 ± 0.09	1.40 ± 0.38	1.20 ± 0.20	1.20 ± 0.20	1.80 ± 0.40	1.80 ± 0.20
	3n Male	0.56 ± 0.13	1.40 ± 0.19	1.60 ± 0.30	1.60 ± 0.20	1.70 ± 0.20	1.70 ± 0.10

*P<0.05; **P<0.01; and ***P<0.001.

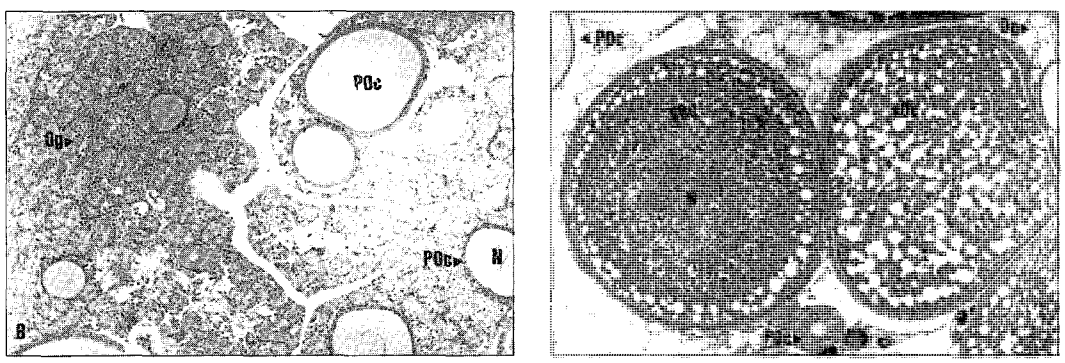

Plate 1. Histological sections of Nile tilapia (*Oreochromis niloticus*) ovaries at 10 months of age (x63). (A) Diploid ovary showing oogonia (Og), primary oocytes or previtellogenic oocytes (POc) and vitellogenic oocytes (VOc), all oocytes with nucleus (N). (B) Triploid ovary containing mostly oogonia (Og) and few small primary oocytes or previtellogenic oocytes (POc).

months), most of the cells were of a similar size with very few undergoing division. In the later stages (eight to 10 months), some of the primary or young previtellogenic oocytes started to show retarded development.

Diploid testes of successive age groups contained highly distinct cysts surrounded by the basal lamina at all stages of development. Spermatogonia, primary and secondary spermatocytes, spermatids and a large number of spermatozoa were observed in all age groups after maturation (Plate 2A). Triploid testes contained mostly cysts with spermatogonia and spermatocytes. In testicular sections of some triploids, germ cells were under active division and had developed into spermatids and spermatozoa: others were blocked during the course of spermatogenesis possibly in prophase 1 of the first meiotic division as suggested by Richter et al. (1987). In later stages (eight to 10 months), most of the cysts began to fuse with degenerating sperm cells resulting

in nearly empty tubules having lightly stained seminal fluid consisting of a few spermatozoa with large nuclei (Plate 2B). Triploid milt contained a few spermatozoa most of them displaying an abnormal or highly abnormal morphology and motility.

Similar observations were made also for the ovaries and testes of triploid individuals of other teleost fishes (see, e.g., Chrisman et al. 1983; Lincoln and Scott 1984; Suzuki et al. 1985; Richter et al. 1987). Vitellogenic oocytes have been observed in triploid ovaries of some species, including *O. aureus* (Penman 1989).

As some triploid males were able to produce motile spermatozoa, these males were crossed with normal females to test the viability of their spermatozoa. The results of 10 different crosses between triploid males and diploid females revealed that triploid spermatozoa were mostly unable to fertilize normal eggs. In a few crosses, where fertilization (2.7-44%) occurred, the hatched larvae were found to be deformed and died before YSR (Fig. 1). Karyotypic analysis revealed that hatched embryos from such crosses were all aneuploid (between 2n=44 and 3n=66). In contrast, all crosses between

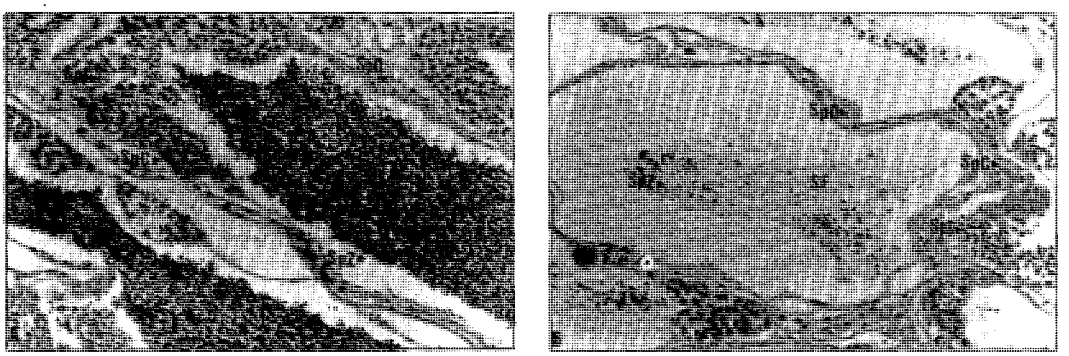

Plate 2. Histological sections of Nile tilapia (*Oreochromis niloticus*) testes at 10 months of age (x250). (A) Diploid testis showing spermatogonia (SpO), spermatocytes (SpC), spermatids (SpT) and a large number of spermatozoa (SpZ). (B) Triploid testis containing cysts mostly with spermatogonia (SpO), spermatocytes (SpC), some spermatids (SpT) and a few spermatozoa (SpZ) in seminal fluid (SF).

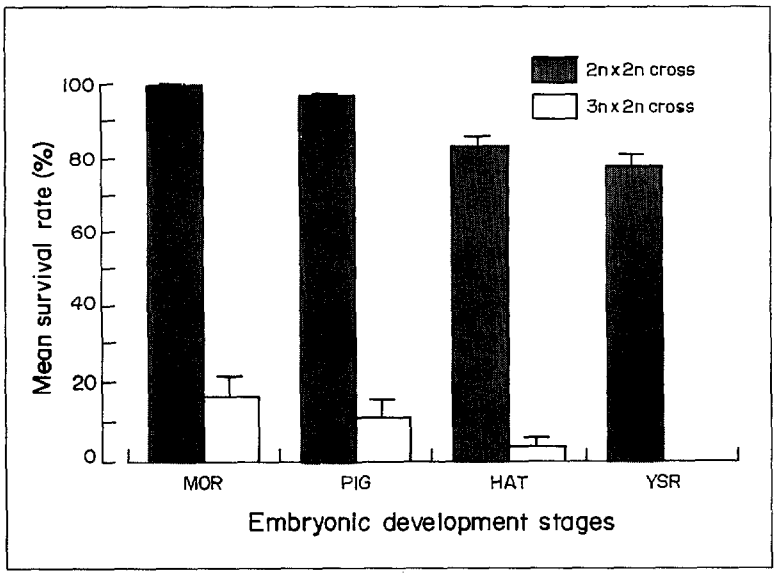

Fig. 1. The mean survival rate of *Oreochromis niloticus* embryos from 10 different crosses between triploid males and diploid females at four development stages: morula (MOR), pigmentation (PIG), hatch (HAT) and yolk-sac resorption (YSR). Scale bars are ± SE.

diploid males and females were normal and about 98-100% eggs were fertilized. Embryo survival to YSR ranged from 62.5 to 93.7% and all individuals were normal diploids (2n=44). For *O. aureus*, Penman et al. (1987) observed no viable progeny from crosses between triploid males and diploid females.

This study has confirmed that both female and male triploid *O. niloticus* are functionally and reproductively sterile. Such reproductive sterility in mixed-sex culture of *Oreochromis* spp. would improve production by preventing precocious sexual maturation, particularly in ponds. The use of sterile triploid fish could also be a way to avoid the risk of gene introgression from farmed stocks into native wild stocks. Widespread use of triploid tilapias will depend upon techniques to produce large numbers at a reasonable cost.

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Aspects of the Reproductive Strategy of *Sarotherodon melanotheron*: Comparison between a Natural Population (Ebrié Lagoon, Côte d'Ivoire) and Different Cultured Populations

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Abstract

The size at first sexual maturity, absolute fecundity and egg and spawn weight were compared in females of *Sarotherodon melanotheron* (Rüppel, 1852) collected in the wild (Ebrié Lagoon) and reared in different lagoon culture conditions: intensive farming in pens and extensive farming in "acadja-enclos" (pens fitted with branches or bamboo poles).

In pens, females reach maturity at a smaller size and produce smaller eggs and in larger quantities than in the wild. In "acadja-enclos" where conditions are intermediate between intensive culture and natural conditions, an intermediate situation is also observed in fecundity and in the size of eggs produced. However, the size at first maturity is similar to that observed in the wild.

In contrast, the relationships between spawn weight and female weight hardly vary with the different growth environments. These results suggest that the quantity of matter produced during a breeding cycle (based on the spawn weight) is a specific constant that may be genetically determined. Environmental conditions have an effect on the gonadal material division process and on the breeding strategy: many small eggs or large ones but few.

Introduction

Sarotherodon melanotheron (Rüppel, 1852) is a typical estuarine species found in large quantities in most West African lagoons and estuaries where it lives and breeds in a wide range of salinity conditions (0-90 ppt; Albaret 1987). Its potential for aquaculture has been noted by several authors (Pauly 1976; Sivalingam 1976; Legendre 1983). However, the different tests done in intensive culture conditions using artificial feeds yielded disappointing results (slow growth and poor feed conversion), making this species at present a poor candidate for this type of culture (Legendre et al. 1989).

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What seems to be giving more promising results is the extensive farming of *S. melanotheron* in "acadja-enclos" (Hem 1992), a farming system derived from the highly productive traditional fishery in acadjas developed in the lagoons of Benin (Welcomme 1972). Acadjas are organized stacks of branches submerged in shallow lagoon waters where some species of the natural stock, including *S. melanotheron*, converge and multiply. The power of these structures to attract fish seems to result, on the one hand, from a significant increase in the surfaces on which microfauna and epiphytes (source of food for fish) develop and, on the other hand, from their function as shelter. Based on the same principle, the objective of the "acadja-enclos" is to promote the growth of natural food in the culture environment, hence considerably reducing the need for artificial feed and, consequently, the costs of production (Hem 1992). The first extensive culture experiments using the "acadja-enclos" technique have given encouraging results: annual fish yields of over 8 $t \cdot ha^{-1}$, of which 60-90% composed of *S. melanotheron*, are possible without adding any artificial feed (Legendre et al. 1989; Hem 1992; Hem and Avit, this vol.).

Considering this new orientation given to the culture of *S. melanotheron*, it may be useful to specify the major characteristics of the reproduction of this species in this particular environment. In the present work, the size at first maturity, the absolute fecundity, the egg and spawn weight have been estimated for two different "acadja-enclos" designs (stacks of branches or bamboo poles). Results are compared to those of a previous analysis of the reproduction of *S. melanotheron* based on samples collected in the wild (Ebrié Lagoon) and in intensive pen culture (Legendre and Ecoutin 1989). Whether in the wild or in culture conditions, sexually mature females of *S. melanotheron* display successive breeding cycles throughout the year without interruption.

Materials and Methods

All culture experiments were conducted at the Layo Aquaculture Station, 40 km west of Abidjan, in an oligo-mesohaline area of the Ebrié Lagoon (Côte d'Ivoire).

Fish Origins and Culture Conditions

Four populations of *S. melanotheron* were studied: one natural population, one population reared in pen using artificial feed and two populations reared in two different "acadja-enclos" with no complementary feed.

Wild specimens were collected in oligohaline areas of the western part of Ebrié Lagoon. They were bought directly from lagoon fishers soon after capture and dissected the same day in the laboratory.

The intensive culture of *S. melanotheron* of different sizes was conducted in association with *Tilapia guineensis* in a 625-m^2 pen without acadja, at an initial stocking density of 5 $individuals \cdot m^{-2}$. Fish were given pelleted feed with 31% crude proteins (Legendre 1986), distributed twice daily six days a week at a daily ration of 5% of total fish biomass. Initially, these fish were captured in the station ponds which they had spontaneously colonized from the lagoon (Albaret and Legendre 1983). Test fish were both individuals that penetrated the ponds while at the fry stage and first generation fish hatched in the ponds.

In the first "acadja-enclos," an artificial reef made of 200 stacks of branches was submerged in an area covering 200 m^2 in a 625-m^2 pen. This structure was stocked with 1,000 individuals of cultured *S.*

melanotheron (initial mean body weight = 40 g) at a density of 1.6 fish·m^{-2}.

The second "acadja-enclos" was made of 4,000 bamboo poles planted vertically in the sediments in an area covering 800 m^2 in a 1,250-m^2 pen. This structure was stocked with 4,000 juveniles of cultured *S. melanotheron* with initial mean body weight of 5 g at a stocking density of 3.2 fish·m^{-2}.

The juveniles used to stock the "acadja-enclos" were produced from broodfish initially captured in the Ebrié Lagoon and frequently renewed with wild individuals.

Sampling and Study of the Fish Reproduction

Monthly samplings of over 30 individuals were taken simultaneously in the wild and in the pen over a period of 16 months, between 1982 and 1983. In the two "acadja-enclos," fish were all harvested in one harvest, 12 months after stocking. In both cases, observations were made on a sample size of approximately 250 individuals. The first "acadja-enclos" was harvested in November 1986 and the second in March 1988.

In all cases, fork length (FL±1 mm) and weight (W±1 g) were estimated for each individual. Sexual maturity was determined by macroscopic examination of the gonads using the scale defined by Legendre and Ecoutin (1989). After dissection, gonads were weighed to the nearest 0.1 g to estimate the gonadosomatic index (GSI = [gonadal weight x 100]/ fish weight).

The size at first sexual maturity (L_{50}) is defined here as the fork length at which 50% of the fish are sexually mature, i.e., their first sexual cycle is at an advanced stage (ongoing vitellogenesis for the females or presence of intratesticular sperm for the males). Moreover, in order to determine the size range in which first maturity is likely to occur, it is useful to specify, in addition to L_{50}, the length of the smallest mature individual as well as the size at which nearly all (95%) the observed fish are at an advanced stage of maturity.

Fecundity, determined from the ovaries of females at the end of the maturation process, represents, in this study, the number of oocytes belonging to the modal group with the largest diameter. This group of oocytes is clearly distinguished from the rest of the egg population and corresponds approximately to the ova that will be spawned. The mean weight of the eggs was determined by weighing (to the nearest 1 mg) 50 oocytes carefully cleared of all traces of superficial moisture using absorbent paper. The spawn weight (i.e., the total weight of oocytes ready to be spawned) was estimated by the product: fecundity x mean weight of one oocyte. The estimation of the spawn weight being relevant only in individuals with completed oocyte development, oocyte mean weight was determined only for females with a GSI>5. No relationship existed between the oocyte mean weight and GSI as defined here in *S. melanotheron* studied here (unpubl. data).

The mean oocyte weight of *S. melanotheron* in the four environments was compared using one-way ANOVA and the Duncan multiple range test. The relationships between fecundity and female weight, on the one hand, and between spawn weight and female weight, on the other, were compared using covariance analysis applied to the slopes of the regressions.

Results

Size at First Sexual Maturity (Fig. 1, Table 1)

In pen culture conditions with artificial feed, females of *S. melanotheron* reach maturity at a much smaller size (L_{50}=140 mm) than that observed in the wild (176 mm). A higher proportion of small, sexually active individuals is also observed in the pen population (Fig. 1). In contrast, the size at first sexual maturity of the populations of the two "acadja-enclos" is high (166 and 189 mm), bracketing the values for females in the natural environment. Results indicate, furthermore, that first sexual maturity occurs at a similar size in males and in females (Table 1).

Fecundity, Egg and Spawn Weight (Figs. 2 and 3, Table 2)

NATURAL AND PEN-CULTURED POPULATIONS

In the wild as in pen culture conditions, the absolute fecundity and the spawn weight are positively correlated with the body weight of the females (Fig. 2). However, in both cases, the estimated correlation coefficients between spawn weight and body weight are higher than between fecundity and weight of female (Table 2). In each environment, individual fecundity variations are compensated, in terms of spawn weight by chances in opposite direction of egg weights. Egg weight and fecundity are thus inversely related such that for equal weight, females showing the highest fecundity produce smaller eggs (Fig. 3, based on the natural populations of females).

There are significant differences in the reproductive characteristics of the natural and pen-cultured populations of *S. melanotheron*. For an equal weight of female, the eggs produced in culture conditions are systematically smaller and produced in larger quantities than in natural conditions. In contrast, relationships between spawn weight and female body weight are equivalent in both environments (Fig. 2 and Table 2).

COMPARISON WITH THE "ACADJA-ENCLOS" POPULATIONS

In the "acadja-enclos" system, contrasting with the previous description, the correlation coefficients between spawn weight and female body weight are not higher than between fecundity and female body weight (Table 2). However, this may be due to the smaller number of females considered in the estimation of spawn weight (females with GSI>5 only).

In both "acadja-enclos" (Fig. 3), the relationships between fecundity and weight of female are similar. In contrast, these relationships are clearly intermediate with those observed in pens and in the wild, differing significantly by the slope or by the position ($P<0.001$). Additional observations (unpublished) have shown that the fecundity of *S. melanotheron* varies with season and is slightly higher in the dry than in the rainy season. Individuals (lagoon and pen populations) considered for this study were sampled throughout the annual cycle—and therefore reflect this seasonal variability—while sampling for the "acadja-enclos" experiments was done at specific intervals. It is clear, however, that the difference in fecundity observed between the females from the "acadja-enclos" and the females from the other two environments exceeds that which could be due to seasonal variability, therefore reflecting a real difference between populations.

The mean egg weight of the females reared in "acadja-enclos" no. 1 (19 mg)

Table 1. Size at first maturity (fork length) observed in *Sarotherodon melanotheron* collected in the wild (Ebrié Lagoon) and in two different culture systems (pens and "acadja-enclos").

Environment	Sex	No. of fish observed	SMI[a] (mm)	L_{50}[b] (mm)	L_{95}[c] (mm)
Lagoon	F	365	146	176	223
	M	96	148	-	-
Pens	F	783	100	140	180
	M	211	105	138	200
"Acadja-enclos" 1	F	170	173	189	220
	M	62	153	170	199
"Acadja-enclos" 2	F	158	161	166	205
	M	91	152	170	203

[a]SMI: smallest mature individual.
[b]L_{50}: size at which 50% of the fish are sexually mature.
[c]L_{95}: size at which 95% of the fish are sexually mature.

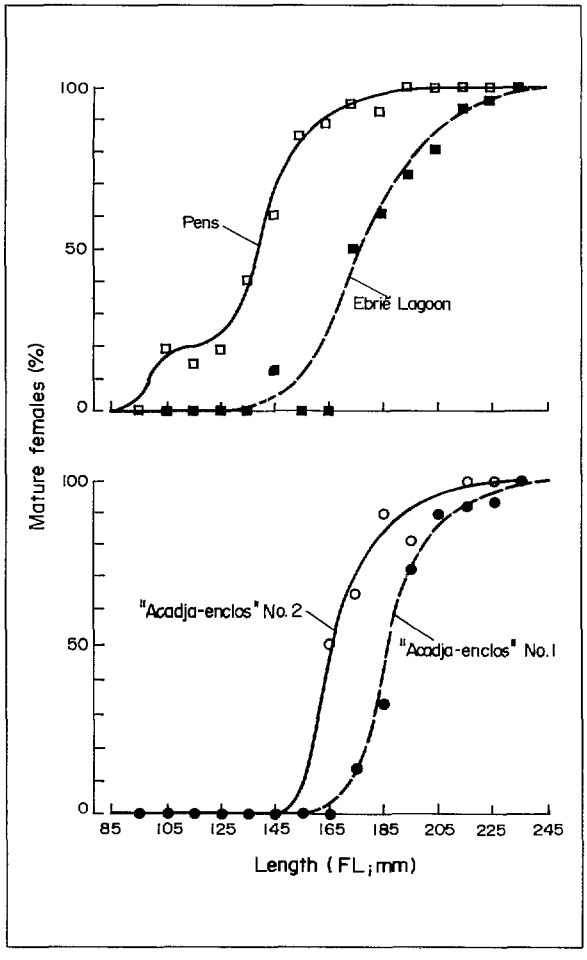

Fig. 1. Size at first sexual maturity in females of *Sarotherodon melanotheron* in different environments.

Table 2. Relationships between fecundity (F) and weight (W) of female; between spawn weight (S) and weight of female; and mean egg weight in *Sarotherodon melanotheron* collected in the wild (Ebrié Lagoon) and in two different culture systems (pens and "acadja-enclos").

Environment	N	Relationship (regression)	r	Mean egg weight (mg)	95% Conf. Int. (±)
Lagoon	31	F=-15.0+1.72 W	0.871		
	31	S=1.60+0.041 W	0.963	28.03	1.90
Pens	46	F=203.9+2.61 W	0.777		
	46	S=0.32+0.045 W	0.939	12.06	1.28
"Acadja-enclos" 1	31	F=132.6+1.81 W	0.881		
	12	S=2.29+0.034 W	0.832	18.94	2.28
"Acadja-enclos" 2	24	F=267.3+1.25 W	0.874		
	18	S=0.38+0.043 W	0.870	15.07	1.74

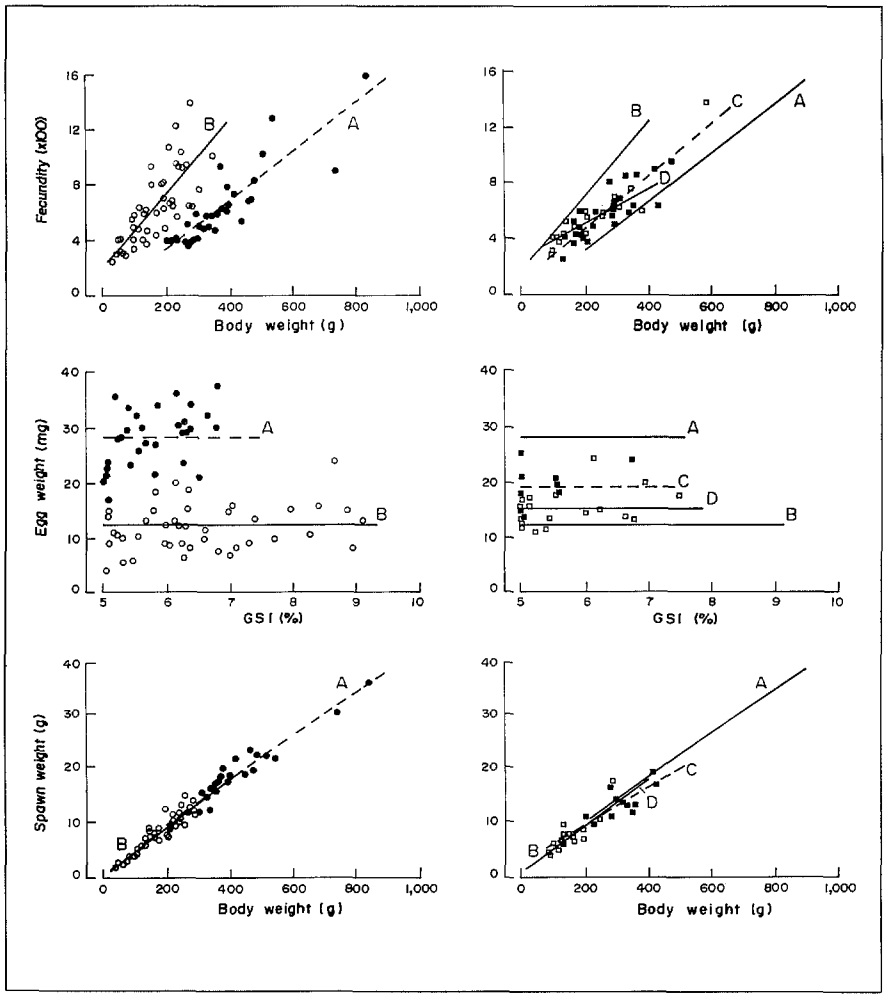

Fig. 2. Comparison of the relationships fecundity-weight of female, egg weight-GSI and spawn weight-weight of female observed for *Sarotherodon melanotheron* in different environments: **A**, natural environment (Ebrié Lagoon); **B**, pens; **C**, "acadja-enclos" no. 1; and **D**, "acadja-enclos" no. 2. Left panels: data points and lines for A and B; right panels: lines for A-B, data points for C and D.

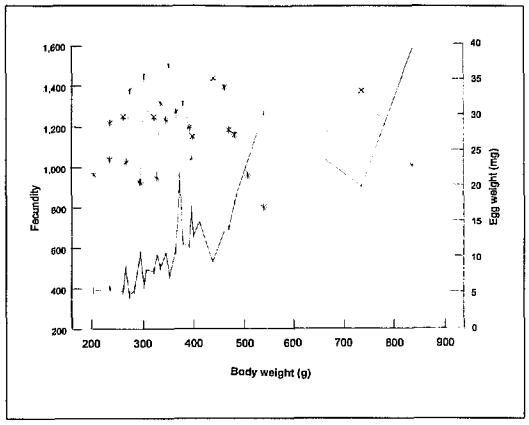

Fig. 3. Relationship between fecundity and egg weight in female *Sarotherodon melanotheron* in the natural environment (Ebrié Lagoon). Each data pair (fecundity, egg weight) placed on the same vertical line corresponds to one female. Individual variations in fecundity and egg weight show a mirror effect.

also shows an intermediate position ($P<0.05$) in comparison with that of females cultured in pens (12 mg) and females in the natural environment (28 mg; Fig. 2). For females reared in "acadja-enclos" no. 2, mean egg weight does not differ significantly from that observed for females cultured in pens or in "acadja-enclos" no. 1. Other observations have shown that egg size tends to increase with the weight of the fish, up to about 100 g, then reaches a plateau. Thus, for the pen population where the sample included few small individuals (Fig. 2), the mean egg weight (12.06 mg±4.20) was slightly underestimated and became 13.35 mg±3.70 when only individuals weighing over 100 g were considered. This, however, does not invalidate our previous conclusions concerning the comparison between different populations.

The relationships between spawn weight and body weight are considered equivalent regardless of the origin of the females (Table 2; Fig. 2) as there is no significant difference between the regression lines estimated for the four environments (natural environment, pen culture with or without complementary feed) either in the slope or in the position. In contrast to fecundity and egg size, spawn weight appears here as a variable that is independent of the environment of the fish.

Discussion

In this study, all fish have the same origin—the Ebrié Lagoon, i.e., do not originate, for the cultured individuals, from a strain of broodfish long isolated from the natural population. In addition, it seems that even when the "acadja-enclos" is artificially stocked, part of the population is composed of wild individuals that entered into the structure as juveniles and grew there until the nets could no longer let them through (Hem, pers. comm.). It is considered, therefore, that all fish studied under this experiment are genetically similar, and the differences observed in their breeding characteristics seem to be adaptive (phenotypic) responses to the different environments.

Size at First Sexual Maturity

In pen culture conditions, the size at first sexual maturity (L_{50}) is much smaller than that observed in Ebrié Lagoon. In contrast, first maturity in the "acadja-enclos" occurs at a size similar to that of the natural population of females, slightly smaller in one instance and slightly bigger in the other.

The fact that the surface areas and the sites for pens and "acadja-enclos" are similar indicates that neither captivity in the Layo site nor available vital space (in terms of enclosed lagoon volume) seems to be responsible for the significant decrease in L_{50} observed in intensive culture.

Lowe-McConnell (1982) reported that, in *Oreochromis niloticus*, natural populations composed of individuals with a low weight for their length tend to reproduce at a size smaller than that observed for populations composed of individuals in better condition. The present study yielded similar results: fish with a low weight for length had a smaller size at first maturity (Fig. 4). Although these results suggest a trophic effect in the determinism of sexual maturity, they do not imply that food supply is the only important factor.

Although authors are not unanimous on the subject (see Noakes and Balon 1982), it is generally agreed that in tilapias, the reduction of the size and age at first sexual maturity is an adaptation to adverse life conditions (Fryer and Iles 1972; Ruwet et al. 1976; Lowe-McConnell 1982). This tends to be supported by the poor condition observed for fish reared in pens which also displayed the lowest L_{50}.

In intensive culture conditions, *S. melanotheron*'s first sexual maturity occurs at the age of six to eight months. But in the absence of data on the growth of this species in Ebrié Lagoon or in "acadja-enclos," it is not clear whether the differences in L_{50} result from a difference in growth or are also coupled with a difference in age at first maturity. Eyeson (1983) reported that when fish are kept in captivity, *S. melanotheron* can be sexually active as early as four to six months at a size as small as 4-5 cm (standard length).

Fecundity and Egg Size

This study shows two distinct fecundity levels with variations observed between individuals of a same population and strong variations observed between populations. At the intraspecific level, results showed that for females of equal body weight, variations in fecundity are coupled with a variation of egg weight in the opposite direction, thus confirming the observations of Peters (1963) on the same species. This also applies to interspecific comparisons: females from populations with the highest fecundity levels also produce the smallest eggs. This balance between fecundity and egg size results in a relationship between individual spawn weight and weight of female that remains unchanged regardless of the population considered. The quantity of matter produced during a breeding cycle (based on spawn weight) is, therefore, a specific constant that may be determined genetically, the environment influencing the division of the gonadal material and the breeding strategy: many small eggs or few large ones.

At the interspecific level, the existence of an opposite relationship between fecundity and egg size is well-known in fish (Bagenal 1978; Mann and Mills 1979; Albaret 1982; Elgar 1990). This balance between the number and size of eggs produced was also shown or suggested for different groups or populations of a same species (Mann and Mills 1979; Springate et al. 1985; de Silva 1986). However, this balance does not seem to exist in all species studied (Mann and Mills 1979). To our knowledge, there is no other clearly demonstrated example where the compensation number/size of eggs observed for various populations of the same species implies a constant spawn weight. Variations in fecundity have sometimes been analyzed in connection with variations in GSI this gives only an approximate measurement of the reproductive effort and can change with the number or the stage of development of the groups of young oocytes which, apart from those that will be spawned, are present in the gonads (Scott 1979; Mann et al. 1984).

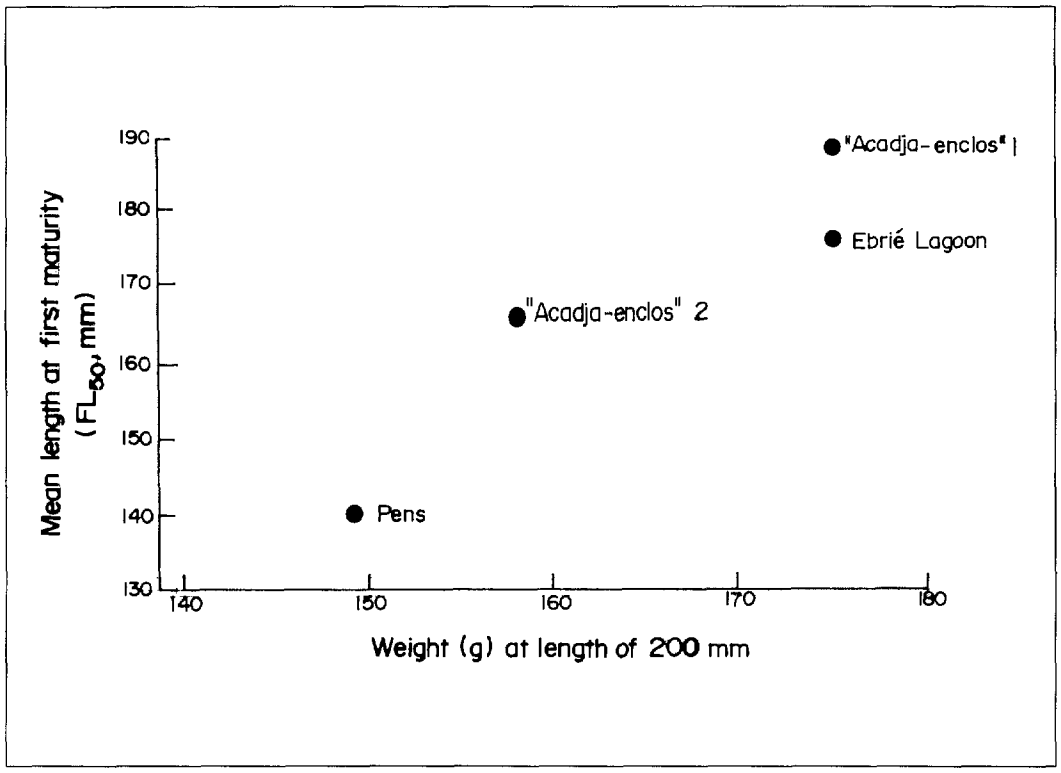

Fig. 4. Relationship between the size at first sexual maturity (L_{50}) and the condition (weight at length) of females of *Sarotherodon melanotheron* in different environments: natural (Ebrié Lagoon) or culture (pens, "acadja-enclos" nos. 1 and 2) environments. The condition is here estimated by the weight of a fish at FL=200 mm. This weight is estimated by the length-weight relationship estimated for each environment.

Three physiological processes are likely to cause variations in fecundity: the rate of oogonia multiplication, the recruitment of oocytes beginning vitellogenesis and the atresia of part of the developing oocytes (Springate et al. 1985). Observations of the ovaries of prespawning females of *S. melanotheron* from the four environments have generally shown atresia. The exact proportion of atresia has not been determined but it is always low, which compares well with the condition reported by Peters (1963) in *Sarotherodon galilaeus*. These observations, however, do not consider the potential extent of atresia at different stages of oogenesis and the significant effect that it can finally have on fecundity.

Townshend and Wootton (1984) reported that in laboratory conditions, the egg size of *Cichlasoma nigrofasciatum* increased with increasing spawning intervals. Thus, in the present study, the higher egg weight and lower fecundity levels observed in wild females could be explained by the combined effect of longer spawning intervals and higher levels of atresia (or the same level over a prolonged period) compared to what has been observed in cultured females. While the mean spawning interval of *S. melanotheron* cultured in tanks is approximately two weeks (Legendre and Trébaol, this vol.), there are no available data on the spawning frequency of this species in the wild. The rate of oocyte recruitment and the atresia level

can, however, vary simultaneously with certain environmental factors. Townshend and Wootton (1984) attributed the low fecundity of *C. nigrofasciatum* reared with restricted feeding regimes both to a decrease in recruitment and to an increase in atresia. At present, the relative importance of these processes in the control of fecundity is not very well-understood in fish and can vary with species (Springate et al. 1985). Several scenarios are therefore possible to explain the responses observed and may involve different rates in oocyte growth. An in-depth, histological comparative study of the ovarian development of *S. melanotheron* maintained in different environments would be necessary to clarify this problem.

Among the external factors likely to have an effect on the production of eggs, feeding has been the subject of the largest number of experiments in fish. A decrease in absolute fecundity is generally observed with a reduction of feeding levels (Bagenal 1969a; Wootton 1979; Billard and de Frémont 1980; Springate et al. 1985). Wootton (1982) indicated, however, that considering the positive relationship between fecundity and size of females, the effect of feed on fecundity can be difficult to separate from that resulting from a simple difference in growth and therefore in fish size. For Cichlidae, Mironova (1977) reported that, in *Oreochromis mossambicus*, a decrease in feeding levels had a limiting effect on growth and reduced the number of eggs produced per spawning, but increased spawning frequency and total number of eggs produced. In *C. nigrofasciatum*, Townshend and Wootton (1984) also observed a decrease in fecundity at the lowest feeding levels. In our study, it is therefore difficult to suggest inadequate feeding to explain both the low condition and the high fecundity of the fish reared in pens. Since high levels of feed (5% of fish biomass) were distributed daily to the fish reared in pens, this raises questions about feed quality rather than quantity. In addition, although the feed is natural and apparently found in sufficient quantity in the "acadja-enclos" and in the lagoon, this does not exclude possible differences in the nature and nutritional quality of the organisms (animals and plants) available for fish in both environments.

The effect of the dietary proteins on the production of eggs and fry was studied recently in various species of tilapias. In *S. melanotheron*, Cissé (1988) did not observe any significant difference either in spawning frequency or in number of eggs produced per spawning with varying protein levels, which, however, could be explained by the small number of fish used in this study. Santiago et al. (1985) and Chang et al. (1988), studying *O. niloticus* and the red hybrid (*O. mossambicus* x *O. niloticus*), respectively, showed a significant increase in the production of fry by broodfish fed with a protein-rich diet. Although there was no direct evidence, these authors attributed this response to an increase in spawning frequency and fecundity due, in turn, to a higher weight of female. In a detailed study, Wee and Tuan (1988) analyzed the reproductive characteristics of *O. niloticus* fed ad libitum with five isocaloric feed containing 20-50% proteins. They showed that fish fed with a lower or intermediate protein diet (20-35%) had a higher fecundity and produced smaller eggs than fish fed with a higher protein diet (42-50%). In addition, fish receiving a feed relatively poorer in proteins had a higher spawning frequency. These results contrast with those of studies previously cited, but tend to confirm the notion of a compensation between fecundity and egg

size, and conform to the general trend of our observations. These results could also suggest that natural feed consumed by *S. melanotheron* in the lagoon or in the "acadja-enclos" has, in fact, a higher protein content than the artificial feed (31%) distributed in the pens.

Other environmental factors such as reduced vital space, increased density or repeated harvests (in pens) may constitute factors of stress and directly or indirectly affect the production of eggs. For example, the varying fecundity levels observed between different Sri Lankan reservoir populations of *O. mossambicus* do not seem to be related to feeding levels, but are positively correlated with the fishing pressure applied to the waterbodies (de Silva 1986).

In addition to feeding levels, the organization of the available vital space constitute the major difference between pens and "acadja-enclos." While pens are limited areas of open waters, the stacks of branches or bamboo poles placed in "acadja-enclos" multiply the hide-outs and shelters which can have an effect on the behavior of this territorial species and on the social interactions among individuals. In this study, since the surface areas covered by "acadja-enclos" and pens are similar, the perception that the fish have of the available vital space seems to be more important than the actual space available. In Ebrié Lagoon, mangroves or bay zones strewn with decomposing branches constitute the preferred biotopes for *S. melanotheron* (Albaret, pers. comm.). Since the "acadja-enclos" can be viewed as an intermediate environment between the natural environment and the pen, it is not surprising that the fecundity of this species is also intermediate in this particular environment.

This discussion implies that the variations observed in the reproductive strategy of *S. melanotheron* are most certainly due to a combination of the different factors mentioned above (and perhaps others) rather than to a single factor. This is also illustrated by the comparison of the reproductive characteristics observed in this species in the natural environment and in "acadja-enclos" no. 1. In this acadja, fecundity (for an equal female weight) is significantly higher than in the lagoon (Fig. 2) while size at first maturity and fish condition (weight for length) are similar (Fig. 4). This suggests, on the one hand, that reduced size at first maturity and high fecundity do not necessarily go together and, on the other hand, that these characteristics may be influenced by different proximate factors.

In aquaculture, early sexual maturity and high fecundity are two negative characteristics for fish in the grow-out phase because of the resulting proliferation of fry and the likely stunting effects. In contrast, these characteristics are positive in tilapia broodfish stock management as the production of fry can be optimized. However, as increased fecundity is negatively related to egg size, a negative effect on the fry survival rate is to be expected. It is well-known that in the wild, larvae from small eggs are smaller and their chances of survival are reduced (Bagenal 1969b, 1978; Mann and Mills 1979). However, in culture conditions where fish are more protected, there is no indication of differences in survival rates between larvae or juveniles produced from eggs of different sizes (Billard and de Frémont 1980; Springate et al. 1985). In *S. melanotheron*, a high rate of survival has been observed in the larvae from the smallest eggs produced by females cultured in pen. Mortality is low during the mouth-brooding phase (Legendre and Trébaol, this vol.) and there is still about 95% fry survival after four weeks of culture in tanks (Legendre 1983).

To conclude, the present results illustrate the remarkable flexibility of the

reproduction of *S. melanotheron* under varying environmental conditions. Important variations are observed for size at first maturity, fecundity and egg size except for spawn weight which remains constant.

In intensive farming (using artificial feed), *S. melanotheron* reaches maturity at a smaller size and produces more eggs and smaller ones than in the wild. In "acadja-enclos" where conditions are intermediate between intensive farming and the natural environment, an intermediate situation is also observed for fecundity and egg size, the size at first maturity remaining similar to that observed in the wild.

However, taken together, the results of this study are not sufficiently explicit to imply any causal effect between the different environmental factors involved and the reproductive characteristics. In tilapias, knowledge on the nature, role and possible interactions between the external factors (biotic and abiotic) involved in the control of the different stages of gametogenesis is still generally insufficient. An experimental approach remains necessary and could greatly contribute to the understanding of the adaptative strategies developed by these species. Furthermore, this approach could also, in time, contribute to an improvement in breeding practices through a better control of the influence these external factors have on fecundity, spawning frequency and first sexual maturity.

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Mouthbrooding Efficiency and Spawning Frequency of *Sarotherodon melanotheron* (Rüppel, 1852) in Culture Environments (Ebrié Lagoon, Côte d'Ivoire)

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LEGENDRE, M. and L. TREBAOL. 1996. Mouthbrooding efficiency and spawning frequency of *Sarotherodon melanotheron* (Rüppel, 1852) in culture environments (Ebrié Lagoon, Côte d'Ivoire), p. 339-348. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

In *Sarotherodon melanotheron*, mouthbrooding efficiency and spawning frequency are two important parameters to consider in the practical management of broodfish reared in captivity. In this species, where males practice mouthbrooding, the number of eggs or fry incubated is positively correlated to male body weight (study made on a population reared in lagoon pens). Mouthbrooding efficiency is discussed in relation to female fecundity, the capacity of the males' mouth, the development stage of the incubated fry and the size ratio between males and females during pairing.

Individual spawning frequency was studied in concrete tanks with isolated pairs and families of different sex ratios. While sex ratios shifted toward the males do not increase spawning frequency significantly, sex ratios shifted toward the females decrease it considerably. The most suitable sex ratio for the production of fry of *S. melanotheron* in captivity is 1:1. Using this sex ratio, mean individual spawning intervals are generally 10-16 days. Annual egg production of this species is estimated, based on long periods of observation (174-587 days) using isolated pairs.

Introduction

Contrary to what has been observed for most cultured fish species, tilapias reproduce spontaneously and rapidly in captivity. Improved reproduction management and planning of fry production for fish culture rely on the assessment of egg production of each species. In sexually mature fish, egg production is measured using the number of fry produced per spawning episode and the spawning intervals.

In mouthbrooders (genera *Oreochromis* and *Sarotherodon*), the number of fry produced in each spawning depends, in turn, on two elements, namely, female fecundity and successful mouthbrooding (Welcomme 1967; Marshall 1979). The latter point is very important when mouthbrooding is practiced by the male as is the case in *Sarotherodon melanotheron*. Here, the mouth capacity can be a limiting factor for the incorporation of the brood in the mouth and for the development of the offspring after hatching (Aronson 1949).

In the present study, efficiency of mouthbrooding was studied in *S. melanotheron* in relation to the fecundity of females, the mouth capacity of the males, the stage

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of development of the incubated fry and the size ratio between males and females during pairing. Spawning frequency was observed in concrete tanks using isolated pairs of fish and families of different sex ratios. Based on these observations, the annual egg production of *S. melanotheron* was estimated.

In this species, contrary to other mouthbrooding tilapias, protective parental behavior is abruptly interrupted upon release of the swim-up fry, which then becomes totally independent (Aronson 1949; Lowe-McConnell 1955).

Materials and Methods

The study was conducted at the Layo experimental fish culture station, 40 km west of Abidjan, in an oligomesohaline part of Ebrié Lagoon (Côte d'Ivoire). In Layo, seasonal salinity ranges between 0 and 10 ppt with a mean monthly water temperature of 27-32°C and pH values ranging between 6.5 and 7.5.

Mouthbrooding

Female fecundity and the number of eggs or fry incubated by the males were studied in a population of *S. melanotheron* of different sizes reared in a 625-m^2 lagoon pen with an initial stocking density of 5 individuals·m^{-2}. The fish were fed with pelleted feed containing 31% crude proteins, distributed at a daily rate of 5% of fish biomass.

Fish fecundity was studied as described in Legendre and Ecoutin (this vol.). Male brooders (n=127), identified underwater—using diving equipment—by the characteristic deformation of their mouth, were captured individually with a handnet and immediately placed in a basin where the offspring was generally spat out right away.

These males were killed soon afterwards, weighed to the nearest 1 g and dissected to count the number of fry swallowed during capture. In some, the mouth capacity was determined by filling the mouth with a silicone paste using a method similar to that described by Drenner (1972). The dry casts were then removed and cleaned while their volume was measured by the displacement of water in a graded test tube.

Eggs and fry were preserved in 4% formalin and counted individually. Six arbitrary stages, visible to the naked eye, were considered to characterize the development stage of each clutch (Table 1). The mean volume of eggs or fry was determined using batches of 50 individuals in a graded test tube and the total volume of the clutch was estimated using the product: number of eggs or fry incubated x mean volume of the fry.

Spawning Frequency

ISOLATED PAIRS

Spawning frequency or the time interval between two successive spawning episodes was first studied in four pairs of fish placed in four different concrete tanks (4 m^2; water depth = 0.5 m) using lagoon water. These pairs, composed of broodfish of different sizes (120-270 g) and given feed containing 31% crude proteins at a daily rate of 3% of the fish biomass, were observed over a period of 174-587 days. Each week, the tanks were drained and spawnings were recorded. Eggs or swim-up fry were removed from the mouth of the males and counted individually. The actual spawning date was estimated based on the development stage of the offspring collected. In all cases, the offspring were removed from the tanks.

FAMILIES OF BROODFISH

In a second experiment conducted in similar conditions over a period of 76 days,

Table 1. Definition of arbitrary stages used for the characterization of ontogenic development of eggs and fry incubated by the males of *Sarotherodon melanotheron*, details of with the mean age, size and volume of eggs and fry: figure in parentheses are ranges of mean values.

Stage	Description	Age (days)[a]	Size (mm)[b]	Volume (ml)
1	From fertilization to formation of melanophores	0-2	3.4 (3.3-3.5)	
2	From first melanophores to pigmentation of the eyes	2-4	3.3 (3.2-3.4)	17.4 (13.9-21.3)
3	From pigmentation of the eyes to hatching	4-6	3.5 (3.1-3.7)	
4	From hatching to first phase of yolksac absorption	-	7.5 (5.5-8.8)	18.4 (15.2-22.7)
5	Second phase of yolksac absorption	-	10.1 (9.0-10.8)	22.7 (18.8-25.0)
6	Swim-up stage	14-15	11.4 (10.7-13.0)	26.0 (19.5-40.5)

[a]Age post-fertilization; based on Shaw and Aronson (1954) and Shaw (1956).
[b]Mean diameters for eggs; total length for fry.

spawning frequency was compared in families composed of 10 individuals of similar weight (200-230 g) with different sex ratios (F:M): 5:5; 9:1; and 1:9 (two replicates per treatment). Two additional pairs were placed in two concrete tanks and observed during the same period. Each week, all tanks were drained and the fish with eggs in their mouth were counted. Several times during draining, some fish spat out the eggs which were immediately cannibalized by the other fish in the tank. Because of this, the number of eggs produced by the families could not be determined. This problem did not occur with the isolated pairs as capture was easier and faster. At the end of the experiment, all individuals were killed for macroscopic examination of the gonads.

Results and Discussion

Mouthbrooding Efficiency

Of the 127 males sampled from the pen population, 30% swallowed part of their brood during capture. This indicates that the stomach of the fish should be routinely examined to obtain an accurate estimate of the number of incubated eggs or fry. One male swallowed nearly half of its brood (207 newly hatched individuals). However, in most cases, the number of eggs found in the stomachs did not exceed 5% of the total offspring.

A positive linear relationship ($r = 0.793$) was found between the number of incubated eggs or fry and the weight of the male brooders (Fig. 1). Covariance analysis was used to compare the regression lines between incubated stock and weight of the males, on the one hand, and fecundity and weight of the females, on the other hand. No difference was found between slopes; in contrast, the Y intercepts differed significantly. The two regression lines can therefore be considered parallel (Fig. 1). This result suggests a preferential size ratio between males and females during pairing, this size ratio being close to parity. The high increase in the number of incubated eggs with male weight shows that the largest males do

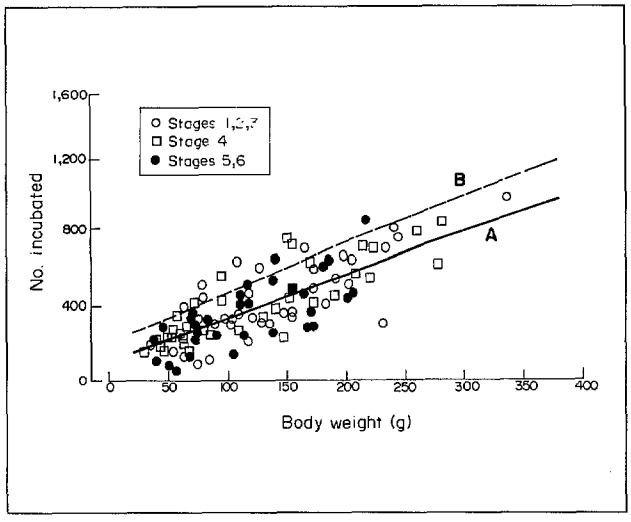

Fig. 1. (A) Relationship between number of eggs or fry incubated (N) and body weight of males (Wm) in *Sarotherodon melanotheron* reared in pens; N = 2.29 Wm + 107.15 (r = 0.793). The development stage of the clutch is indicated for each individual. (B) Relationship between fecundity (F) and body weight of females (Wf) in *S. melanotheron* reared in pens; F = 2.61 Wf + 203.91 (r = 0.777).

not mate spontaneously with the smallest females.

When the incubated stocks are examined with regard to their development stage (see Table 1), the scattergrams appear to be similar for eggs and for swim-up fry (Fig. 1), suggesting a very limited loss in eggs and fry during incubation. This was confirmed by estimating, for each individual, the ratio (R) between actual incubated stock and stock estimated by the general model describing the increase in average size of clutch with male weight (Fig. 1): R = observed stock/(2.29 Wm + 107.15). Results showed that "R" remains close to 1 (Table 2) regardless of the development stage of eggs and fry. This confirms that embryonic mortality is very low during the mouthbrooding period in *S. melanotheron*.

In *Oreochromis leucostictus*, where the females incubate the eggs, Welcomme (1967) defined mouthbrooding efficiency as the ratio of incubated stock (fertility) and number of eggs initially produced (fecundity).

In *S. melanotheron*, as incubation is practiced by the male, the estimation of mouthbrooding efficiency must be based on hypothetical size ratios between males and females during pairing. Since we have already observed that the preferential size ratio is close to parity, three hypothetical ratios were considered: (1) males and females of equal body weight; (2) males with a body weight 25% higher than that of the females; and (3) males with body weight 25% lower than that of the females.

In case no. 1 (equal weights), mouthbrooding efficiency is minimal (60%) when the male is small (25 g). Efficiency gradually increases with male weights of about 150 g, but does not exceed 80% in males of 400 g (Fig. 2).

When the male is slightly larger than the female (case no. 2), mouthbrooding efficiency improves significantly, reaching a value of 100% for males with body weight > 300 g. In contrast, when male weight is lower than female weight (case no. 3), mouthbrooding efficiency decreases significantly, never exceeding 65% (Fig. 2).

In *S. melanotheron*, mouthbrooding efficiency is similar to that observed in *Oreochromis macrochir* (60-100% depending on female sizes; Marshall 1979) and significantly higher than that reported for *O. leucostictus* (where it is never > 50%; Welcomme 1967). It should, however, be noted that both studies were conducted on mouthbrooding females captured in the wild with a seine net, and that the eggs and the fry that may have been

Table 2. Values of "R" (observed incubated stock/ estimated incubated stock [see text]) in relation to the development stage of eggs and fry incubated.

Development stage	R	No. of clutches observed
1	0.99	16
2	1.01	15
3	1.01	18
4	1.06	38
5	0.87	11
6	0.98	26

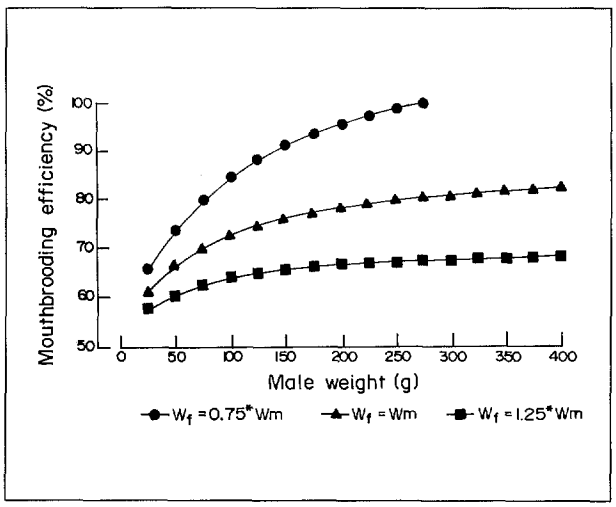

Fig. 2. Mouthbrooding efficiency in *S. melanotheron* based on different hypothetical ratios between the weight of males (Wm) and the weight of females (Wf) during pairing.

swallowed were not counted. Therefore, it is possible that the broods incubated by these species were underestimated.

In *S. melanotheron*, mouth capacity is related to male weight by a positive linear relationship (r = 0.927; Fig. 3). The comparison of this relationship with the increase in mean volume of eggs produced in relation to female weight (Fig. 3) shows that mouth capacity increases faster with male weight than the spawn volume increases with female weight. The spawn volume of females with body weight < 100 g does not differ noticeably from the mouth capacity of males of similar weight. The mean buccal volume of males with body weight < 50 g is too small to allow the complete development of the average clutch produced by a female of similar weight. Only males with body weight > 150 g have a buccal volume always higher than the mean clutch volume at the end of its development (Fig. 3). Therefore, when pairing is between males and females of equal weight, mouth capacity constitutes a limiting factor for mouthbrooding only in males of body weight < 150 g.

The percentage of the male mouth capacity occupied by the clutch (clutch volume x 100/mouth capacity) was determined for 30 male brooders of different

sizes (Fig. 4). This proportion is higher (about 40-90%) in males of body weight < 150 g than in larger males, in which it never exceeds 60%. These observations provide a reasonable explanation of the fact that mouthbrooding is less efficient in males with body weight < 150 g than in males with higher body weight (Fig. 2). These observations also tend to confirm that, in a population of individuals of different sizes, pairing preferentially takes place between males and females of similar size. As mentioned previously (Fig. 1), the largest males do not mate spontaneously with the smallest females. The fact that the proportion of the mouth occupied by the clutch does not exceed 60% in males with body weight > 150 g (Fig. 4) confirms that males neither mate spontaneously with females that are much bigger than they are.

In the wild (Ebrié Lagoon), where the size at first sexual maturity is higher than in culture conditions (Legendre and Ecoutin, this vol.), mouthbrooding efficiency is likely to be higher. The fork length of the smallest mature male observed in the lagoon environment reached 148 mm (80 g) against 105 mm (22 g) in pen culture. Because of the limited mouth capacity of the small males, the stunting (or sexual precocity) observed in culture conditions should result in lower yields of fry per single spawn.

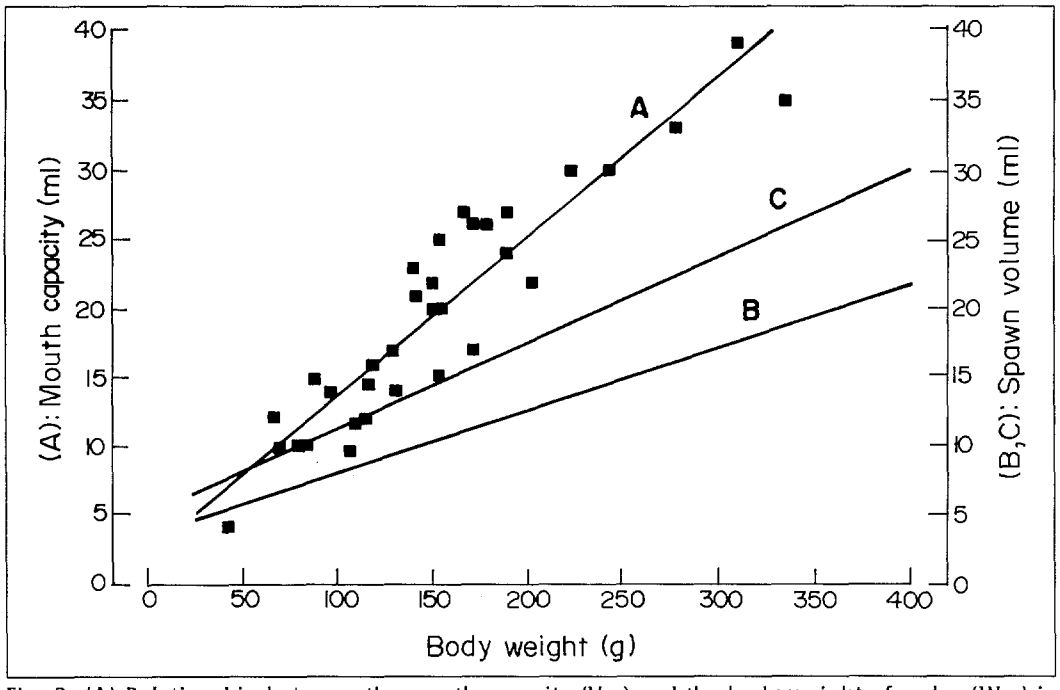

Fig. 3. (A) Relationship between the mouth capacity (Vm) and the body weight of males (Wm) in *Sarotherodon melanotheron*. Vm = 0.114 Wm + 2.21 (r = 0.927). (B) Eggs: mean increase in spawn volume with weight of female. Spawn volume = fecundity x mean volume of one egg (17.4 µl). (C) Swim-up fry: mean increase in spawn volume with weight of female. Spawn volume = fecundity x mean volume of one fry (26.0 µl). In B and C, fecundity is estimated by the relationship F = 2.61 Wf + 203.91, where Wf = female body weight (Fig. 1).

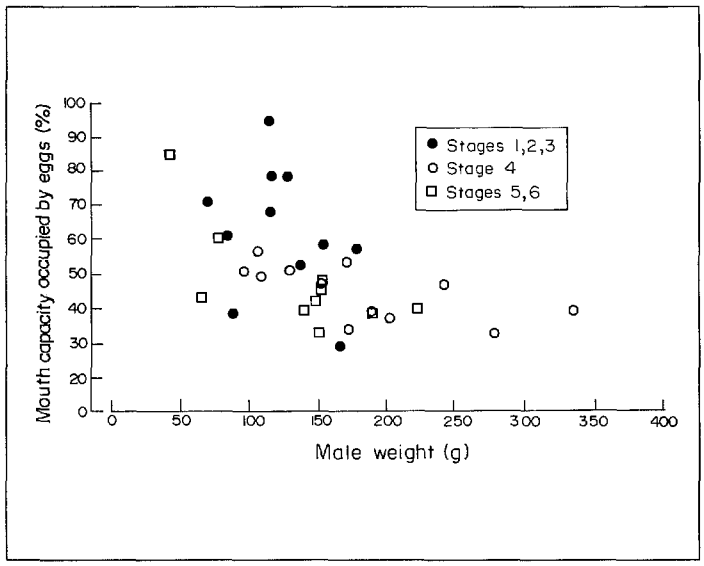

Fig. 4. Percentage of the mouth capacity occupied by eggs or fry incubated in relation to male body weight in *S. melanotheron*. The development stage of the clutch is indicated for each individual.

Spawning Frequency and Number of Eggs Collected

In isolated pairs, the mean spawning interval is generally 10-16 days (Tables 3 and 4). Only one pair composed of a large female and a small male showed higher mean intervals (25 days; Table 3). For all pairs combined, 6 and 39 days were the extreme values in the range. Therefore, the spawning frequency of *S. melanotheron* reared in 2-m^3 tanks is very regular compared to the values observed in other species of tilapias such as *O. niloticus* or *O. vulcani* (Mires 1982). Aronson (1945) reported that the spawning intervals of *S. melanotheron* reared in aquarium varied between 8 days and one year with a mode of 15 days, the latter value being in agreement with our observations.

The routine counting of clutches from each pair of the experiment showed that compared to the number of eggs or fry produced at longer spawning intervals, shorter cycles (six to eight days) never imply a significant reduction in eggs or fry production, therefore reflecting a faster vitellogenesis which involves total spawn rather than partial spawning occurrence. In general, the mean spawning interval is shorter in *S. melanotheron* (two weeks approximately) than in the species of *Oreochromis* (generally, four to six weeks; Baroiller and Jalabert 1989). In *Oreochromis*, it is established that the presence of eggs or fry in the mouth of the females has an inhibiting effect on the development of oocytes (Smith and Haley 1988). In *O. niloticus*, the frequent removal of incubating eggs is one of the methods used to increase spawning frequency and fry production (Verdegem and McGinty 1987).

The average number of eggs collected per spawning episode for the different pairs observed varied between 368 and 718 (Table 3). This quantity is closely related to the size of the males used in this experiment and does not seem to be much affected by female fecundity. Indeed, the relationship (Fig. 1) between the incubated stock and the weight of males appears highly suitable for use as a model for estimating the production of eggs by the broodfish of *S. melanotheron* reared in culture conditions (Table 3). When the weight of the males (about 200 g) is higher than the weight of females (about 150 g), the annual production of eggs for a pair

Table 3. Spawning frequency and number of eggs collected in isolated pairs of *S. melanotheron* in concrete tanks. Comparison between observed and estimated productions of eggs.

Pair no.	Observation period (day)	Mean body weight of broodfish (g)		No. of spawns observed	Mean spawning interval (day)	Total number of eggs collected	Number of eggs collected per year	Average number of eggs per spawn	Estimate of egg no.	
		Female		Male					incubated per male[a]	produced per female[b]
1	587	172	194	43	14.0±1.7	24,754	15,392	576	551	653
2	475	164	130	31	15.8±3.5	15,473	11,889	499	405	632
3	398	152	269	34	12.1±0.9	24,425	22,400	718	723	601
4	174	243	118	8	24.8±7.5	2,942	6,171	368	377	838

[a]Average number estimated using the relation between the number of eggs or fry incubated (N) and the weight of males (Wm): $N=2.29\ Wm + 107.15$ ($r=0.793$).
[b]Average number estimated using the relation between fecundity (F) and weight of females (Wf): $F=2.61\ Wf + 203.91$ ($r=0.777$).

Table 4. Spawning frequency in *S. melanotheron* for families of different sex ratios over a period of 76 days in concrete tanks. Values are the means of two replicates.

Composition of the families (No. females/no. males)	Average no. of spawns observed	Mean no. of spawns per female	Mean spawning interval (day)
1/1	6	6[a]	13.1[a]
5/5	29	5.8[a]	13.2[a]
1/9	7.5	7.5[a]	10.6[a]
9/1	14	1.6[b]	49.5[b]

Values with the same letter in a same column are not significantly different at the 5% significance level.

varies between 15,000 and 23,000 (Table 3). However, it should be noted that in the same culture conditions, the spawning frequency of *S. melanotheron* is higher in the dry season (two to three spawns per month) than during the rainy season (1.5 spawns per month; Legendre and Ecoutin 1989).

In 2-m^3 tanks, the spawning frequency per female in an isolated pair or in a family of 10 broodfish with equal sex ratios is identical (Table 4). Annual production of eggs in such a family can be directly estimated using the model linking the number of eggs or fry incubated to male weights. When the sex ratio is strongly in favor of males (9:1), the mean spawning interval, although slightly lower, does not differ significantly from that observed in families with equal sex ratios (Table 4). In contrast, when the sex ratio is in favor of the females (1:9), the spawning interval is considerably increased (50 days on average). The macroscopic examination of the gonads of these females at the end of the experiment showed that in 25% of the cases, post-vitellogenesis oocytes present in the ovaries showed high levels of atresia. This suggests that females are capable of completing normal cycles of vitellogenesis, but that lack of males in sufficient number regularly causes the total reabsorption of the oocytes ready for spawning. It is also possible that in this particular situation, the spawning frequency was underestimated: 29% of the observed incubations were done by females (with nonfertilized eggs); however, females have a strong tendency to swallow the eggs that they incubate (Aronson 1949).

Taken together, these results indicate that the most suitable sex ratio for the production of eggs and fry in *S. melanotheron* reared in captivity is 1:1. A higher male sex ratio does not significantly increase individual spawning frequency while a higher female sex ratio decreases it considerably.

From a practical point of view, the abovementioned problem of cannibalism on eggs and fry spat out by the mouthbrooders during draining indicates that this particular system, with weekly collection of the offspring, is poorly adapted for the efficient management of broodfish. In the future, this problem should be solved by maintaining fish in hapa-like structures which are easier to handle (Hugues and Behrends 1983) and allow rapid and simultaneous capture of all broodfish.

Conclusion

In *S. melanotheron*, mouthbrooding efficiency is reduced in males with body

weight < 150 g compared to larger individuals. Smaller males have a limited mouth capacity which can hardly take in all the eggs produced by the smallest females. Due to this physical constraint, mouthbrooding efficiency is significantly improved when a female mates with a larger male. Egg and fry mortalities appear to be low during the mouthbrooding phase. When creating broodfish families, it is recommended not to use males with body weight < 150 g and to always choose males larger than females.

In captive *S. melanotheron*, spawning intervals are about two weeks. The most suitable sex ratio for the constitution of families of broodfish for egg and fry production in culture conditions is 1:1. Observations on egg production by isolated pairs over a long period of time indicate that the relationship between the number of eggs and fry incubated, and the weight of the males can be used to predict and plan the production of fry in a fish farm.

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A Search for Sex-specific DNA Regions in *Oreochromis niloticus*

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Abstract

A study was undertaken to identify sex-specific regions of the genome in *Oreochromis niloticus*, using probes which give sex-specific hybridization patterns in other organisms: pDP1007, a human Y chromosome-specific probe sequence and pUGD0600, a W chromosome-specific sequence in chickens. DNA was extracted from sibling males and females, and hybridized to the probes after cutting with a number of restriction enzymes. No sex-specific patterns were observed but the results suggested further lines of comparative work using similar techniques.

Introduction

Since the work of Hickling (1960), sex-determination mechanisms in tilapias have been studied by intra- and interspecific crosses (Jalabert et al. 1974; Majumdar and McAndrew 1983; Hanson et al. 1983); hormonal sex reversal and progeny testing (Clemens and Inslee 1968; Jalabert et al. 1974; Mair et al. 1987; Gilling et al., this vol.); and induction of diploid gynogenesis (Chourrout and Itskovich 1983; Penman et al. 1987; Avtalion and Don 1990; Mair et al. 1991a, 1991b). In *O. mossambicus* and *O. niloticus*, there is evidence for a basic system of female homogamety (XX) and male heterogamety (XY), whereas *O. aureus* demonstrates female heterogamety (WZ) and male homogamety (ZZ) but inconsistencies in the sex ratios from both sex reversal (Calhoun and Shelton 1983) and gynogenesis (Avtalion and Don 1990) experiments have resulted in other models of sex determination being put forward (Avtalion and Hammerman 1978; Hammerman and Avtalion 1979; Avtalion and Don 1990).

Sex-specific DNA sequences have been observed in a number of organisms ranging from *Schistosoma* spp. to humans (e.g., Kodama et al. 1987; Walker et al. 1989; Page et al. 1990).

Recent studies have attempted to find sex-specific genomic DNA banding patterns in fish using a variety of sex-specific and tandem repeat probes from other phyla and synthetic oligonucleotide repeat sequences. Ferreiro

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et al. (1989) hybridized Page's mammalian Y chromosome-specific probe (Page et al. 1990) to trout and sturgeon genomic DNA and observed nonsex-specific banding patterns. Lloyd et al. (1989) found that GATA-GACA repetitive sequences, first isolated as a female-specific satellite DNA fraction in the banded krait (Bkm sequences; Singh et al. 1980), cross-hybridized to trout genomic DNA but gave nonsex-specific banding patterns that revealed fingerprint polymorphisms. Nanda et al. (1990), using a (GATA)4 oligonucleotide probe, found a male-specific simple tandem repeat locus in outbred populations of the guppy (*Poecilia reticulata*), but this was only observed in two out of eight laboratory strains. Devlin et al. (1991) have recently succeeded in isolating a Y chromosome-specific DNA probe for *Oncorhynchus tshawytscha* using subtractive hybridization.

In this study, two labelled probes, a tandem repeat probe cloned into vector pUGD0600, which is sex-specific in *Gallus* spp. (Kodama et al. 1987), and a human Y chromosome-specific probe cloned into vector pDP1007 (Page et al. 1990), were hybridized to genomic DNA from *O. niloticus* to investigate their specificity in discriminating between males and females. Both probes are nonradioactively labelled. In the past, nonradioactive labelling has not proved sensitive enough for routine detection of single copy sequences. However, the chemiluminescent labelling technique used in this study promises sensitivity equal to that of P^{32}. Moreover, radiochemical facilities are not required and these chemiluminescent probes are stable for up to one year compared to a few days for radioactively labelled probes.

This study was specifically aimed at finding simple differences in the genomic DNA of male and female *O. niloticus*, and assessing the feasibility of using chemiluminescent probes in work with fish DNA.

Materials and Methods

Samples

Work was carried out on an *O. niloticus* (Lake Manzala) stock maintained at Swansea and originally obtained from Stirling University. Blood samples were taken from the caudal vein under anesthetic and fin clippings from the caudal and dorsal fins. Seven males and 10 females were used in hybridization experiments with probe pDP1007. With probe pUGD0600, five males and five females were used.

DNA Extraction

High molecular weight DNA samples were extracted from blood and fin samples by overnight digestion in proteinase K (Boehringer Mannheim GmbH; 10 $\mu g \cdot ml^{-1}$) in STE DNA extraction buffer (0.1M NaCl, 0.05M Tris base and 0.1M EDTA) and 0.5% SDS (sodium dodecyl sulfate) followed by phenol/chloroform extraction and precipitation by isopropanol. DNA sample concentration was determined by fluorimetry (Hoefer Scientific Instruments, TKO 100 mini-fluorimeter).

Electrophoresis and Blotting

DNA samples of 10-20 µg were digested overnight with *EcoR*1 and run on a 0.8% agarose gel for eight hours in TEA (0.4M Tris Base, 0.2M Na acetate, 0.02M EDTA and 0.18M NaCl) buffer and then denatured before posiblotting for one hour (Stratagene) onto a positively charged nylon membrane (Boehringer Mannheim GmbH) and fixed by baking at 120°C for 25 minutes.

Probes

Two probes were used: plasmid pDP1007 (Page et al. 1990), which contains a 1.3kb *Hind*III fragment of the human ZFY gene, cloned into the *Hind*III site of pUC13—the probe sequence was recovered from a low melting point agarose gel after digestion with *Hind*III; and plasmid pUGD0600 (Kodama et al. 1987) containing a W chromosome-specific 0.7kb repeat unit from the female White Leghorn chicken in the Sal 1 site of pUC9—the probe was recovered by digestion with PstI and *EcoR*1. Both probes were labelled by random priming with dioxigenin-duTP (Boehringer Mannheim GmbH).

Hybridization

Filters were prehybridized for three hours in a solution of 50% (w/v) formamide; 2% (w/v) blocking reagent (Boehringer Mannheim GmbH), added from a 10% sterile stock solution; 0.02% (w/v) SDS; 0.1% (w/v) N-lauroyl-sarcosine; and hybridized according to a protocol supplied by Boehringer for 16 hours at 42°C with 30 µl (approximately 300 ng) of digested/labelled probe in 4 ml of prehybridization solution. Filters were then washed at low (2xSSC, 0.1% SDS; 2x10 minutes at room temperature: 1xSSC, 0.1% SDS; 2x20 minutes at 60°C) or high (2xSSC, 0.1% SDS; 2x10 minutes at room temperature: 0.5 or 0.1xSSC, 0.1% SDS; 2x20 minutes at 68°C) stringency. Digested/labelled probes were detected using AMPPD (Tropix Inc., Bedford, Massachusetts, USA) as a chemiluminescent substrate for anti-dioxigenin-AP-conjugate and visualized by auto-radiography (10-80 minutes exposure).

Results and Discussion

When hybridized to *O. niloticus* DNA (*EcoR*1 digest, Fig. 1), the probe cloned into plasmid pDP1007 gave a single

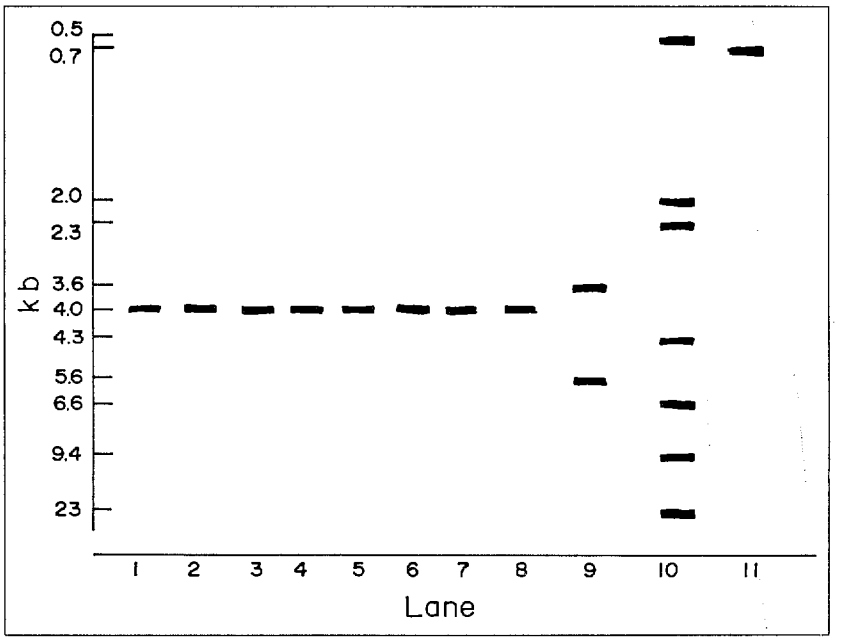

Fig. 1. Results of hybridization with the probe sequence cloned in plasmid pDP1007. Lanes 1-4 contain *EcoR*1 restricted male *O. niloticus* DNA; lanes 5-8 contain *EcoR*1 restricted female *O. niloticus* DNA. Lane 9 is male human DNA restricted with *EcoR*1. Lane 10 is lambda DNA restricted with *Hind*III and digested/labelled. Lane 11 is the probe sequence.

4kb band in both male and female individuals, while an *EcoR*1 digest of male human DNA gave two bands at 3.6 and 5.6kb. The 3.6kb band corresponds to the Y-specific band seen by Page et al. (1990). Ferreiro et al. (1989) observed no variation in banding patterns between male and female rainbow trout when Taq I, Hae III and KpnI digests of trout genomic DNA were hybridized to pDP1007.

When the sequence cloned into plasmid pUGD0600 was hybridized to *O. niloticus* genomic DNA, a strong signal throughout each lane on the filter occurred even at high stringency with no clear bands visible. This is a repeat sequence probe and the results from its use point to the presence of many repeat sequences scattered throughout the genome.

While no sex-specific patterns were observed, the presence in *O. niloticus* of a sequence homologous to that of the human ZFY gene was demonstrated even under high stringency wash conditions. Thus, although the molecular basis of the sex determining "switch" is different in fish and mammals, the ZFY gene is conserved between these lineages. It has also been demonstrated that nonradioactive chemiluminescent probes are capable of detecting single copy sequences in tilapia.

In taxonomic groups where the evolution of sex has been well-studied (e.g., mammals and reptiles), similarities have been observed in the organization and evolution of sex chromosomes (Jones and Singh 1985; Page et al. 1990). This is particularly true of the accumulation of repeat sequences on the heterologous sex chromosome (Y chromosome in mammals; W chromosome in birds). The conservation of sequences involved in sex determination may be determined by cross-taxonomic hybridization of sex-specific probes. This approach will prove useful in the study of the evolution of sex-determining mechanisms. Both probes used in this study cross-hybridize widely and give sex-specific patterns in the taxa from which they were isolated.

A more immediate approach to studying the molecular basis of sex determination in the tilapias may be the isolation of tilapiine sex-specific sequences using subtractive hybridization techniques (Devlin et al. 1991).

Acknowledgements

Thanks are due to Dr. S. Mizuno for providing probe pUGD0600 and Dr. D. Page for the provision of probe pDP 1007. Thanks also to Cath Fisher for help with many of the techniques used. This work was supported by the Overseas Development Administration, London.

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Comparative Growth of Hybrids (F_1, F_2 and F_3) of *Oreochromis niloticus* (L.) and *O. macrochir* (Blgr.)

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MICHA, J.-C., R. CUVELIER, CH. TILQUIN, B. MURAILLE, M. BOURGOIS and U. FALTER. 1996. Comparative growth of hybrids (F_1, F_2 and F_3) of *Oreochromis niloticus* (L.) and *O. macrochir* (Blgr.), p. 354-360. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Based on the hypothesis of an introgression with slowed growth between two mouthbrooding species of *Oreochromis niloticus* and *O. macrochir* introduced into different African lakes, we performed a series of hybridizations up to the third generation then compared, in controlled conditions (aquarium), the growth of the individual juveniles (from 5 g to ±100 g) from parental strains and different progenies. Results show that the heterosis effect in the F_1, F_2 and F_3 hybrids is relatively low and no more determining than the intraspecific variation. However, the pure *O. niloticus* strain grows faster than either *O. macrochir* or the different hybrid strains, except for the F_1 hybrid *O. niloticus* female x *O. macrochir* male (NM). This seems to confirm the hypothesis of an introgression of part of the *O. macrochir* gene pool into the *O. niloticus* gene pool. However, the presence of two hybrid subgroups with different growth rates does not seem to be confirmed by our aquarium growth results, which are limited to the early stages (7 to 100 g).

Introduction

In the 1950s, the production results of farmed tilapia credited this animal for being the miracle fish capable of putting an end to malnutrition problems in developing countries (Micha 1974; Powles 1987). Amidst the general enthusiasm, various species—not always correctly identified—were introduced into many African lakes. The idea was not a bad one, at least for lakes with vacant ecological niches for this type of species. Unfortunately, insufficient knowledge of tilapia taxonomy, the absence of fauna inventories in the different lakes and ignorance of the mechanisms of speciation resulted in a disruption of the biogeography of this African genus and, in many cases, in hybridization between native and introduced species.

In general, hybridization is a technique used by farmers to select faster-growing strains. "Semi-natural" hybridization in lakes—Lake Itasy in Madagascar (Daget and Moreau 1981) and Lake Ihema in Rwanda (Plisnier et al. 1988)—is seen as a failure of specific reproductive isolation that often leads to a deterioration in the genetic stock of the parental species. Thus in Lake Itasy, which harbored a flourishing population of previously introduced *Oreochromis macrochir*, the additional introduction in 1961 of *O. niloticus* resulted in the disappearance of *O. macrochir* in favor of *O. niloticus*. In Lake Ihema, the introduction of *O. macrochir* (circa 1970), after the introduction of *O. niloticus* (circa 1950), led to the appearance of hybrids (circa 1979). Fish counts from 1983 to 1987 (Kiss 1976; Plisnier 1984; Mughanda and Micha 1988) indicate that the phenotype of *O. niloticus* decreased in size during this period (from 30 to 20% of individuals), while the phenotype *O. macrochir* remained at 60%, and the other individuals showed a variety of intermediate types (hybrid swarms: 10 to 20%). The hybridization pattern between these two close parent species introduced in two different lakes therefore seems to have evolved along different paths.

To test this hypothesis of introgression, using a comparison of the morphological characteristics and growth of hybrids, controlled aquarium experiments were performed hybridizing F_1, F_2 and F_3 generations and their backcrosses, and measuring and comparing growth performance of fry.

Materials and Methods

The broodfish of *O. niloticus* (16 individuals, including eight males) came from the Egyptian strain bred at Auburn University and introduced to Rwanda. The broodfishes of *O. macrochir* originated from the Kigembe fish-farm in Rwanda.

The growth tests and the preliminary stages of the experiments (reproduction, nursing and pre-experimental growth) were conducted in the M. Huet laboratory facilities of the College of Agriculture Science at the Louvain Catholic University (UCL), Louvain-la-Neuve (Kestemont et al. 1989), where there are many 200-, 100- and 70-l aquaria (L: 58 cm, W: 48 cm and H: 50 cm). The water was maintained at 28±2°C and a 12-hour (0700-2100) photoperiod using three 40-watt fluorescent bulbs was applied. The aquaria were drained daily to remove organic residues from the bottom. During experiments, the oxygen concentration varied between 70 and 100% saturation, while the nitrate concentration never exceeded 1 $mg \cdot l^{-1}$.

To obtain the desired hybridizations (the female sibling is conventionally indicated first), the arena system of Haller and Parker (1981) was adapted by equipping aquaria (200 and 100 l) with two plastic-coated lattice partitions separating the females' areas (Fig. 1). A third partition with openings allowed the passage only of females smaller than the males. This system protected females that were not ready to spawn from the unceasing attacks of the territorial male. Each aquarium contained a group of four fish: one male and three females. The condition of the females was monitored several times daily. As soon as one of them was incubating (mouth enlarged with eggs, feeding stopped), the male was removed. Six to seven days later, the two other females were also removed. Swim-up fry appeared two to three days later. Ten days later, the fry were separated from their mother and placed in pre-growout aquaria (50/aquarium), where they were fed ground

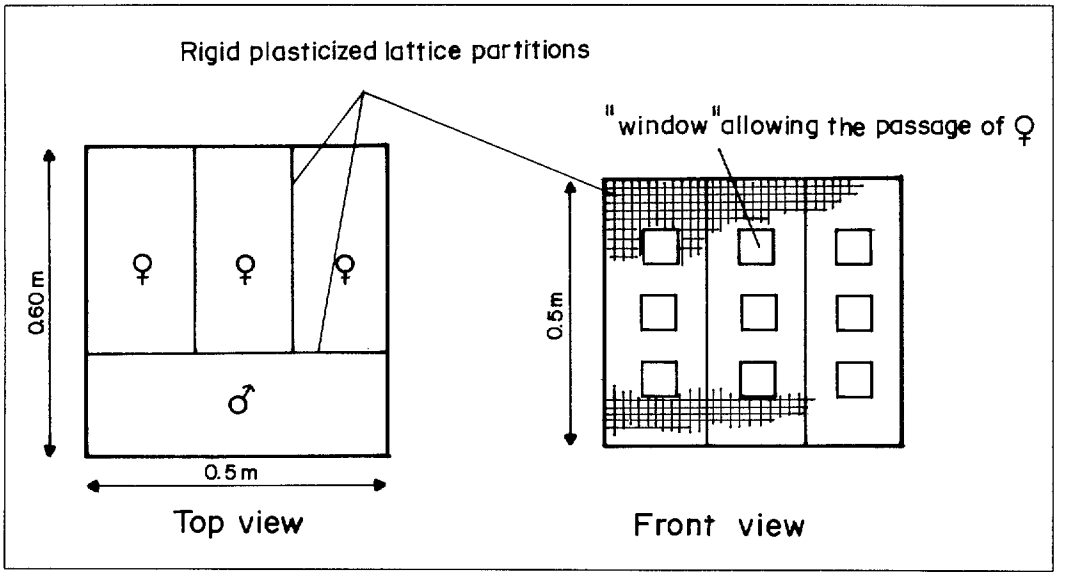

Fig. 1. Arena system of Haller and Parker (1981) adapted to the scale of the aquarium.

pellet (Trouvit K30) at 6% of the fish biomass in three daily rations. For growth rates, two groups of 16 individuals per aquarium (70 l), with 7.0 g mean body weight and individual tags (burned with a pin head), were monitored over 10 weeks. Each week, all fish were measured (standard length to the nearest 0.1 mm) and weighed individually after wiping on paper towels (weight in g to the nearest 0.1 g). The sex of each individual was determined by binocular examination of the genital papilla.

The increase in weight was estimated using the standard exponential model (Wheaterley 1976), and the growth comparison of the different strains was done using the mixed nested ANOVA I or II (Dagnelie 1975). The "aquarium" random variable consists of two levels that are further subdivided into four or six levels of the "strain" independent variable. This is an unbalanced equation, given that the different combinations of the two factors comprise a different number of observations (random number of females among the 16 individuals in each aquarium). For this analysis, we used the Statistical Analysis System (SAS) software of the UCL Calculation Center at Louvain-la-Neuve.

Results

Comparative Growth of Pure Strain Broodfishes and F_1 Hybrids

The parental strains (*O. niloticus* and *O. macrochir*) and the F_1 hybrids from the crosses of *O. niloticus* female x *O. macrochir* male (NM) and of *O. macrochir* female x *O. niloticus* male (MN) were monitored over the 10-week experiment. The mean body weight of the fish increased from 7.0 g to nearly 100 g.

The statistical analysis revealed no significant difference between aquaria of the same strain (replicate). This result allowed us to gather the data for each strain from the two aquaria and to test the strain effect by comparing a bigger number of observations with greater accuracy. Only the male data were used to test the different strains (*O. niloticus*: 11 males, *O. macrochir*:

18 males; and F_1 Nfemale x Mmale: 32 males, F_1 Mfemale x Nmale: 18 males). The growth results, presented in the form of linear regressions (weight vs. time) (Fig. 2), showed that:

- F_1 Nfemale x Mmale (NM) hybrids have the highest growth rate;
- the *O. niloticus* (M) strain ranks in second;
- F_1 Mfemale x Nmale (MN) hybrids rank third; and
- the *O. macrochir* (M) strain has the poorest growth rate.

The four-level (four strains) ANOVA I statistical test confirmed that:

- F_1 Nfemale x Mmale (NM) hybrids have a growth rate significantly higher than *O. niloticus* (P=0.05);
- F_1 Nfemale x Mmale (NM) hybrids have a growth rate significantly higher than their reciprocal hybrid (P=0.01);
- F_1 Mfemale x Nmale (MN) hybrids have a growth rate significantly higher than *O. macrochir* (P=0.01); and
- *O.* macrochir has a growth rate significantly lower than all the other strains.

Furthermore, it must be noted that the cross Nfemale x Mmale yielded a homogeneous hybrid progeny (100% male), while the opposite cross, female *O. macrochir* and male *O. niloticus*, yielded a heterogeneous progeny with 75% males and 25% females, each sex showing two clearly different phenotypes (pink-red and gray in equal proportions).

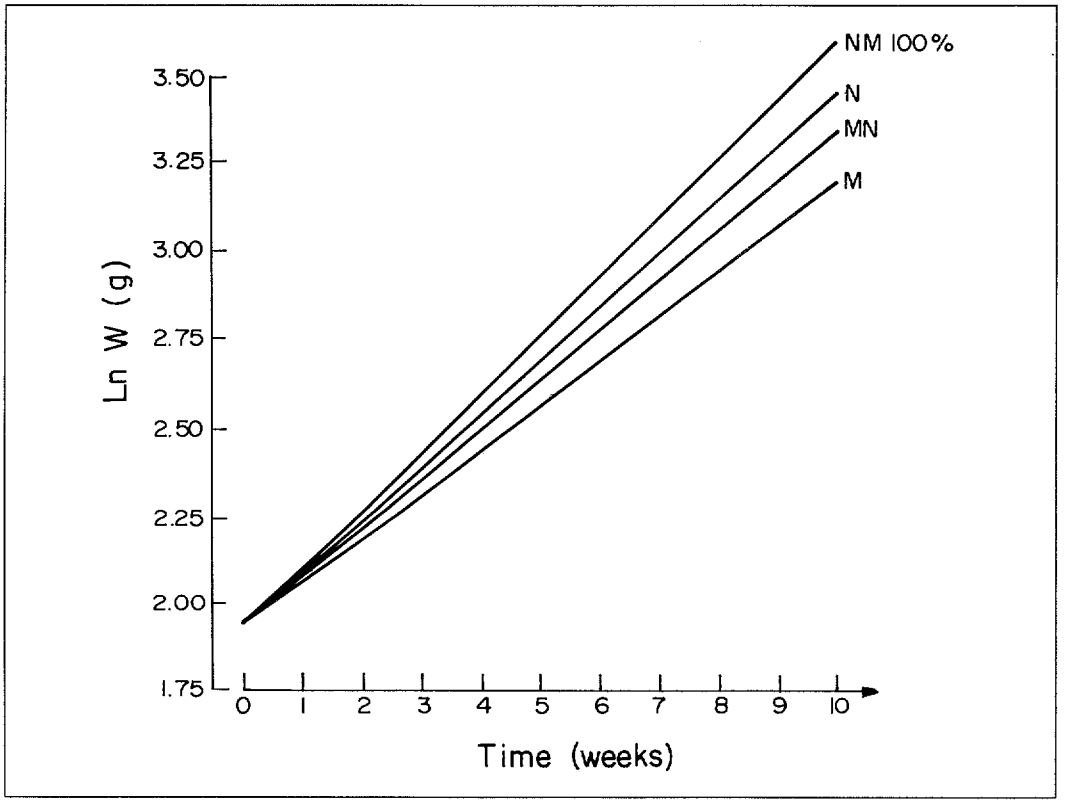

Fig. 2. Regression lines of ln weight (g) vs. time for the four *Oreochromis niloticus* (N) strains, *O. macrochir* (M), F_1 hybrids (Nfemale x Mmale: 100% males) and F_1 reciprocal hybrids (Mfemale x Nmale).

Comparative Growth of Pure Strain Broodfishes, F_2 Hybrids and Backcrosses

In the second generation, three types of crosses were performed:

- female hybrid x male hybrid, gray variety (g): Mng x Mng,
  red variety (r): Mnr x Mnr;
- pure *O. macrochir* female x male hybrid: MM x NM, MM x MN; and
- female hybrid x pure male: MN x NN, MN x NM.

The cross of pure female x male hybrid of *O. niloticus* was not successful. The pure intraspecific crosses of *O. niloticus* (N) and *O. macrochir* served as reference. The comparative growth of the pure strains, second generation hybrids and backcrosses (Table 1) showed that the red-colored F_2 hybrids (MNrMNr) and the backcross (MMNM) between *O. macrochir* females and NM hybrids, as well as the pure strain of *O. niloticus* (N), had significantly higher growth rates than the other crosses. Ranking next were the two gray-colored MNgMNg crosses and the MNMM cross. The growth rates of the MMNN and MNNN crosses, which are backcrosses between females of *O. macrochir* and MN males and between MN females and males of *O. niloticus*, respectively, can be described as relatively low. Finally, the pure strain of *O. macrochir* (M) showed a significantly lower growth rate than F_2 hybrids and backcrosses.

Comparative Growth of Pure Strain Broodfishes and F_3 Hybrids (Backcrosses)

For the third generation, a multitude of crosses were possible. However, according to the description of the introgression phenomenon, the crosses that occur were backcrosses between various levels of hybrids and parental species. There were therefore two types of crosses to conduct:

Table 1. Comparisons of weight increase expressed as slopes and their standard deviations in *Oreochromis niloticus* (N) and *Oreochromis machrochir* (M). Items MN_rMN_r to M refer to parental strains, F_2 hybrids (MN_rMN_r and MN_gMN_g, with r and g referring to red and gray, respectively) and backcrosses with pure (underlined) females (MNNM, NNMN) or males (MNMM, MNNN). Items MNNNNN to N refer to male fingerlings from parental strains (N,M) and two F_3 hybrids between hybrid females and pure (underlined) males (MNNNN and MNMMMM).

	Slopes		
Item	Mean	SD	CV (%)
MN_rMN_r	0.0224	0.0032	14.3
MMNM	0.0223	0.0031	13.9
N	0.0218	0.0036	16.5
MN_gMN_g	0.0182	0.0038	20.9
MNMM	0.0181	0.0039	21.6
MNNN	0.0176	0.0048	27.3
MMMN	0.0174	0.0034	19.5
M	0.0155	0.0036	23.2
MNNNNN	0.0223	0.0032	14.4
MNMMMM	0.0230	0.0043	18.7
M	0.0232	0.0043	18.5
N	0.0256	0.0035	13.7

- pure female x male hybrid; and
- female hybrid x pure male.

However, the cross of female *O. niloticus* x male *O. macrochir* is very difficult to produce (Falter, this vol.) and the cross between pure females of *O. niloticus* and male hybrids of *O. macrochir* has not taken place in the aquarium (problem of aggressive behavior). We therefore limited ourselves to backcrosses of female hybrids x pure males. The chosen female hybrids (MNMM and MNNN) were second generation backcrosses in which the female was also a hybrid. The comparative growth of the male fry of the four strains (Table 1) showed *O. niloticus* in first place, followed by *O. macrochir*. Next came the F_3 hybrids, the two genotypes of which seemed to show no difference in growth rate. The statistical analysis revealed a significant difference only between the genotype *O. niloticus* (N) and the three other genotypes.

Discussion

A heterosis effect with respect to growth, brought about by interspecific hybridization in the genus *Oreochromis*, has already been shown in several crosses. *O. niloticus* is involved in several of these: with *O. hornorum* (Pruginin 1968); with *O. mossambicus* (Avault and Shell 1966, Kuo 1969); and with *O. macrochir* (Jalabert et al. 1971). Our results show, like those of Jalabert et al. (1971), that the cross of *O. niloticus* and *O. macrochir* also exhibits a heterosis effect and all the more so because the female belongs to the faster-growing species (*O. niloticus*). This could reflect a direct maternal effect (Falter and de Jaegere 1989).

The genotype *O. niloticus* was significantly different from the other genotypes. Moreover, the genotype MNNNNN that was most similar to the genotype *O. niloticus* showed a growth rate much closer to that of the genotype *O. macrochir*.

The absence of replicates in crosses, means, however, that caution is needed in drawing conclusions.

Finally, the genotype *O. macrochir* generally showed the lowest growth rate compared to the F_1 and F_2 hybrids, but it showed no difference compared to the F_3 genotypes (backcrossed).

These results tend to support the Daget and Moreau (1981) hypothesis of introgression of the gene pool of *O. macrochir* into that of *O. niloticus*, which is the only remaining phenotype in Lake Itasy, but the results cannot explain the strange persistence in Lake Ihema of *O. macrochir* (60% of tilapia catches) and the low representation of *O. niloticus* (20%).

Finally, we must emphasize the importance of environmental factors that were not considered here, but that obviously create different life conditions that can modify the dynamic balance between species within a defined biocenosis.

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Comparison of Growth Performance and Electrophoretic Characteristics of Three Strains of *Oreochromis niloticus* Present in Côte d'Ivoire

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Abstract

Growth performance and characterization of three strains of *Oreochromis niloticus* present in Côte d'Ivoire were compared through a study of enzyme polymorphism: the "Bouake" strain at the Institut des Savanes (IDESSA) research station in Bouake; the same strain kept in confinement for the past six years by a small-scale fish farmer in Western Côte d'Ivoire (Daloa); and a strain (named "Burkina Faso") from the Volta basin recently introduced at the IDESSA station in Bouake. The results of tests run for a duration of four months indicated that the growth performance of the three strains met the requirements of commercial aquaculturists (mean individual growth ≥ 2.5 g·day^{-1}). All three strains were identified and differentiated using electrophoretic techniques. Results showed high levels of polymorphism, particularly in the "Bouake" strain kept at IDESSA.

The authors conclude that all three strains show good potential for aquaculture. The strains present at the IDESSA station ("Bouake" and "Burkina Faso") are also polymorphic stocks that can be used in genetic improvement programs.

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Introduction

The Institut des Savanes (IDESSA) research station (formerly the "Centre technique forestier tropical" [CTFT]) in Bouake, Côte d'Ivoire is at the origin of many transfers of *O. niloticus* not only to different African countries but also to Brazil (Lazard 1990). From Brazil, the "Bouake" or "Côte d'Ivoire" strain was transferred to Auburn University (Alabama, USA) (Lovshin and da Silva 1975). Individuals of *O. niloticus* originally from Côte d'Ivoire also occur in Israel (Hulata et al. 1985).

Results from experiments using the Ivorian strain at Auburn University and in Israel showed the relatively low fecundity of this strain compared to a Ghanaian strain (Hulata et al. 1985; Smitherman et al. 1988), difficulties in improving its growth performance through selection (Teichert-Coddington 1983) and inferior growth potential compared to an Egyptian strain (Khater 1985). A review of the current status of these strains of *O. niloticus* in Côte d'Ivoire was found necessary and it is in this context that the growth performance of the three Ivorian strains were tested at the IDESSA station in Bouake.

Materials and Methods

Origin of the Test Strains

The three test strains are the following:

1. The "Bouake" (Bk) strain resulting from the progressive mixing at the IDESSA station in Bouake of fish introduced in 1957 from Burkina Faso (Volta basin) and in 1968 from Uganda, but originally from the Nile basin. It is this mixed or "synthetic" strain that was transferred to other countries and introduced in vast areas of the Ivorian water system. As the Bk strain is the result of different genetic contributions, it may have been less homogeneous in the past than it is today. Therefore, differences may be found in the characteristics of the fish of this strain depending on the time of transfer.
2. The "Daloa" (Da) strain derived directly from the Bk strain. It is used by rural fish farmers in Midwestern Côte d'Ivoire where the strain was introduced several years ago. The fish on these very small farms live in confinement which may lead to genetic drift.
3. The "Burkina Faso" (BF) strain introduced at the Bouake station in 1987 from the Kou valley (Volta basin) to renew, if necessary, the Bk strain (Lazard 1990).

Evaluation of the Growth Performance of the Three Strains in the Context of an Experiment for the Production of Table Fish

TEST FISH

Manually sexed male fingerlings were used for this experiment. Whereas the fingerlings of the Bk and BF strains were nursed at the Bouake station under comparable conditions, the fingerlings of the Da strain were introduced already sexed from the fish farms in Midwestern Côte d'Ivoire. The nursing conditions for the Da strain were different from those adopted for the two other strains but one cannot tell the impact this may have had on the differences in growth observed between strains.

PONDS AND EXPERIMENTAL DESIGN

Nine 50-m^2 ponds were used for this experiment (four-month test period) at the Bouake station using a randomized block experimental design (three replicates

per "strain"). A summary evaluation of the pond fertility was done to determine block allocation before the beginning of the tests. To this end, male fingerlings of *O. niloticus* Bk were stocked in each pond at a density of 0.8 ind·m^{-2} and reared for a duration of one month during which no treatment was applied to the ponds. Pond production varied from a maximum of 530 g·pond^{-1}·month^{-1} (1,272 kg·ha^{-1}·year^{-1}) to a minimum of 150 g·pond^{-1}·month^{-1} (360 kg·ha^{-1}·year^{-1}). Ponds with the three highest-ranking yields were assigned to Block 1; the three following yields to Block 2; and the last three to Block 3. The allocation of the different strains within each block was done by drawing of lots.

Stocking was done with 110 male fingerlings (2.2 ind·m^{-2}) with individual mean body weight of 28.55 g for the Bk strain, 31.79 g for the Da strain and 39.61 g for the BF strain.

FISH FEED

Throughout the experiment, the fish received an industrial pelleted feed produced in Côte d'Ivoire ("2GE" from FACI[c] containing 30% protein, 10% of which was of animal origin). The feed was distributed ad libitum via feeders at equal daily rates in all ponds. Feeding rates were adjusted to the lowest consumption rates among the nine ponds, and were increased or decreased depending on the consumption levels recorded at the end of the day.

The relationship between the mean monthly feeding rates per pond and the estimated mean fish biomass per pond (estimation based on an evaluation of all ponds) gave feeding rates of 4.7, 2.6, 1.8 and 1.6% of the fish biomass for each of the four months of experimentation, respectively.

[c]FACI: Fabrique d'aliment de la Côte d'Ivoire (Feed Producers of Côte d'Ivoire).

TEST HARVESTS

Twenty-five to 55% of the fish were harvested in each pond every month using a seine net to estimate the increases in individual weight.

FINAL HARVEST

All ponds were totally drained after 122 days of experimentation. The fish from each pond were counted and weighed together.

Electrophoretic Characterization of the Three Strains

Thirty individuals per strain were randomly sampled at the end of the culture experiment. Samples of muscle, eye and liver tissues were taken from each individual and deep-frozen. These samples were pounded in distilled water (muscle 1 g·ml^{-1}, eye 1 g·0.5 ml^{-1}) and centrifuged to 5,000 r·min^{-1} for 30 minutes. For the eye tissues, one-third chloroform was added to eliminate glycoproteins and glycolipids that can induce distortions during enzyme separation.

This study was done using electrophoretic techniques on starch gel. The separation and staining techniques used were modified from Krieg (1984) and McAndrew and Majumdar (1983), following a protocol established by Rognon and Guyomard (this vol.). Eighteen enzyme systems coded by 30 loci were analyzed (Table 1).

Results

Comparison of Growth Performance

Complete results are presented in Table 2. Table 3 gives the mean daily individual weight gain (MDWG), mean yields and mean feed conversion ratios (FCR).

Table 1. List of the enzyme systems studied: location in the tissues, structure and the enzyme separation buffer used. Tissues: L = liver, M = muscle, E = eye. Enzyme separation buffers: MC2 = morpholin citrate-pH 6.2 (gel = 5%), MC4 = morpholin citrate-pH 6.6 (gel = 10%), RW = ridgway, TEB = tris-EDTA-borate.

System	Locus	Tissues	Buffer
AAT	1*	LME	MC2
	2*	LE	MC2
	3*	ME	MC2
ADH	1*	L	RW
AK	1*	M	MC2
CK	1*	ME	RW
	2*	E	RW
EST	2*	ME	RW-TEB
LBP	1*	M	MC4
	2*	L	MC4
LH	1*	M	MC2
G3PDH	1*	M	MC4
	2*	LM	MC4
GPI	1*	ME	RW
	2*	LE	RW
IDDH	1*	L	RW
IDHP	1*	L	MC4
	2*	M	MC4
LDH	1*	ME	RW
	2*	LME	RW
	3*	E	RW
MDH	1*	ME	MC2
	2*	LME	MC2
	3*	M	MC2
MEP	1*	M	MC4
	2*	LM	MC4
MPI	1*	M	TEB
PGDH	1*	LM	MC4
PGM	1*	M	TEB
SOD	1*	L	RW

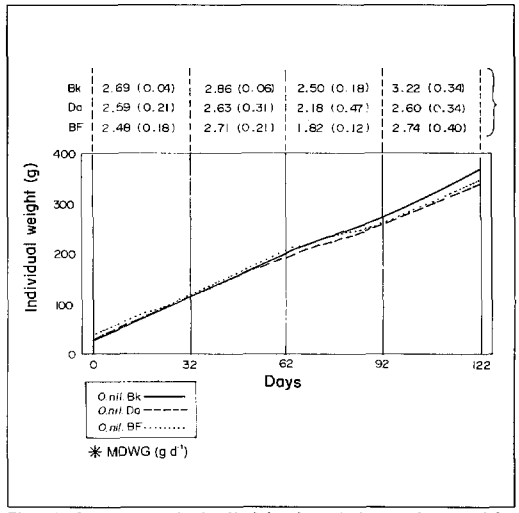

Fig. 1. Increases in individual weight and monthly growth for the three strains of *Oreochromis niloticus*. Standard deviations are given in parentheses.

Fig. 1 shows increases in mean individual weight and mean daily weight gain recorded during the monthly test harvests and the final harvest.

All three strains gave satisfactory individual growth results although the Bk strain scored higher with 2.85 $g \cdot day^{-1}$ and a peak >3 $g \cdot day^{-1}$ during the fourth month of experimentation. Relatively weak FCR (Bk 1.58; Da 1.72; and BF 1.85) were records.

Yields were compared in terms of growth and survival rates through statistical analysis. The analysis of variance and Student's t-test showed highly significant differences (at the 1% significance level) between the yields of the Bk strain (20,376 $kg \cdot ha^{-1} \cdot year^{-1}$), on one hand, and those of the Da and BF strains (18,812 and 17,742 $kg \cdot ha^{-1} \cdot year^{-1}$, respectively), on the other. The differences in yield between the Da and BF strains were at the significance level of 5%.

Comparison of the Electrophoretic Characteristics

Allelic frequencies at the polymorphic loci, polymorphism and heterozygocity are presented in Table 4. Eight loci are polymorphic in the Bk strain, four in BF and five in Da.

The stock used for aquaculture in Daloa is originally a strain of *O. niloticus* from Bouake which was dispersed all over Côte d'Ivoire in the context of projects for the development of inland aquaculture. Compared to the other strains, that from Da shows the highest rate of heterozygosity (7.32% versus

Table 2. Comparison of the growth performance of three Ivorian cultured strains of *O. niloticus*. Results from a four-month culture experiment in 50-m^2 ponds at the IDESSA station in Bouake.

Parameters	Test strains/replicates								
	O. niloticus "Bouake" (Bk)			*O. niloticus* "Daloa" (Da)			*O. niloticus* "Burkina Faso" (BF)		
	Block 1	Block 2	Block3	Block 1	Block 2	Block 3	Block 1	Block 2	Block 3
Date of stocking	03/18/91	03/18/91	03/18/91	03/15/91	03/15/91	03/15/91	03/15/91	03/15/91	03/15/91
Date of harvest	07/22/91	07/22/91	07/22/91	07/22/91	07/22/91	07/22/91	07/22/91	07/22/91	07/22/91
Number of days of experimentation	122	122	122	122	122	122	122	122	122
Number of fish stocked	110	110	110	110	110	110	110	110	110
Stocking density (ind·m^{-2})	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2
Number of fish at harvest	100	100	100	105	107	99	97	101	98
Survival (%)	90.90	90.90	90.90	95.45	97.27	90.00	88.18	91.82	89.09
Initial mean individual weight (g)	27.64	28.20	29.82	30.82	32.55	32.00	39.73	40.20	38.91
Final mean individual weight (g)	370.5	360.90	384.40	326.86	342.81	341.52	362.06	336.22	336.22
Mean daily weight gain (MDWG) (g·day^{-1})	2.810	2.727	2.906	2.427	2.543	2.537	2.642	2.426	2.437
Total initial weight (kg)	3.04	3.10	3.28	3.39	3.58	3.52	4.37	4.42	4.28
Total final weight (kg)	37.05	36.09	38.44	34.38	36.68	33.81	35.12	33.96	32.95
Production (kg)	34.01	33.00	35.16	30.93	33.10	30.29	30.75	29.54	28.67
Yield (kg·ha^{-1}·$year^{-1}$)	20,350	19,739	21,038	18,707	19,807	18,124	18,399	17,673	17,155
Quantity of feed distributed (kg)	53.9	53.9	53.9	53.9	53.9	53.9	53.9	53.9	53.9
Feed conversion ratio (FCR)	1.58	1.68	1.53	1.74	1.63	1.78	1.75	1.82	1.88
Production of fry (kg)	-	-	-	2.72	-	-	-	-	-

Table 3. Summary comparison of the mean growth performance of three populations of *O. niloticus* during a four-month culture experiment in nine 50-m² ponds at the IDESSA station in Bouake.

Parameters	*O. niloticus* "Bouake" (Bk)	*O. niloticus* "Daloa" (Da)	*O. niloticus* "Burkina Faso" (BF)
Survival (%)	90.2 (0)[a]	94.24 (3.09)	89.69 (1.55)
MDWG (g)	2.81 (0.07)	2.50 (0.05)	2.50 (0.10)
Yield ($kg \cdot ha^{-1} \cdot year^{-1}$)	20,376 (531)	18,812 (719)	17,742 (510)
FCR	1.58 (0.04)	1.72 (0.06)	1.82 (0.05)

[a]Standard deviations are indicated between parentheses.

Table 4. Allele frequencies at polymorphic loci, mean heterozygosity (H) and mean polymorphism (P=95 and 99%) for the three strains of *O. niloticus* used in the growth experiments in ponds.

Locus	Allele	*O. niloticus* Bk (30)	*O. niloticus* BF (30)	*O. niloticus* Da (30)
sAAT*	*100	0.600	0.467	0.683
	*55	0.400	0.533	0.317
FH*	*120	0.500	0	0
	*100	0.500	1	1
IDDH*	*169	0.100	0.500	0
	*100	0.750	0.150	0.533
	*16	0.150	0.350	0.467
LDH-2*	*100	0.950	0.767	0.733
	*52	0.050	0.233	0.267
MDH-3*	*119	0.017	0	0
	*100	0.983	1	1
MEP-1*	*125	0.050	0.183	0.517
	*100	0.950	0.817	0.483
MEP-2*	*120	0.183	0	0
	*100	0.817	1	1
MPI*	*107	0.100	0	0.250
	*100	0.900	1	0.750
H (%)		6.96	5.86	7.32
P95 (%)		23.33	13.33	16.67
P99 (%)		26.67	13.33	16.67

6.96% and 5.86% for Bk and BF, respectively) although levels of polymorphism are lower in this strain than in the Bouake stock.

Discussion

These growth performances obtained in pond-culture conditions characterized by poor water renewal and the absence of artificial aeration, compare well with the growth of *O. niloticus* recorded in the specialized literature (Mélard 1986).

The low FCR reflect not only the good potential of the test strains, but also good feed quality and efficient distribution and feeding techniques.

While the differences in growth performance between the Bk and BF strains are most probably due to differences among these strains, a more cautious approach is needed regarding the differences observed in the Da strain. These may be due to the fact that the nursing

conditions (very long period of nursing with feed of poor quality) for the Da strain were less favorable than for the other strains.

Heterozygosity was higher in the three populations studied than in the natural populations from the Nile (McAndrew and Majumdar 1983), the Volta or the Niger water systems (Rognon and Guyomard, this vol.). These observations can be explained by the history of the Bk strain originally constituted by mixing from 1968 onward (see above) of two populations of *O. niloticus* originally from the Volta and the Nile basins, respectively. Other cultured populations of Côte d'Ivoire, originally of the Bk strain and used for aquaculture or for stocking hydroelectric reservoirs, have also shown similar levels of heterozygosity and polymorphism (Rognon and Guyomard, this vol.).

By contrast, in cultured stocks reared in other countries, very variable heterozygocity levels have been observed, such as 1.35% for one population in Thailand (Macaranas et al. 1986) or 8.88% in Japan (Basio and Taniguchi 1984). The high heterozygosity levels that are often observed in stocks of *O. niloticus* used for aquaculture in the Philippines are due to a genetic introgression by *O. mossambicus* (Taniguchi et al. 1985; Macaranas et al. 1986).

Conclusion

The growth performance and the electrophoretic characteristics of the three cultured strains of *O. niloticus* tested in our study do not reflect any loss in genetic variability in comparison with the original natural populations. Founding and maintaining these stocks in captivity seem to have had little negative impact (such as founder effect, bottlenecks and inbreeding) on genetic variability. In terms of growth performance and genetic variability, the Bouake strain dominates all others.

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Genetic Differentiation in Several Stocks of *Sarotherodon melanotheron* and *Tilapia guineensis* from Côte d'Ivoire, Senegal and Gambia

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POUYAUD, L. and J.F. AGNESE. 1996. Genetic differentiation in several stocks of *Sarotherodon melanotheron* and *Tilapia guineensis* from Côte d'Ivoire, Senegal and Gambia, p. 368-376. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.-B. Amon Kothias and D. Pauly (eds). The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Enzyme protein polymorphism was studied in 12 stocks of *Tilapia guineensis* (238 individuals) and seven populations of *Sarotherodon melanotheron* (221 individuals) from Côte d'Ivoire, Senegal and Gambia. Of the 28 loci coding 15 enzyme systems, 17 were found to be polymorphic in *T. guineensis* and 11 in *S. melanotheron*. In both species, the Senegalese stocks are clearly differentiated from the Ivorian populations. Within each group, each population is clearly distinguishable. The high polymorphism observed in the stocks of both species warrants their use in programs of genetic improvement through selection or interspecific crossbreeding.

Introduction

Sarotherodon melanotheron and *Tilapia guineensis* are two species of lagoon tilapia found along the West African coastal zone, from Senegal to Congo. They can live in freshwater or in saltwater (salinity of 100 ppt). *S. melanotheron* is mainly phytophagous. In Côte d'Ivoire, it is used in acadja-based farming systems. Because of their high resistance to salinity, both species offer possibilities for crossbreeding with species that grow fast but have poor salinity tolerance, like *Oreochromis niloticus*. The aim of this study was to increase knowledge on the genetic polymorphism of both species and their use in interspecific breeding programs.

Materials and Methods

Four hundred and fifty-nine individuals from 19 populations were studied. Sampling areas are shown in Fig. 1 (note that all sampling sites for Senegambia are in Senegal, except for Banjul, Gambia). Upon capture, the specimens were kept in ice for a few hours then dissected in the laboratory. Eye, muscle and liver tissues (approximately 1 cm^3) were taken from each individual and each tissue sample was preserved in liquid nitrogen until it was analyzed. Enzyme electrophoreses using starch gel were conducted following the protocols of Pasteur et al. (1988) and McAndrew and Majumdar (1983).

Table 1 shows the enzyme systems investigated, the buffers used and the

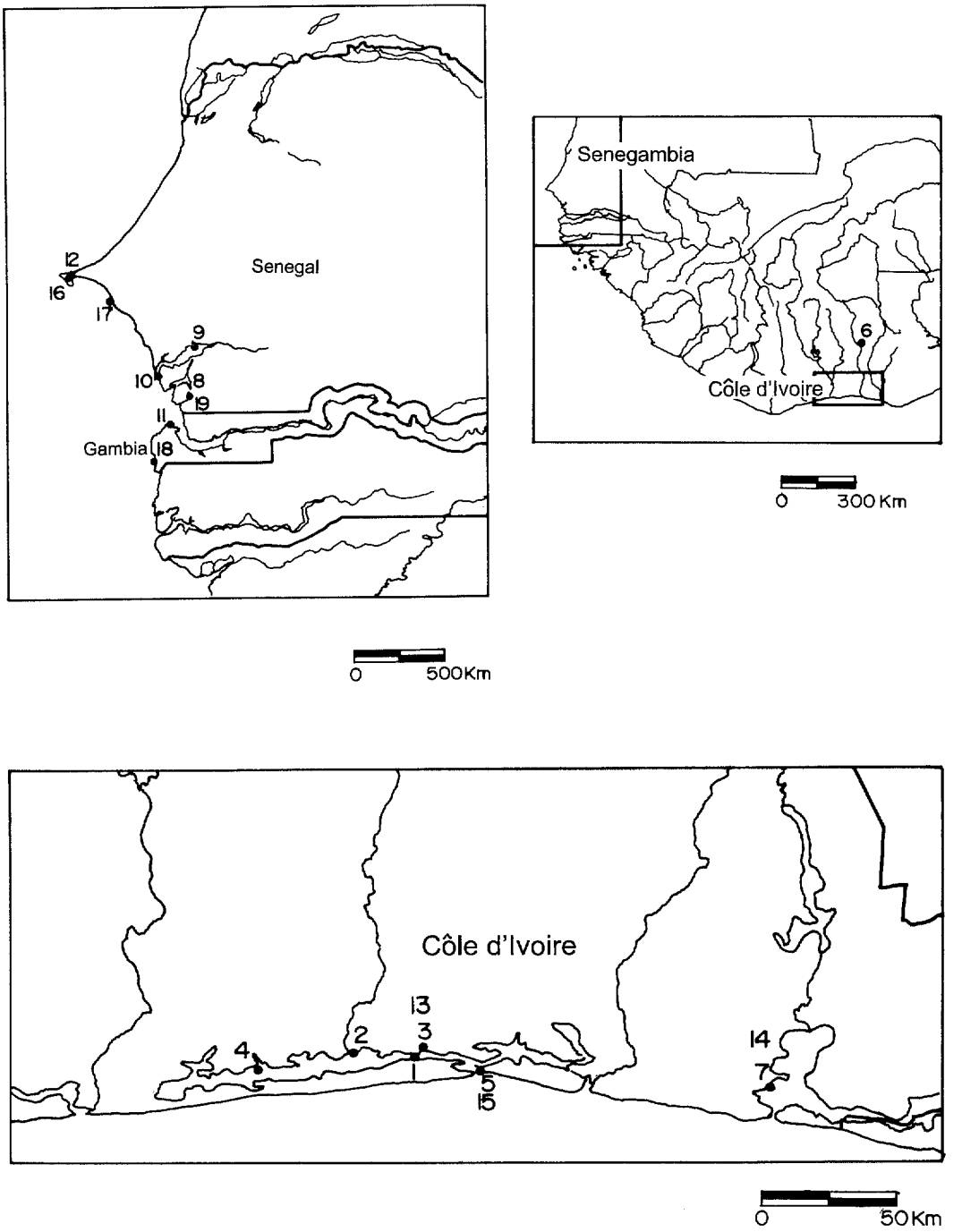

Fig. 1. Location of sampling areas. For *Tilapia guineensis*, in Côte d'Ivoire: (1) Adiapote, (2) Layo, (3) Adiopodume, (4) Tupah, (5) Bietry, (6) Abenguru Bridge and (7) Anga; in Senegambia: (8) Missirah, (9) Fundiugne, (10) Jiffer, (11) Banjul and (12) Pikine. For *Sarotherodon melanotheron*, in Côte d'Ivoire: (13) Adiopodume, (14) Anga and (15) Bietry; in Senegambia: (16) Dakar, (17) Somone, (18) Banjul and (19) Missirah.

Table 1. Enzyme systems, organs (L=Liver; M=muscle; and Y=eye) and buffers used during the analysis. The systems were coded following the recommendations of Shaklee et al. (1989). The buffers are those of McAndrew and Majumdar (1983), Basio and Taniguchi (1984) and Pasteur et al. (1988).

System	Enzyme no.	Locus	Tissues	Buffer
AAT	2.6.1.1	*AAT-1*	L M Y	TC 6, 7
		AAT-2	L Y	TC 6, 7
		AAT-3	M Y	TC 6, 7
ADH	1.1.1.1	*ADH-1*	L	MC 2
AK	2.7.4.3	*AK-1*	M	MC 2
ALP	3.1.3.1	*ALP-1*	L	TC 6, 7
CK	2.7.3.2	*CK-1*	M Y	MC 2
		CK-2	Y	MC 2
EST	3.1.1.-	*EST-1*	L	POULIK 1/2
		EST-2	Y	RW, TEB 8, 6
		EST-3	Y	TEB 8, 6
		EST-4	L	POULIK 1/2
FBP	3.1.3.11	*FBP-1*	M	MC 2
		FBP-2	L	MC 2
FH	4.2.1.2	*FH-1*	M	MC 2
GPI	5.3.1.9	*GPI-1*	M Y	RW
		GPI-2	L Y	RW
IDHP	1.1.1.42	*IDHP-1*	L	MC 2
		IDHP-2	M	MC 2
LDH	1.1.1.27	*LDH-1*	M Y	MC 2
		LDH-2	L M Y	MC 2
		LDH-3	Y	MC 2
MDH	1.1.1.37	*MDH-1*	M Y	MC 2
		MDH-2	L M Y	MC 2
PGM		*PGM-1*	M	TEB 8, 6
PROT		*PROT-1*	M	MC 2
		PROT-2	M	MC 2
SOD	1.15.1.1	*SOD-1*	L	MC 2

organs in which the various loci are expressed. The nomenclature is that proposed by Shaklee et al. (1989).

The electrophoretic data were analyzed using the BIOSYS 1 program of Swofford (1989). The genetic distance index used was calculated following Nei (1978).

Results and Discussion

Tables 2 and 3 present the allele frequencies and the heterozygosity estimated for the different test populations.

In *T. guineensis*, observed heterozygosity varied between 0.015 (Lake Pikine, 12) and 0.132 (Adiapote, 1). On the whole, these are comparable to the values found in previous studies of other tilapia species (McAndrew and Majumdar 1983; Basiao and Taniguchi 1984).

Very few significant differences were found between the allelic frequencies observed and those expected following Hardy Weinberg's equilibrium hypothesis. These can be considered as panmictic populations.

The dendogram (Fig. 2) generated using Nei's genetic distance matrix (Table 4) shows the grouping of the Ivorian lagoon populations and of the Senegambian lagoon stocks. Only two stocks are outside these groups: the Pikine stock (12) and the Abenguru Bridge stock (6). Both are characterized by the lowest heterozygosities (0.015 for the Pikine stock and 0.019 for the Abenguru Bridge stock). These low H values partly explain the greater genetic distances observed between both stocks and the geographic groups from which they originate.

The Pikine stock was captured in a pond fed by the water-table also supplying the nearby suburbs of Dakar. When water level is low, the surface area and volume of water available to fish decrease considerably (depth < 1 m/km^2 of surface). Consequently, predation by birds and humans is high.

At the end of the low water level period, the population size is thus low. These regular bottlenecks may explain the very low rate of polymorphism observed in this population.

The Abenguru Bridge stock shows two diagnostic alleles (*LDH-1 B* and *LDH-3 C*), that is, alleles that are specific to this population and that are absent in the potentially sympatric populations of Ebrié Lagoon: Adiapote (1), Layo (2), Adiopodume (3), Tupah (4) and Bietry (5). There is a gene flow barrier between this population and those occurring downstream from the Comoe basin. The Senegambian stocks seem well differentiated from each other, often by several diagnostic alleles: *FBP-1 D* and *PGM C* for the Pikine stock, *AAT-2 E* (Fundiugne, 9 and Banjul, 11), *EST-4 D* (Missirah, 8; Fundiugne, 9; and Pikine, 12), *FH-1 D* (Missirah, 8; Fundiugne, 9, Banjul, 11), *PGI-2 F* (Missirah, 8; Fundiugne, 9; Jiffer, 10; and Banjul, 11) and *PGM-1 B* (Missirah, 8 and Banjul, 11).

In *S. melanotheron* stocks, observed heterozygosity varied between 0.035 (Anga, 15) and 0.071 (Dakar, 16). There was no significant difference between the allelic frequencies observed and those expected following Hardy Weinberg's equilibrium hypothesis.

The dendogram (Fig. 3) generated using Nei's genetic distances (Table 5) shows the grouping of the Ivorian stocks and the Senegambian stocks. In the Ivorian group, two populations of Ebrié Lagoon, Adiapote (13) and Bietry (15), are grouped together, in contrast to the Aby Lagoon population (Anga, 14). There are diagnostic alleles in all these stocks: *AAT-3 C* and *PGI-2 E* (Anga, 14), *FH B* (Bietry, 15), *PROT-1 D* (Dakar, 16), *FBP-1 A* and *PGM C* (Somone, 17), *PGI-2 D* (Banjul, 18) and *IDH-2 A* (Missirah, 19).

Table 2. Genic frequencies observed at polymorphic loci and sample size (N) of *Tilapia guineensis*. List of stations, in Côte d'Ivoire: (1) Adiapote, (2) Layo, (3) Adiopodume, (4) Tupah, (5) Bietry, (6) Abenguru Bridge and (7) Anga; in Senegambia: (8) Missirah, (9) Fundiugne, (10) Jiffer, (11) Banjul and (12) Pikine. The loci *AAT-1*, *CK-1*, *CK-2*, *FBP-2*, *GPI-2*, *IDHP-2*, *PROT-2* and *SOD* are monomorphic for the same allele in the samples.

Populations (N) Locus	Alleles	1 (7)	2 (10)	3 (10)	4 (18)	5 (13)	6 (19)	7 (47)	8 (14)	9 (33)	10 (3)	11 (34)	12 (30)
AAT-2	*A*	.79	.85	.90	.86	.81	.00	.97	.32	.42	.83	.47	.05
	B	.21	.15	.10	.14	.19	1.00	.03	.68	.56	.17	.51	.95
	E	-	-	-	-	-	-	-	-	.02	-	.02	-
AAT-3	*A*	1.00	.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.97	1.00
	D	-	.06	-	-	-	-	-	-	-	-	.03	-
ADH	*A*	.64	.45	.44	.61	.50	-	.89	1.00	.91	1.00	1.00	.92
	D	.36	.55	.56	.39	.50	1.00	.11	-	.09	-	-	.08
AK	*A*	1.00	1.00	1.00	.94	1.00	1.00	.96	1.00	1.00	1.0	1.00	1.00
	B	-	-	-	.06	-	-	.04	-	-	-	-	-
ALP	*A*	1.00	1.00	.95	1.00	1.00	1.00	.93	1.00	1.00	1.00	1.00	1.00
	D	-	-	.05	-	-	-	.07	-	-	-	-	-
EST-1	*A*	.21	.65	.55	.50	.45	-	.89	-	-	-	-	-
	B	-	-	-	-	-	.36	-	.25	.03	.17	.22	.07
	D	-	-	-	-	-	.64	.02	.75	.97	.83	.78	.93
	E	.79	.35	.45	.50	.55	-	.09	-	-	-	-	-
EST-4	*A*	1.00	.90	1.00	.92	1.00	1.00	.95	.75	.97	1.00	1.00	.97
	C	-	.10	-	.08	-	-	.05	-	-	-	-	.02
	D	-	-	-	-	-	-	-	.25	.03	-	-	.01
FBP-1	*A*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-
	D	-	-	-	-	-	-	-	-	-	-	-	1.00
FH	*A*	.50	.80	.70	.93	.50	1.00	.89	.86	.97	1.00	.75	1.00
	D	-	-	-	-	-	-	-	.14	.03	-	.25	-
	E	.50	.20	.30	.07	.50	-	.11	-	-	-	-	-
GPI-2	*A*	1.00	1.00	1.00	1.00	.69	-	.99	.64	.50	.17	.63	1.00
	C	-	-	-	-	-	-	.11	-	-	-	-	-
	F	-	-	-	-	-	-	-	.36	.50	.83	.37	-
	H	-	-	-	-	.31	1.00	-	-	-	-	-	-
IDHP-1	*A*	1.00	1.00	1.00	1.00	1.00	1.00	.94	1.00	1.00	1.00	1.00	1.00
	E	-	-	-	-	-	-	0.06	-	-	-	-	-
LDH-1	*A*	1.00	1.00	1.00	1.00	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00
	B	-	-	-	-	-	1.00	-	-	-	-	-	-
LDH-3	*A*	1.00	1.00	1.00	1.00	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00
	C	-	-	-	-	-	1.00	-	-	-	-	-	-
MDH-1	*A*	.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	B	.07	-	-	-	-	-	-	-	-	-	-	-
MDH-2	*A*	.57	.85	.75	.75	.83	1.00	.97	1.00	1.00	1.00	1.00	1.00
	B	.14	.15	.12	.25	.12	-	.02	-	-	-	-	-
	C	.29	-	.13	-	.05	-	.01	-	-	-	-	-
PGM	*A*	1.00	1.00	1.00	1.00	1.00	1.00	.99	.38	1.00	1.00	.19	-
	B	-	-	-	-	-	-	-	.62	-	-	.81	-
	C	-	-	-	-	-	-	-	-	-	-	-	1.00
	G	-	-	-	-	-	-	.11	-	-	-	-	-
PROT-1	*A*	.50	.75	-	.42	.55	-	.30	.96	-	.17	.17	-
	C	-	.20	.03	.05	1.00	-	-	.14	-	.05	-	-
	D	.29	-	.80	.50	.25	-	.68	.04	.86	.83	.78	1.00
	F	.21	.25	-	.05	.15	-	.02	-	-	-	-	-
	H	.132	.086	.080	.089	.127	.019	.048	.101	.059	.051	.077	.015

Table 3. Genic frequencies observed at polymorphic loci and sample size (N) of *Sarotherodon melanotheron* in Côte d'Ivoire: (13) Adiopodume, (14) Anga and (15) Bietry; and in Senegambia: (16) Dakar, (17) Somone, (18) Banjul and (19) Missirah. The loci *AAT-1*, *AK*, *FBP-2*, *CK-1*, *CK-2*, *EST-2*, *EST-3*, *GPI-1*, *IDHP-1*, *LDH-1*, *LDH-2*, *LDH-3*, *MDH-1*, *MDH-2*, *PROT-2* and *SOD* are monomorphic for the same allele in all populations.

Populations (N) Loci	Alleles	13 (13)	14 (40)	15 (44)	16 (27)	17 (29)	18 (32)	19 (36)
AAT-2	*A*	-	-	-	1.00	.95	.97	.97
	B	1.00	1.00	1.00	-	.05	.03	.03
AAT-3	*A*	-	-	-	.50	.10	.03	.07
	B	1.00	.81	1.00	.50	.90	.97	.93
	C	-	.19	-	-	-	-	-
ADH	*A*	-	.01	.04	-	-	.05	.18
	B	1.00	.99	.96	1.00	1.00	.95	.82
ALP	*A*	1.00	1.00	1.00	.35	.55	.56	.51
	B	-	-	-	.65	.45	.44	.49
EST-1	*A*	.11	.89	.40	.35	.02	.53	.21
	B	.89	.11	.60	.65	.98	.47	.79
FBP-1	*A*	-	-	-	-	.14	-	-
	B	1.00	1.00	1.00	1.00	.86	1.00	1.00
FH	*A*	1.00	1.00	.11	1.00	1.00	1.00	1.00
	B	-	-	.89	-	-	-	-
GPI-2	*B*	.04	.03	.07	1.00	1.00	.95	1.00
	C	.96	.86	.93	-	-	-	-
	D	-	-	-	-	-	.05	-
	E	-	.11	-	-	-	-	-
IDHP-2	*A*	-	-	-	-	-	-	.07
	B	1.00	1.00	1.00	1.00	1.00	1.00	.93
PGM	*B*	1.00	1.00	1.00	1.00	0.90	1.00	1.00
	C	-	-	-	-	.10	-	-
PROT-1	*A*	.35	.03	-	.40	.0	.09	.01
	B	.58	.89	.99	.52	.90	.89	.18
	C	.07	.08	.01	.06	-	.02	.81
	D	-	-	-	.02	-	-	-
	H	.020	.032	.024	.067	.049	.054	.063

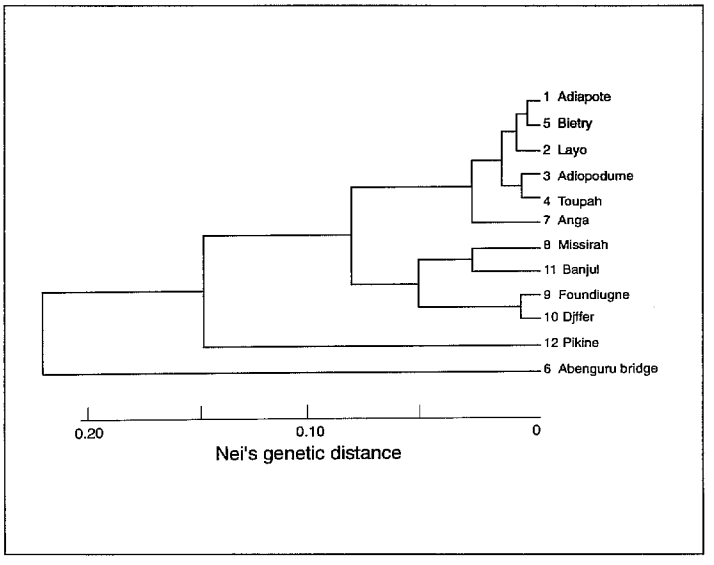

Fig. 2. Dendogram showing the genetic relationships between the various populations of *Tilapia guineensis*. This dendogram was generated using Nei's distance matrix (1978) (Table 4) and the BIOSYS 1 program of Swofford (1989).

Table 4. Nei's genetic distance (1978) for the various stocks of *Tilapia guineensis* obtained from the allelic frequencies (see Table 2) using the BIOSYS 1 program of Swofford (1989).

Populations	1	2	3	4	5	6	7	8	9	10	11
1 ADIAPOTE	-										
2 LAYO	.987	-									
3 ADIOPODUME	.985	.977	-								
4 TUPAH	.990	.993	.994	-							
5 BIETRY	.997	.994	.995	.999	-						
6 ABENGURU	.770	.785	.788	.782	.805	-					
7 ANGA	.964	.976	.983	.988	.970	.753	-				
8 MISSIRAH	.918	.927	.891	.923	.921	.786	.913	-			
9 FUNDIUGNE	.923	.914	.929	.940	.926	.816	.940	.947	-		
10 DJIFFER	.920	.914	.933	.939	.927	.787	.943	.941	.992	-	
11 BANJUL	.911	.901	.918	.923	.911	.777	.925	.972	.968	.961	-
12 PIKINE	.833	.822	.848	.850	.829	.729	.850	.880	.904	.871	.908

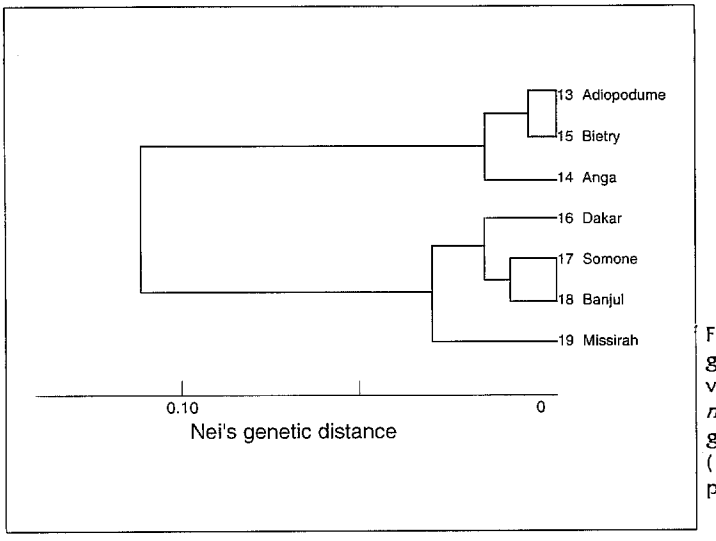

Fig. 3. Dendogram showing the genetic relationships between the various populations of *Sarotherodon melanotheron*. This dendogram was generated using Nei's distance matrix (1978) (Table 5) and the BIOSYS 1 program of Swofford (1989).

Table 5. Nei's genetic distance (1978) for the various stocks of *Sarotherodon melanotheron* obtained from the allelic frequencies (see Table 3) using the BIOSYS 1 program of Swofford (1989).

Populations	13	14	15	16	17	18
13 ADIOPODUME	-					
14 ANGA	0.972	-				
15 BIETRY	0.992	0.990	-			
16 DAKAR	0.898	0.888	0.894	-		
17 SOMONE	0.918	0.893	0.917	0.983	-	
18 BANJUL	0.913	0.919	0.924	0.985	0.989	-
19 MISSIRAH	0.902	0.881	0.894	0.974	0.974	0.973

The significant differentiation observed between these populations supports Trewavas (1983), who distinguished several subspecies in West Africa. The populations of Banjul (18) and of Missirah (19) may be identified as the subspecies *S. melanotheron heudelotii*, and the population of Dakar (16) as *S. melanotheron paludinosus*. No morphometric study has ever been conducted on the individuals of Somone (17). All the Ivorian stocks, Adiopodume (13), Anga (14) and Bietry (15), may be identified as the subspecies *S. melanotheron melanotheron*.

The genetic variability in both *T. guineensis* and *S. melanotheron* shows a sharp differentiation between the Ivorian and Senegambian stocks. This significant differentiation warrants performance comparisons among strains from both regions.

Some populations of both species show high heterozygosity rates (0.132 for the Adiopote stock [1] of *T. guineensis* and 0.067 for the Dakar stock [16] of *S. melanotheron*). Individuals from these stocks could be used in interspecific crossbreeding where they would be likely to contribute many new genes.

Lastly, the high level of polymorphism observed warrants further studies in other sampling areas (Guinea, Cameroon and Congo) using other genetic techniques

such as sequencing of mitochondrial DNA.

Acknowledgements

This study was funded by a grant from the Groupement de coopération scientifique sur les bases biologiques de l'aquaculture and from ORSTOM. The authors wish to thank J.J. Albaret, P.S. Diouf and A. Pariselle for their help during fish capture.

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Growth and Gonadal Development of Triploid Tilapia (*Oreochromis niloticus*)

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Abstract

Triploidy was induced by heat-shock in 50 egg batches of tilapia. Determination of triploidy was done by chromosome examination during the embryonic phase. For growth and gonadal development studies, a treatment was chosen which gave 100% triploid embryos in the samples used for chromosome examination. After optimizing temperature treatment, time of exposure and duration of shock, growth and gonadal development of triploids till the age of 220 days were studied in 18 full-sib families. Half of each full-sib family was maintained as a diploid control over the whole period. At the 336th day, half of each batch (diploids and triploids) was killed and the following parameters were examined for each individual fish: body weight, body length, sex, gonadal weight and development, and number of chromosomes. The procedure was repeated on the 178th and the 220th day. In total, 1,304 diploid and 1,228 triploid fishes were analyzed. In general, triploids showed poorer growth performance than diploids. At all ages, the gonadal development in the triploid groups was significantly poorer than in diploids. Triploids proved incapable of producing viable progenies.

Introduction

Stunting is the main problem in tilapia production, caused by early and uncontrolled reproduction before fish achieve marketable size. Triploidy of fish could be a simple solution. Few attempts have been made to produce triploid tilapia (Valenti 1975; Chourrout and Itskovich 1983; Penman et al. 1986; Pandian and Varadaraj 1990). Comparative studies on gonadal and growth development of diploid and triploid tilapia have been carried out so far only with small stocks of fish and for relatively short periods. This study aimed to optimize a technique to obtain triploid Nile tilapia (*Oreochromis niloticus*) and to test triploid tilapia for growth and gonadal development in comparison with diploid full-sibs for a period including sexual maturation.

Materials and Methods

O. niloticus (Lake Manzala, Egypt) received from the Institute of Aquaculture, Stirling, Scotland, were used as broodstock. In total, 40 females and 40 males derived from this broodstock served as parents in this experiment. The studies were carried out in the recirculation system of the Institute of Animal Husbandry and Genetics, University of Göttingen under

standardized environmental conditions as described by Kronert et al. (1989).

Egg batches were produced by single-pair matings carried out in 125-l aquaria, applying a 12L/12N photoperiod regime. Immediately after deposition of the first few eggs on the bottom of the tank, the females were removed from the tanks, anesthetized and stripped. Eggs were collected in small sieves (75 cc) without water contact. Milt was obtained by dry stripping. For fertilization, milt was diluted 1:1 with 0.9% NaCl solution and mixed by drawing it up into a pipette repeatedly. The eggs and sperm solution were gently shaken. Immediately thereafter, the egg batches were covered with tap water at 31°C with pH 8.1. After addition of water, the egg batches were constantly stirred for 30 seconds and then divided into two groups. For each trial, one group was triploidized while the other group served as a diploid full-sib control.

For retention of the second polar body, a heat shock treatment was applied. After testing with modification of the treatments described by Chourrout and Itskovitch (1983) and Penman et al. (1986) on 32 batches, the following method was chosen based on experience of successful triploidization and hatching with 18 batches (see Puckhaber 1992). Four minutes after fertilization, the sieves with batches to be triploidized were carefully transferred to a constant temperature water bath at 41°C, then slightly stirred and after 4.5 minutes returned to the plastic bowl. After 15 minutes, eggs from control and treated groups were counted and transferred to the hatching unit.

For artificial hatching of the eggs, a special incubator was used at 27°C with constant water inflow. Further information concerning this hatching unit is given by Habitsky-Biester (1987). For detecting triploidy in the treated egg batches, chromosome examinations were carried out on embryos and adult fish. Ten embryos out of each treated batch were examined. Only batches in which all 10 embryos proved to be triploid were chosen for rearing.

For chromosome examination, a technique used by Kligerman and Bloom (1977) was modified as follows: 40-hour-old embryos were kept for 4 hours in 0.4% colchicine solution at 26°C; then moved to a Petri dish containing 0.7% NaCl solution and dissected. Afterwards, they were transferred to a 1.1% sodium citrate solution for 10 minutes. Thereafter, the samples were fixed in an ethyl alcohol (3): acetic acid (1). (vol:vol) solution. This fixative was changed twice after 30 minutes, respectively. Afterwards the embryonic tissue in the fixative was left overnight at -20°C. For the dissociation of cells, a 50% acetic acid solution was used. After 5 minutes, the cell suspension was mixed in a pipette and dropped on to a prewarmed slide (50°C). The air-dried slides were then stained with Giemsa.

For chromosome examination of treated adults, colchicine solution (2%) was injected into the dorsal muscle (2 mg·1,000 g^{-1}) 4 hours before the fish were killed. Gill tissue was then taken and treated like the embryonic tissue described above. Feeding and rearing of the diploid and triploid full-sib groups were as described by Kronert et al. (1989). A summary is shown in Table 1.

To ensure the same environmental conditions for triploids and their corresponding diploid full-sib groups, stocking densities were equalized by random sampling every two weeks. At the age of 136 and 178 days, half of each diploid and triploid group, and on the 220th day the rest of each group, were killed. The following parameters were recorded: body weight, total length, sex, gonadal weight, stage of gonadal development

Table 1. Rearing and feeding regimes of diploid and triploid *Oreochromis niloticus* families in 80-l tanks.

Age (days)	No. of full-sibs per family Diploid/Triploid	Daily ration as % (body weight)$^{0.9}$	Crude protein content (%)
24 - 37	150 /150	9	50
38 - 93	120 /120	5	50
94 - 135	80 / 80	3	25
136 - 177	40 / 40	3	25
178 - 220	20 / 20	3	25

and chromosome number. The gonadal development was classified according to Kronert et al. (1989) and Oldorf et al. (1989) (Table 2). A total of 2,532 fishes was examined, 1,304 of which were diploids and 1,228 triploids. For testing reproductive capability, one male and one female from each batch were kept and tested in the spawning area.

Results and Discussion

A total of 18 full-sib families was studied over 220 days. The average sex ratio (male:female) was 1:0.86 in diploids and 1:0.87 in triploids. The chromosome set examination of the 1,228 triploidized fish revealed, in three batches, one fish to be diploid (0.24%). As these were single cases, an escape from the diploid control groups during handling is the most likely explanation. The modified heat shock method resulted in 100% triploidization and may be used for large-scale triploidization, even under tropical farm conditions.

Growth Comparison

In general, triploids showed poorer growth performance than diploids, as also observed by Penman et al. (1986). Even after sexual maturation, growth performance of triploids was not found to be better than that of diploids as observed, for example, in trout (Thorgaard 1986). However, growth differences between diploid and triploid full-sibs varied distinctly among batches. Minimum and maximum weights between triploids and diploids within batches were in the range of +36% to -48%.

Fig. 1 shows the average growth development of the groups examined, at three ages. At all ages, the triploids had significantly ($P<0.01$) lower body weights than the diploid control groups. Body weight of triploid males was on average 38% lower than that of diploid males. For females, a mean body weight difference of only 29% was found between diploids and triploids. Females in both (diploid and triploid) groups showed a poorer average growth rate than males, except triploids on the 136th day. The average weight difference between males and females in the triploid groups was lower (13.3%) than in the diploid groups (24.6%) ($P<0.01$).

Gonadal Development

Under our laboratory conditions, spawning activities as well as egg deposition of diploids were suppressed by the limited space. This was indicated by the gonadal development of diploid females. At the age of 220 days, 75% of diploid females were found to be overripe (maturity stage 6 = spent).

Table 2. Gonad development stages in female and male tilapia.

Stage	Females (Kronert et al. 1989)
1. immature/inactive	no eggs visible
2. inactive-active	<20 eggs visible, size <0.2 mm
3. active	>20 eggs visible, size <0.2 mm
4. active-ripe	eggs yellow, size 0.2 - 1.1 mm
5. ripe/ripe-running	eggs yellow, size > 1.1 mm
6. spent	absorption of yolk material, eggs white
Stage	**Males (Oldorf et al. 1989)**
1. immature	thread-like, colorless
2. inactive	translucent, wider than above
3. inactive-active	flesh colored, still thin
4. active	white/yellowish, thickened, no milt apparent when cut
5. active-ripe	cream-colored, thick and enlarged
6. ripe	distended fully over length of visceral cavity, milt evident when testis cut
7. ripe-running	white/silvery, milt runs freely under pressure

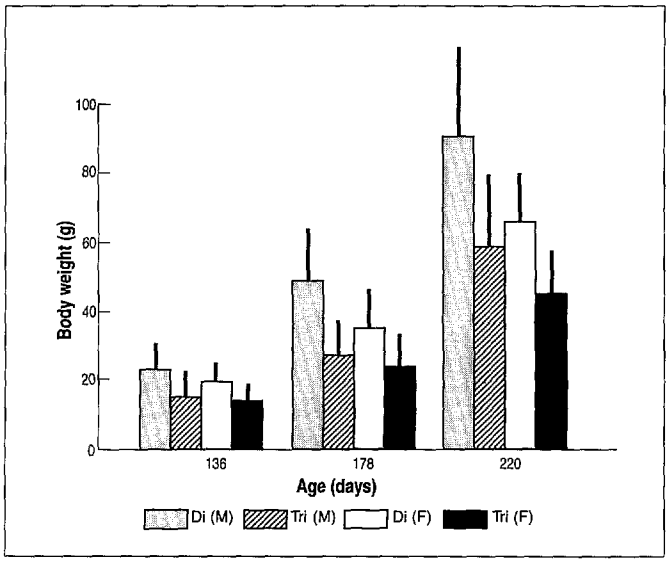

Fig. 1. Growth of diploid (Di) and triploid (Tri) *Oreochromis niloticus* (M=males; F=females) (and standard errors) as a function of age.

Fig. 2 illustrates the average gonadal development of the diploid and triploid groups. At all ages, the gonadal development in the triploid groups was significantly poorer ($P<0.01$) than in the diploid full-sib control groups. The differences in gonadal development between diploids and triploids were more pronounced in females (60.6%) than in males (34.7%). The testes of triploid males appeared to be shorter and more translucent than those of diploid males. Gonads of triploid males classified "active" contained a clear liquid in which only a few deformed spermatozoa were found. The triploid females showed only ova with underdeveloped white oocytes. The mean gonadosomatic index (GSI) shown in Fig. 3 also proved to be significantly lower ($P<0.01$) in triploids than

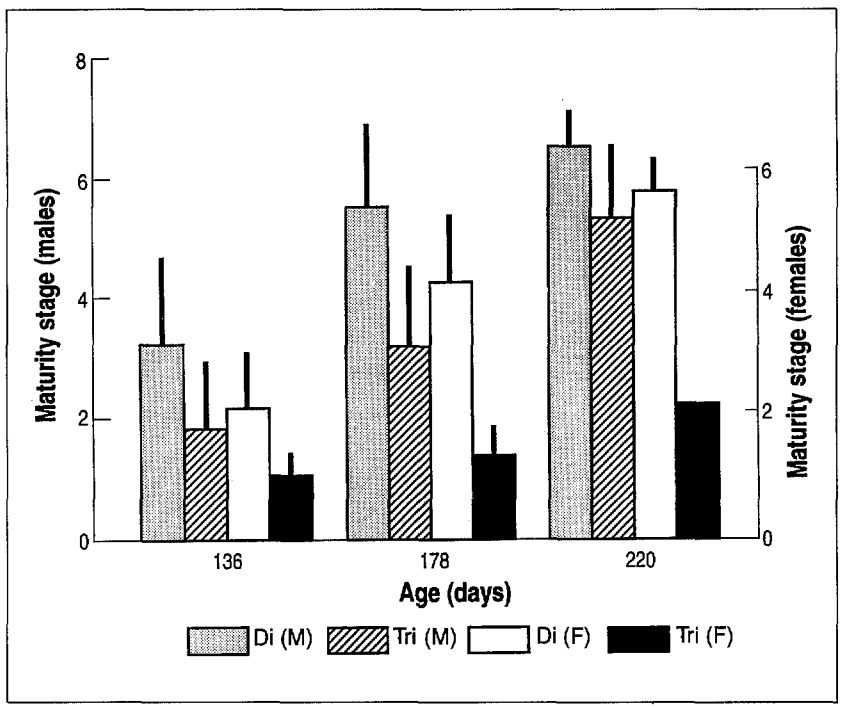

Fig. 2. Gonad development (and standard errors) in diploid (Di) and triploid (Tri) *Oreochromis niloticus* (M=males; F=females) as a function of age. The maturity stages correspond to the gonad development stages in Table 2.

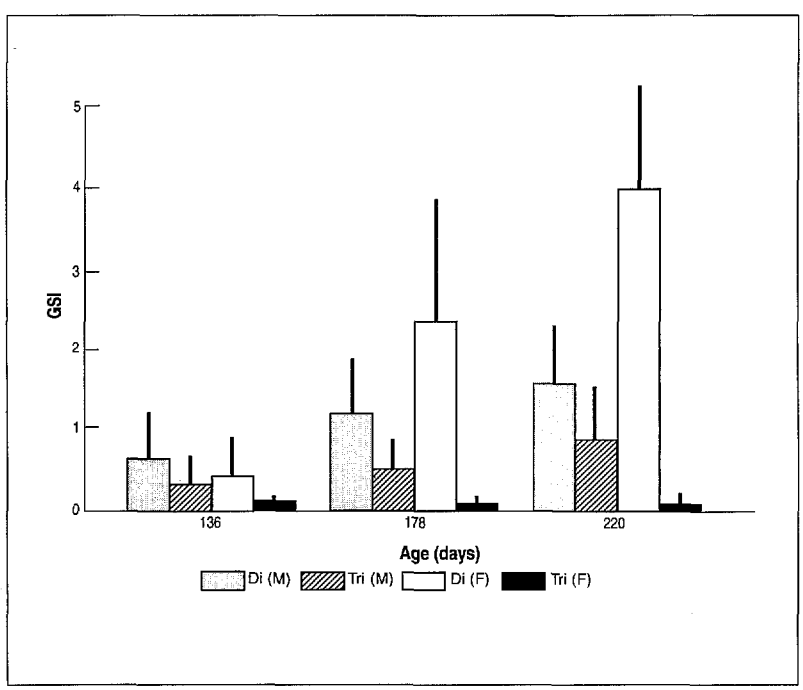

Fig. 3. Gonadosomatic index (GSI and standard errors) of diploid (Di) and triploid (Tri) *Oreochromis niloticus* (M=males; F=females) as a function of age.

in their diploid full-sibs. All tests showed a lower average GSI (49.4%) for triploid males than for diploid males. In females, the GSI difference was even more distinct (91.3% lower for triploids than for diploids).

Sterility

For testing sterility, triploid fish were paired with diploid partners. The pairs of triploid females and diploid males were observed for three months; no spawning occurred during this period. Mating of triploid males with diploid females resulted in spawning in all cases, but gave no viable larvae. Triploid *O. niloticus* thus proved incapable of producing viable progenies.

More testing of triploids will now be started under practical extensive pond conditions. When compared to diploids, the advantage of functional sterility of triploids may result in better growth performance than under these laboratory conditions. Studies of net carcass and meat quality of triploids will be included. Special attention will be given to pond productivity.

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Implications of Reproductive Behavior of Captive *Oreochromis* Broodstock on the Quality of Their Fry

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RANA, K.J. 1996. Implications of reproductive behavior of captive *Oreochromis* broodstock on the quality of their fry, p. 383-390. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Parental behavior in tilapias is generally considered to have evolved to enhance the survival of the progeny. Under cultured conditions, however, breeding conditions and aspects of their reproductive behavior may be altered and this may affect the quality of the brood. Aspects of the breeding behavior were therefore investigated under hatchery conditions in *Oreochromis niloticus* and *O. mossambicus* to determine the influence of male spawning frequency on the fertility rates of eggs; the influence of female brooding on fry quality; the growth of artificially incubated and naturally brooded siblings; and the influence of delayed initial feeding on fry quality. In both species, the number of developing eggs in a clutch declined rapidly with the increase in male spawning frequency on a given day. With four spawnings per day, the fertility rates achieved by the males declined from over 90% to below 30%. The relationships between the buccal cavity, total egg and fry volumes suggest that the egg and fry volumes are considerably lower than the buccal cavity volumes. For 11-12 cm *O. niloticus* and *O. mossambicus* females' egg clutch volumes were only 10-15% and 15-40% of the buccal volumes, respectively. Losses of newly hatched fry increased linearly between five and eight days postspawning and up to 25% of the clutch were damaged. The types of fatal damage are presented.

In all clutches, the artificially reared fry were significantly heavier and longer ($P<0.05$) than their naturally reared siblings. This difference depended on the timing of the initial release and differences of up to 200% were observed. Delaying initial feeding beyond six days posthatching decreased growth significantly ($P<0.05$). This decrease was greater if the fry originated from smaller broodstock and hence smaller eggs. The implications of these findings are discussed in relation to hatchery production.

Introduction

In *Oreochromis* species, mate selection and successful prespawning courtship between a male and female can lead to a temporary pair bonding and eventual spawning. The female remains in the males' courtship territory and will release several batches of eggs (20-50 eggs/batch) which are immediately fertilized by the male, over a period of 45-120 minutes (Trewavas 1983). On completion of spawning, the female leaves the spawning site to rear her clutch. Under hatchery conditions, however, aspects of this behavior may be perturbed such that the overall production of fry may be reduced. Specific aspects of the reproductive behavior under captive conditions were therefore identified for investigation (Fig. 1).

To ensure their paternity of offspring, *Oreochromis* males establish and vigorously defend nest sites on common breeding grounds. A prespawning female, on the other hand, visits breeding grounds to select one of a few males to fertilize her clutch. Consequently,

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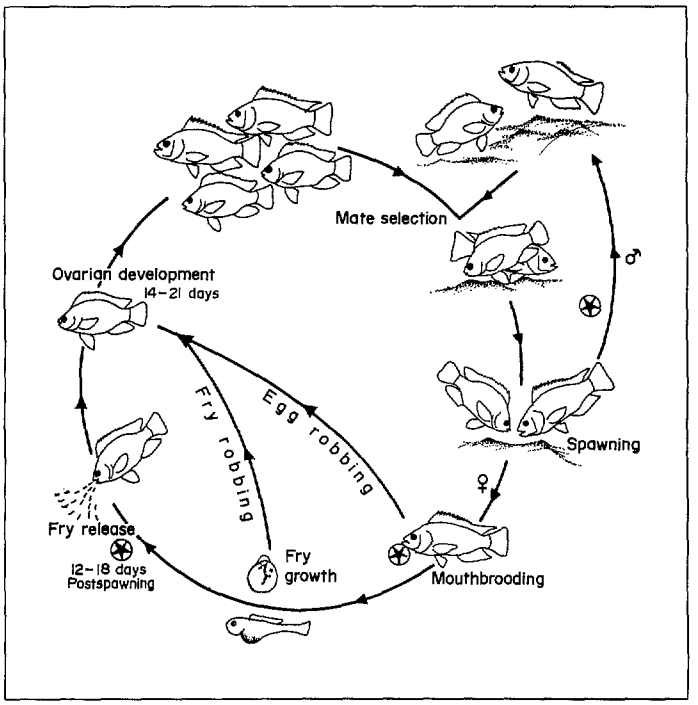

Fig. 1. Breeding events in *Oreochromis* species.

infighting among males may result in a few dominant males fertilizing eggs of a disproportionately large number of females.

To sire as many offspring as possible, one would expect the male to shed sufficient milt to maximize the fertilization in each batch of spawned eggs and to ensure the female deposited all the eggs in his nest.

The first trial described here was therefore conducted to test the hypothesis that if the male fertilizes several batches of eggs, the fertilization rates of subsequent batches of eggs may decline.

The female, however, collects the eggs into her buccal cavity and leaves the spawning site to rear her clutch, during which time the viable eggs hatch and the total volume of the clutch increases. The second trial was therefore conducted to determine if buccal volume was a limiting factor as suggested in earlier studies by Aronson (1949) and Baerends and Baerends van-Roon (1950), and to evaluate the implication of oral rearing under hatchery conditions on fry damage.

The duration of buccal rearing, especially the time at first release of fry in *Oreochromis* species, can vary among females and between spawnings of the same female (Rana 1986, 1990). Clearly, if the final release of fry is delayed beyond their first feeding stage, their growth and survival potential may be reduced.

In view of the possible implication of the above interacting factors of reproductive behavior on egg viability and the quality of the fry, in terms of survival and growth, trials were conducted to determine the effects of:

1. male spawning frequency on fertility rates of egg clutches;
2. buccal volume and rearing on fry survival;
3. mouthbrooding on the growth of naturally reared fry when compared to their artificially reared siblings; and
4. delayed initial feeding on the growth and survival of fry of *O. niloticus* and *O. mossambicus*.

Materials and Methods

Effect of Male Spawning Frequency on Fertility Rates of Naturally Spawned Egg Clutches

Males and females were conditioned separately in 1-m^2 tanks and were fed three times a day on a daily ration of 1-2% of their body weight. The females were starved 24 hours prior to the commencement of each trial. Eight females and one dominant male were selected and transferred to a 3-m^2 spawning tank containing 35 cm of water. Females were identified with a numbered tag and were color-coded with subcutaneous injections of Alcian blue dye. The fish were fed ad libitum three times a day and observations were made daily for spawning activity and the sequence noted in which individually tagged females spawned. Twelve hours after the last spawning of the day, the eggs from all the females were separately removed, number identified and then returned to the spawning tank. A random sample of about 100 eggs from each clutch was preserved in Bouin's fluid and the number of developing eggs noted. Each trial, which lasted for 5-18 days, was repeated four times for each species.

Fry Losses Associated with Mouth-brooding

Tagged and color-coded females (12-18 months of age) were stocked in 1- and 3-m^2 breeding tanks (water depth 35 cm) at a sex ratio of 3 females:1 male (8 fish) and 100-l glass aquaria (4 fish), fed 1-2% body weight per day and held at 28°C.

Tanks were observed several times a day and spawning dates of individual fish were recorded. Brooding females were allowed to rear their clutches for between 5 to 12 days. The hatchlings were then carefully removed in a double netted net; an outer 1-mm and inner 5-mm mesh to facilitate the separation and collection of eggs and hatchlings from parent with minimal damage. The clutch was then placed in a 15-cm diameter Petri dish containing clean water. Damaged fry were then quickly separated and examined under a dissecting microscope. Damaged fry with a heart beat were assumed to be an artifact of handling and therefore these were added to the undamaged total. Five brooders were sampled for each brooding period.

Estimation of Buccal Cavity and Clutch Volumes

Buccal cavity volumes were determined indirectly by water displacement of cavity casts made from expandable foam (Handy foam plus-FEB [Great Britain] Ltd., Manchester, England).

Various sizes of females were killed with an overdose of benzocaine, tagged for later identification and their weights and standard lengths recorded. Expandable foam was injected into all areas of the oral cavity and the mouth was held shut with a bent syringe needle and allowed to set for 90 minutes. During the setting stage, a syringe needle with the tip bent to form an "L" was inserted into the body of the cast to facilitate later handling. The casts were then removed by cutting along the floor of the buccal cavity and lower jaw, washed and dried at room temperature. Triplicate estimates of volumes of the casts were determined by weighing the displaced water on a tared top-pan balance.

To determine egg numbers, eggs from varying sizes of females were placed in a Petri dish and photocopied. Egg volumes were determined by the displacement method using a 5-ml

graduated measuring cylinder to an accuracy of 0.1 ml. The volume of 100 swim-up fry from these egg clutches were similarly measured and maximum clutch volumes were calculated, assuming all eggs developed into fry.

Growth of Artificially and Naturally Reared Siblings

Spawning tanks of broodstock were set up in 1-m^2 tanks as described in the section on fry losses above. In addition, when the broodstock were fed, ground and sieved (250-500 µm), feed containing 40% protein (Pellet No. 4, Edward Baker Ltd., Bathgate, Scotland) was also added to the tanks in excess as a source of food for any fry that may be released. Brooders were observed daily for spawnings. Brooders were gently guided into a large net and their mouths quickly held shut to prevent release of eggs. The mouth of each female was then gently partially opened to release about 100 eggs before returning her to rear the remainder of her clutch naturally. The date of spawning and tag identification was noted. To minimize the mixing of fry released from different females, eggs of brooders spawned within two days of the last recorded spawning were removed and discarded. The eggs released were artificially incubated in round-bottomed incubators (Rana 1986). Swim-up fry were stocked at 10 per liter and fed to excess four times per day on the same feed as used in the spawning tanks.

Feeding of the artificially reared fry was terminated when their naturally reared siblings were first observed to be released from the female. The naturally reared fry were carefully collected and they and their artificially reared siblings were held separately for four to five hours in clean water to evacuate their gut contents. They were then killed in benzocaine and the standard lengths of duplicate random samples of 20 fry were measured (to 0.1 mm), and their mean moisture (%) and dry weights (to 0.1 mg) determined.

Results and Discussion

Effect of Male Spawning Frequency on the Fertility Rates of Naturally Spawned Clutches

These studies suggested that although a male may readily court and successfully mate with several females in a day, his ability to maximize fertilization rates declines with an increase in spawning frequency.

In both species, between two and four spawnings per day were obtained (Tables 1 and 2). In both species, the proportions of developing eggs declined rapidly with an increase of spawning frequency, irrespective of male age; in the case of *O. niloticus*, from 96% in the first spawning to 22% in the fourth spawning. A similar trend was observed for *O. mossambicus*. Unfortunately, data on only four males are available, but the trend in fertilization rates are consistent for all males studied (Tables 1 and 2).

Fry Loss and Types of Damage Associated with Mouthbrooding

In both species, the cumulative losses of fry increased linearly up to eight days postspawning (Fig. 2). Thereafter, the rate of losses decreased, and by day 10, were nearly zero. By day 12, between 25 and 30% of the batch were fatally damaged. Numbers of damaged fry were not significantly correlated with fry numbers (r^2=0.042 and 0.167 with df=15 and 18, respectively, $P>0.05$).

Table 1. Effects of repeated matings by *Oreochromis niloticus* males on the viability of naturally spawned egg clutches.

Male no. (age)	Date of spawnings	Number of matings/day	Spawning order of females[a]	Egg viability (% of clutch)		
				Nonviable eggs (%)[b]	Unfertilized eggs (%)[c]	Developing eggs (%)[d]
1 (26 months)	2/24/84	4	4,383[e]	3	1	96
			4,377	4	16	80
			4,883[e]	5	26	69
			4,384	8	70	22
	4/3/84	2	4,375	0	0	100
			4,381	3	27	70
	8/3/84	2	4,377	4	-	96
			4,384	7	21	72
2 (8 months)	6/7/84	3	4,894	5	11	84
			4,895	4	33	63
			4,891	3	90	7

[a]Tag no. of individual females ranked in the sequence of their spawning.
[b]Defined as those eggs in which the germinal disc was not visible.
[c]Defined as eggs remaining in the germinal disc stage.
[d]Eggs in blastula-embryonic shield stage.
[e]Refers to a female which spawned twice.

Table 2. Effects of repeated matings by *Oreochromis mossambicus* males on the viability of naturally spawned egg clutches.

Male no. (age)	Date of spawnings	Number of matings/day	Spawning order of females[a]	Egg viability (% of clutch)		
				Nonviable eggs (%)[b]	Unfertilized eggs (%)[c]	Developing eggs (%)[d]
1 (16 months)	4/27/84	4	4,820	2	6	92
			4,890	5	15	80
			4,441	3	41	56
			4,277	2	70	28
	4/29/84	2	4,272	6	14	80
			4,068	2	30	68
2 (9 months)	2/6/84	2	4,882	2	19	89
			4,889	4	33	63
	10/7/84	2	4,889	1	14	85
			4,881	2	27	71

[a]Tag no. of individual females ranked in the sequence of their spawning.
[b]Defined as those eggs in which the germinal disc was not visible.
[c]Defined as eggs remaining in the germinal disc stage.
[d]Eggs in blastula-embryonic shield stage.

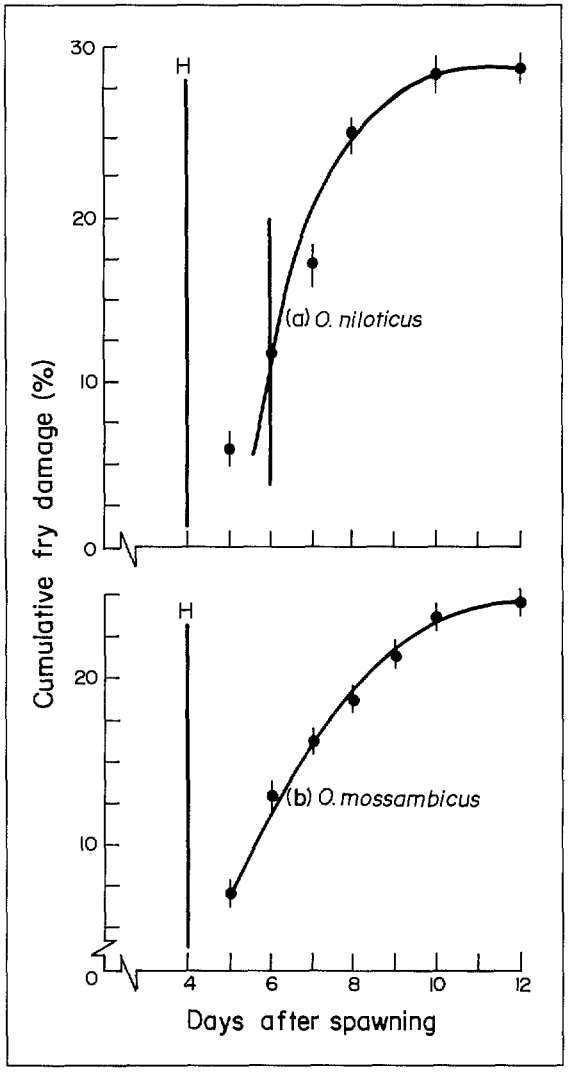

Fig. 2. Physical damage to fry within the buccal cavity associated with natural rearing under hatchery conditions: (a) *Oreochromis niloticus* and (b) *O. mossambicus*. ● denotes average and vertical bars show the range of fry damage during each brooding period. Note the large range for *O. niloticus* at six days was due to the inclusion of two parents kept in 100-l aquaria in which high numbers of fry were damaged; the remaining broodfish were kept in 1- and 3-m tanks.

Displacement of the yolksac accounted for 81-87% of the total damaged fry. In addition to this, 11-16% of the fry had eye damage and 5% had crushed heads.

These types of damage were probably due to physical injuries sustained during the churning of the clutch by the female. Under normal conditions, the churning frequency decreases from 95 to 105/min to 25-30/min by the third day of spawning and decreases to 3-8/min towards the later stages of rearing (Rana 1986). When females are harassed by dominant fish in a confined environment, increases in churning frequency and opercular movement may result in the fragile fry being damaged by the pharyngeal teeth.

Differences between the expected egg numbers and the numbers of fry reared have been attributed to buccal volume adjustments (Aronson 1949; Lowe-McConnell 1959; Riedel 1965). Based on the measurements of buccal cavity cast volumes, manual stripping to avoid partial spawning and maximum swim-up fry volume, this study suggests that even though the clutch volumes increase from 90 to 100% by the swim-up stage, they are well below buccal cavity volumes. It is therefore unlikely that buccal volume is a limiting factor for mouthbrooding (Fig. 3).

Comparison of Naturally and Artificially Reared Siblings

The weights of artificial and natural siblings are shown in Fig. 4. The time of initial release of fry varied between 11 and 18 days postspawning. In all clutches, artificially reared *O. niloticus* and *O. mossambicus* fry were significantly longer and heavier ($P<0.05$) than their naturally reared siblings. Artificially reared fry were between 14 and 211% heavier than naturally reared siblings: the

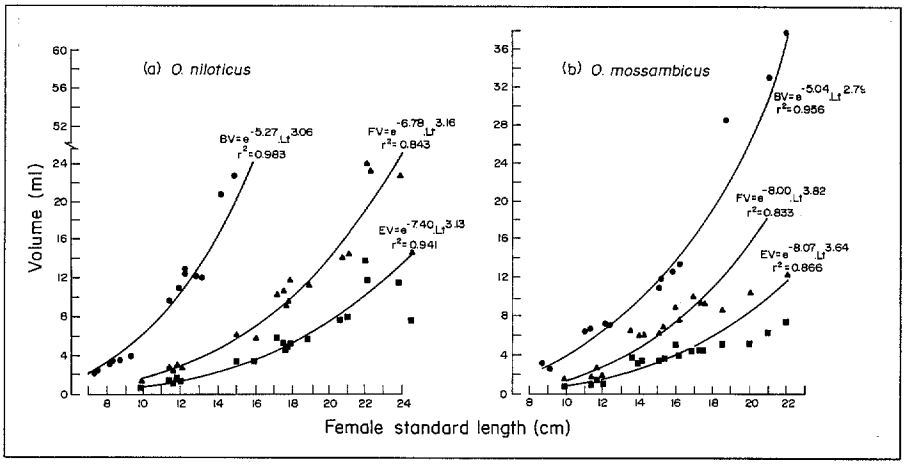

Fig. 3. Comparison between the buccal volume (BV), total egg volume (EV) and total fry volume (FV) of females of various sizes: (a) *Oreochromis niloticus* and (b) *O. mossambicus*. Curves relate to: ● buccal volume; ■ egg volume; and ▲ fry volume. Equations given are based on the natural logarithmic transformation of data.

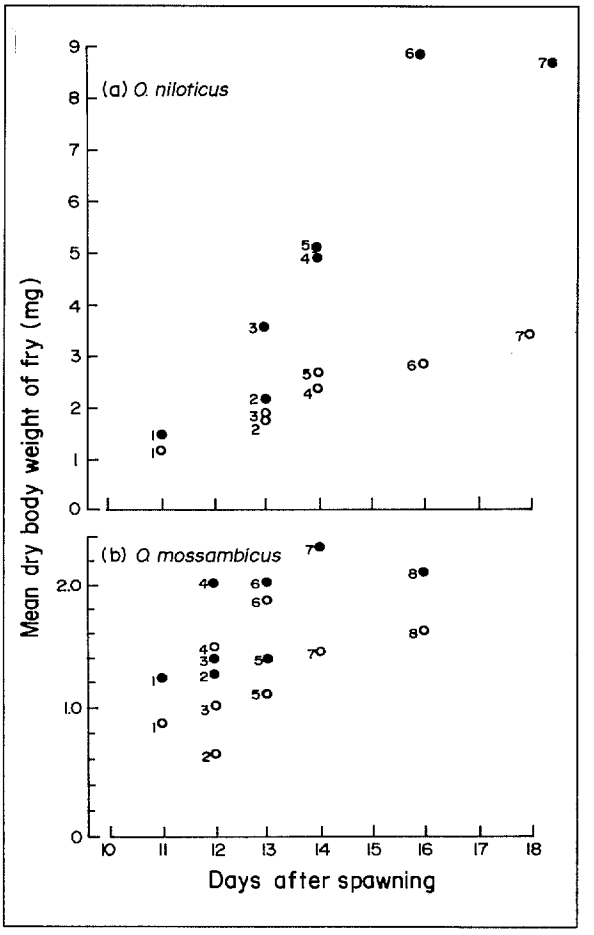

Fig. 4. Comparison between the mean body (fry less yolk) weights of artificially and naturally reared "siblings" from the same clutch: (a) *Oreochromis niloticus* and (b) *O. mossambicus*. O and ● refer to naturally and artificially reared "siblings," respectively.

later the initial release, the greater the difference. In both species, the onset of feeding commences between five and six days posthatching (9-10 days postspawning) at 27-28°C (Rana 1985, 1986). In these trials, the first release times ranged from 11 to 18 days postspawning and consequently first-feeding opportunities were delayed for varying periods of time resulting in lost growth opportunities. Delaying the initial feeding of artificially reared fry beyond six days significantly decreased ($P<0.05$) the growth of fry, especially if they originated from small eggs (Rana 1990).

The above trials suggest that breeding behavior under captive conditions may affect the quantity and quality of tilapia eggs and fry. Fertilization rates and, hence, the proportion of viable larvae may be influenced by the male spawning frequency. In addition, the number of damaged larvae can be increased if the brooders are kept in suboptimal conditions. The quality of the fry in terms of growth may be reduced if the initial and irreversible release of fry is delayed beyond the onset of feeding.

Acknowledgements

This study was made possible by a grant awarded to the author by the African Education Trust and by facilities provided by the British Overseas Development Administration at the Institute of Aquaculture, University of Stirling, Scotland.

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Observations on Intergeneric Hybrids in Tilapias

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Abstract

This study describes the hatchability, growth, sex ratios and reproductive behavior of intergeneric hybrids between *Tilapia zillii* (Linneus) and *Sarotherodon galilaeus* (Linneus), and between *T. zillii* and *Oreochromis andersonii* (Castelnau), *O. aureus* (Steindachner), *O. macrochir* (Boulenger), *O. mortimeri* (Trewavas), *O. mossambicus* (Peters), *O. niloticus* (Linneus), *O. placidus* (Trewavas), *O. spilurus* (Günther) and *O. tanganicae* (Günther). Only hybrid crosses using *T. zillii* as the maternal parent were successful.

Mean fertilization rates of the pure *T. zillii* cross and the hybrid crosses were similar and ranged from 91 to 98%. Hatching rates varied from 67 to 91%. The growth of the juveniles varied among the hybrids, but the growth patterns were similar to *T. zillii*. The sex ratios of the hybrids also showed marked differences and ranged from all males in the *T. zillii* x *O. andersonii* and *O. tanganicae* to all females in the *T. zillii* x *O. niloticus*, *O. placidus* and *O. aureus*. By convention, the female parent is named first. The hybrids in all crosses produced mature gonads. The characteristics of the eggs in all crosses were closer to the maternal parent.

Introduction

In recent years, many approaches have been used to resolve the problems of relatively low-farmed tilapia yields and the low proportion of marketable fish caused by uncontrolled breeding in poorly managed tilapia production systems. To minimize or eliminate this unwanted reproduction, the central aim of all approaches has been to produce all-male tilapia seed for culture. Techniques such as hand-sexing, x-rays, chemical sterilization, interspecific hybridization and hormone therapy have been used with varying degrees of success. Hybridization has also generated considerable interest and controversy concerning the sex-determining mechanism of tilapias.

In the last decade, interspecific hybridization between mouthbrooding species such as *Oreochromis niloticus*, *O. aureus*, *O. mossambicus* and *O. urolepis hornorum* has been widely used to produce predominantly male offspring for culture (Hulata et al. 1983).

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These species have compatible breeding behavior, readily hybridize under culture conditions and therefore are easily produced and studied. In contrast, there appears to be little information on the biology of *intergeneric* tilapia hybrids, especially hybrids between the substrate spawners and mouthbrooders. Investigations on intergeneric hybrids between the female *Tilapia tholloni* and the males *O. niloticus* and *O. mossambicus* suggested that all F_1 hybrids were female and that reciprocal crosses were unsuccessful (Heinrich 1967; Bauer 1968). A study by Fishelson (1988) on intergeneric hybrids between *Sarotherodon galilaeus* and *O. niloticus* suggests that even though these hybrids were easy to produce, their reproductive performance was reduced due to various levels of gonadal deformity.

In view of the incompatibility of the breeding behavior of substrate spawners and mouthbrooders, their progeny may not breed naturally. Previous studies have indicated biased sex ratios in some crosses and such hybrids may help to elucidate the basis of sex determination in tilapias.

In the present study, intergeneric hybridization between the substrate spawning *T. zillii* and 10 mouthbrooding tilapias were conducted to determine if such crosses are able to produce viable offspring and to gain information on growth, sex ratios and reproductive biology of these hybrids.

Materials and Methods

Procurement of Tilapia Eggs and Sperms

The substrate spawner *T. zillii* served as the dam in all the intergeneric hybridization trials. A different female was used for each trial; the numbers of crosses and trials for a given species pair are given in the Results Section (Tables 3 and 4). Six attempts to produce hybrids from all the reciprocal crosses were unsuccessful.

Single pairs of *T. zillii* (100-200 g) were introduced in 140-l glass aquaria supplied by a recirculatory system maintained at 28°C and checked daily for spawning activity. Prespawning females were removed and held in a covered 10-l plastic aquarium containing clean aerated water at 28°C.

Three spermiating *S. galilaeus*, *O. andersonii*, *O. aureus*, *O. macrochir*, *O. mortimeri*, *O. mossambicus*, *O. niloticus*, *O. placidus*, *O. spilurus* and *O. tanganicae* were transferred into separate containers until required.

Manual Stripping and Fertilization of Gametes

To avoid cross-contamination of sperm during hybridization, disposable pipettes and Petri dishes were used for each cross and were immediately discarded. Approximately 150-300 eggs were stripped onto labelled 25x5 cm-perspex slides and spread into a monolayer with a fine paint brush. Milt was collected from three conspecific males, pooled in a Pasteur pipette, and then spread over the eggs and activated with warm water (28°C). Samples of the diluted milt mixture, collected from the slide, were then checked under a microscope to ensure the presence of sufficient motile sperm. After 5 minutes, the eggs were gently rinsed with water (28°C) and incubated in individual 2-l containers of a recirculatory incubating system containing UV-treated water (Rana 1986).

Since the eggs were sequentially stripped from the female for each cross, the pure *T. zillii* cross was carried out last to ensure that all the eggs stripped from the female were viable.

Fertilization rates in all the crosses were determined within 24 hours. The number of pigmented eggs in a random sample of 50-100 eggs was counted on each slide using a dissecting microscope. The eggs were kept submerged in a Petri dish for this procedure before being returned to the incubator.

The time to 50% hatching was noted for each cross. After completion of hatching, each slide was shaken to dislodge the larvae. To estimate the hatching rate, the numbers of unhatched and empty egg shells adhering to the slide were counted. The pre-swim-up larvae (three-day-old) were transferred and reared in 20-l plastic tanks before being used in growth trials.

Survival, Growth and Sex Ratios of Intergeneric Hybrids

Thirty fry from each of the above crosses were transferred to duplicate 30-l plastic rearing tanks served by a recirculatory system. They were fed three times a day at a daily rate of 10% body weight on a trout feed containing 40% protein (No. 4, Edward Baker Ltd., Bathgate, Scotland). Random samples of 10 fry were bulk-weighed every two weeks and feeding rations adjusted, after accounting for mortalities. The growth trials were terminated after eight weeks.

The validity of significance was established using ANOVA after arc sine transformation of data. The fish were weighed and each cross transferred to 60-l tanks for ongrowing to sexual maturity and then killed in an overdose of benzocaine. The fish (50-150 g body weight) were sexed by examination of the genital papillae, internal identification of the gonads and, in uncertain cases, by gonadal squash preparation. Fish were weighed and gonads were removed, then weighed and representative samples fixed for histology.

Breeding of Hybrids

For each hybrid cross, hybrid females were introduced, with either male *T. zillii* or a male of the *Oreochromis* species used as the hybrid parent, into 120-l aquaria for observation of spawning activity.

Results and Discussion

Fertilization, Hatching Rates and Morphological Differences

Mean fertilization rates of the pure *T. zillii* and the intergeneric hybrids were similar ($P<0.05$) and ranged from 91 to 98%. The hatching rates, however, were variable and ranged from 67% in the *T. zillii* x *O. mossambicus* cross to 91% in the pure *T. zillii* (Table 1). Hatching times of the hybrids were similar and eggs from all the crosses hatched within 48 hours of fertilization. The length of the hybrids and *T. zillii* larvae at hatching were not significantly ($P<0.05$) different and ranged from 4.8 to 5.0 mm.

T. zillii and all the hybrid larvae possessed two pairs of head glands, but the amount of mucus produced by the hybrids was reduced resulting in the hybrids being free of the substrate a day earlier. The intensity of melanophore pigmentation on the yolksac epithelium also varied between the hybrids, but *T. zillii* controls showed the greatest intensity. The hybrids and *T. zillii* fry began feeding four to five days posthatching (at 28°C).

Early Fry Survival and Growth

The survival and growth of the various hybrids are given in Table 2. The mortality patterns between the replicates were similar, suggesting a genetic basis. Survival of fry between hybrids

Table 1. Means (and SE) for the fertilization and hatching rates of intergeneric hybrids between *Tilapia zillii* females and males of various *Sarotherodon* and *Oreochromis* species.

Male parent	Fertilization rate (%)	Hatching rate (%)
T. zillii[c] (control)	96.8 (1.4)	91.0 (3.29)
S. galilaeus[c]	98.7 (0.86)	87.7 (2.82)
O. andersonii[b]	91.1 (4.54)	89.0 (5.02)
O. aureus[d]	95.0 (2.9)	88.0 (2.65)
O. macrochir[c]	93.3 (3.75)	78.3 (8.43)
O. mortimeri[b]	96.0 (1.84)	84.5 (8.48)
O. mossambicus[d]	93.8 (2.85)	67.1 (3.10)
O. niloticus[d]	93.5 (2.05)	78.6 (2.45)
O. placidus[b]	95.0 (1.70)	85.0 (4.03)
O. spilurus[d]	95.0 (1.7)	83.5 (4.25)
O. tanganicae[a]	92.1 (-)	87.4 (-)

a: N=1; b: N=2; c: N=3; and d: N=4 (no. of attempts).

Table 2. Means (and SE) for the growth characteristics of intergeneric hybrid tilapia juveniles: all with *Tilapia zillii* as the female parent and various *Sarotherodon* and *Oreochromis* species as the male parents.

Male parent	Initial weight[1] (g)	Final weight[1] (g)	SGR (SE)[1,2] (%·day^{-1})	Survival[1,2] (%)
T. zillii (control)	0.076	2.38	6.4[a]	81.5[cd]
	(0.003)	(0.035)	(0.099)	(1.61)
S. galilaeus	0.132	3.49	6.1[a]	81.5[cd]
	(0.027)	(0.11)	(0.179)	(1.66)
O. andersonii	0.072	3.24	7.1[ab]	98.5[d]
	(0.006)	(0.057)	(0.198)	(1.5)
O. aureus	0.087	2.49	6.2[a]	98.5[d]
	(0.004)	(0.134)	(0.198)	(1.50)
O. macrochir	0.096	3.06	6.18[a]	76.5[cd]
	(0.006)	(0.10)	(0.18)	(3.5)
O. mortimeri	0.093	3.19	6.55[a]	50[ab]
	(0.007)	(0.028)	(0.049)	(9.9)
O. mossambicus	0.170	5.02	6.25[a]	37[a]
	(0.003)	(0.30)	(0.148)	(-)
O. niloticus	0.058	3.68	7.65[b]	48.5[a]
	(0.002)	(0.636)	(0.247)	(1.5)
O. placidus	0.115	3.33	6.25[a]	61.5[abc]
	(0.002)	(0.010)	(0.049)	(8.5)
O. spilurus	0.070	2.29	6.45[a]	88.5[cd]
	(0.002)	(0.042)	(0.049)	(11.5)
O. tanganicae	0.093	3.79	6.9[ab]	78.5[bcd]
	(0.003)	(0.071)	(-)	(1.5)

[1]Means based on duplicate treatment.
[2]Means within columns having the same letters are not significantly ($P<0.05$) different.

at the end of the trial varied significantly ($P<0.05$). Hybrids with *O. mossambicus* and *O. niloticus*, and *O. andersonii* and *O. aureus* had the lowest and highest survival rates, respectively. Despite these differences, the growth rates of the hybrids at the end of the trial were not significantly ($r=-0.145$, $df=21$, $P<0.05$) correlated with the survival rates.

Mean initial weight of the different hybrid groups varied between 0.06 and 0.17 g and were significantly correlated with the final weight ($r=0.712$, $df=21$, $P<0.05$) and weight gain ($r=0.699$, $df=21$, $P<0.05$). This association presents a problem since these growth estimators may result in growth being biased by variation in initial weights. In an attempt to reduce this bias, weight data were transformed and growth of the hybrids was compared as specific growth rate (Table 2). Hybrids of *O. tanganicae*, *O. andersonii* and *O. niloticus* showed the highest growth rates. The high apparent growth rates of *O. niloticus* hybrids, however, may have been influenced by their low survival rate.

Reproductive Behavior, Gonadal Development and Sex Ratios

All the hybrid females lacked the typical breeding behavior of either of their parental species and no spawning occurred in the breeding tanks. One "accidental" spawning did occur with a *T. zillii* x *O. mossambicus* female. This female was held on her own in a plastic tank and therefore no information on the viability of the eggs was obtained. The eggs were laid on the tank bottom. They were partially adhesive and similar to those of a substrate spawner. In addition, the female guarded and periodically fanned the eggs.

From the observations performed to determine the sex of the hybrids, not all fish could be classed as male or female. In some crosses, between 1 and 6% of the fish were sterile (Table 3). These fish lacked any gonadal tissue and contained instead a clear, viscous fluid.

Depending on their paternal species, the sex ratios of hybrids fall into three main groups: predominantly male, pre-

Table 3. Sex ratios of intergeneric tilapia hybrids having *Tilapia zillii* as the female parent and various *Sarotherodon* and *Oreochromis* species as the male parents.

	Trial 1			Trial 2			Trial 3		
Male parent	M	F	S	M	F	S	M	F	S
T. zillii (control)	15	8	-	10	16	-	14	34	-
O. niloticus	0	21	3	0	23	1	0	27	2
O. placidus	0	32	2	0	46	1	0	65	2
O. mortimeri	0	27	4	-	-	-	2	27	1
O. aureus	2	52	1	-	-	-	0	53	-
O. mossambicus	5	17	1	0	50	-	0	33	-
O. spilurus	7	36	-	-	-	-	0	25	-
S. galilaeus	9	24	-	-	-	-	12	13	-
O. macrochir	81	36	-	-	-	-	-	-	-
O. tanganicae	31	0	-	-	-	-	22	0	-
O. andersonii	52	0	-	-	-	-	49	0	-

M: male; F: female; and S: sterile fish (see text).

dominantly female and an approximately equal number of both sexes (Table 3). It is difficult to see any obvious pattern related to the presumed sex-determination system from other studies (McAndrew 1993) as *O. macrochir* and *O. aureus* (homogametic males) occur in different groups. There is no firm evidence that the observed sex ratios are based on any phylogenetic relatedness, as suggested by Sodsuk and McAndrew (1991) who found *O. andersonii* to always be closely grouped with *O. mossambicus* and *O. mortimeri*. In this study, *O. andersonii* appears to be clearly separated from the two other species.

The gonads of hybrids, where recognizable, were of particular interest. In all cases, the gonadosomatic indexes of both sexes were significantly ($P<0.05$) lower than those of pure *T. zillii* progeny, and ranged between 0.005 and 0.36% for the males and 0.32 and 1.74% for the females (Table 4). The majority of the hybrid female ovaries contained relatively few oocytes compared to those of pure *T. zillii*. The majority of the gonads examined contained previtellogenic oocytes up to stage 3, and few gonads contained mature oocytes at stages 5 and 6. Even though the testes of the hybrids with *O. andersonii* and *O. tanganicae* were significantly smaller than those of pure *T. zillii*, they contained gametes at all stages of development. In some fish, mature spermatozoa were present in the tubules of the testes.

It is difficult to derive any hypothesis from this study on sex determination in tilapia. Hybrid sex ratios are a notoriously difficult analytical character (Majumdar and McAndrew 1983). As a result of the difficult statistical problems in identifying and separating expected sex ratios and our limited knowledge on the genetic mechanisms involved in sex determinations in pure species, the consequences for hybridization are impossible to predict. The observed sex ratios, the reduced reproductive performance and the phytophagous feeding habit of the substrate

Table 4. Means (and standard deviations) for the gonadosomatic indexes (GSI) of intergeneric tilapia hybrids from *Tilapia zillii* female parents and various *Sarotherodon* and *Oreochromis* species as male parents.

Male parent	No.	Sex	Mean body weight (g)	GSI* (%)
T. zillii (control)	11	M	78.5 (36.2)	1.47 (0.47)[a]
	6	F	51.5 (23.3)	2.8 (0.20)[1]
S. galilaeus	7	M	101.7 (20.6)	0.36 (0.30)[b]
O. andersonii	10	M	136.6 (42.6)	0.02 (0.013)[b]
O. aureus	21	F	101.2 (46.6)	1.26 (0.96)[1]
O. macrochir	33	M	142.7 (52.7)	0.19 (0.05)[b]
	8	F	125.0 (31.9)	0.50 (0.56)[2]
O. mortimeri	14	F	103.9 (22.8)	0.67 (0.22)[2]
O. mossambicus	6	M	67.0 (34.4)	0.02 (.005)[b]
	5	F	64.1 (55.5)	0.32 (0.10)[2]
O. niloticus	16	F	132.4 (29.4)	1.74 (1.28)[1]
O. placidus	11	F	53.7 (16.7)	0.35 (0.12)[2]
O. spilurus	25	F	74.4 (38.8)	0.51 (0.53)[2]
O. tanganicae	10	M	75.5 (30.5)	0.05 (0.026)[c]

*Means with different letters (for males) and numbers (for females), respectively, are significantly ($P<0.05$) different.

spawner parent may, in themselves, be of some advantage in possible future uses of these hybrids.

Acknowledgements

The authors are grateful for the facilities provided by the British Overseas Development Administration and to Prof. R.J. Roberts for his financial support.

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Study of Genetic Variation in Farmed Populations of Some Species of the Genus *Oreochromis*

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Abstract

An electrophoretic study of enzyme polymorphism was conducted on five species of tilapia of the genus *Oreochromis: O. niloticus, O. aureus, O. mossambicus, O. urolepis hornorum* and *O. macrochir.* For *O. niloticus*, five farmed populations were analyzed, as well as two natural populations from the Volta and the Niger rivers. Eighteen enzyme systems, coded by 30 loci were studied. Heterozygosity and genetic distances between the different samples were calculated. The study of the genetic diversity of *O. niloticus* shows the resemblances and dissimilarities that exist among these populations, as well as the maintenance of variability in farmed stocks. Analysis of the results obtained for one of the populations of *O. aureus* and *O. mossambicus* suggests a case of genetic introgression due to the continued use of these populations in interspecific hybridization. An analysis was conducted to estimate phylogenetic relationships between the different populations.

Introduction

The production of tilapia has made great strides in subtropical and tropical countries. Some 20 species, often originating from introductions (Welcomme 1988; Lazard 1990), are used for aquaculture with *Oreochromis niloticus* as main species. It seems that most of these stocks have not been produced and managed in a rational way for aquaculture (Wolfarth and Hulata 1983; Thys van den Audenaerde 1988). They have often been developed from broodstock taken from a limited number of sites, sometimes even a single site, and in small numbers. Moreover, some farmed populations have had a quite complicated history, with successive transfers multiplying the risks of genetic bottlenecks. Thus, the genetic potentialities of these species are perhaps not totally exploited and some genetic deterioration may have occurred over generations.

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Finally, cases of genetic introgression have been described (Taniguchi et al. 1985; Macaranas et al. 1986) in aquaculture strains, whether due to interspecific hybridization programs or to the introduction of exotic individuals into farming areas. This situation has led to an increased interest over the last few years in describing the genetic diversity of the principal species of tilapia used for aquaculture to identify the different populations with aquaculture potential and to improve the management of culture stocks.

In Côte d'Ivoire, farmed populations of *Oreochromis* are all of exotic origin, although *O. niloticus* exists in a few rivers belonging to the Niger and Volta river basins. The first introductions were made by the Tropical Forestry Technical Center (CTFT) in 1957 (Lazard 1990) at the Bouake station (currently IDESSA[b]), and have continued, particularly in the search for species adequate for lagoon aquaculture, up until the 1980s. The preliminary results of the study on genetic diversity in these Ivorian populations, using the electrophoretic characterization of enzyme polymorphism, are presented here.

Materials and Methods

Randomly selected individuals were harvested from 11 farmed populations belonging to five species of *Oreochromis*, as well as from two natural populations of *O. niloticus* (Table 1).

Samples of muscle, liver and eye tissue were taken from each individual and deep-frozen to -20°C. Samples were homogenized in distilled water (muscle 1 $g \cdot ml^{-1}$, liver 1 $g \cdot 1.5\ ml^{-1}$ and eye 1 $g \cdot 0.5\ ml^{-1}$) and centrifuged to 5,000 $r \cdot min^{-1}$ for 30 minutes. For the eye tissue, chloroform was added to eliminate the glycoproteins and glycolipids that can cause distortions during enzyme separation.

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Electrophoretic analyses were done on 12% starch gels. The separation and staining techniques used were those described by Krieg (1984) and McAndrew and Majumdar (1983). Eighteen enzyme systems coded 30 loci were analyzed (Table 2). The designation and numbering of the different loci are as proposed by Shaklee et al. (1990). The numbering of the different loci in a given enzyme system is based on their mobility, that closest to the cathode receiving number one. For each locus, the most frequent allele in the Bouake strain of *O. niloticus* (the point of reference) received the index 100, the other alleles being designated according to their relative mobility. Alleles migrating to the cathode are numbered according to the same principle, their index being preceded by a negative sign.

Allelic frequencies were estimated from the breakdown of the alleles. For polymorphic loci, the agreement to Hardy Weinberg's equilibrium was verified by the exact test (BIOSYS-1/1.7) of Swofford and Selander (1989). Polymorphism was calculated taking into account that a locus is polymorphic when the frequency of the most common allele is lower than 0.95 (P95) or 0.99 (P99). Genetic distances and heterozygosity were calculated following Nei (1975). Based on the genetic distance matrix, a dendogram was created with the Kitsch program of Phylip (Phylip Package), using the methods of Fitch and Margoliash (1967) and the least squares method, with constant evolution rates. Finally, a hierarchical breakdown of genetic diversity following Nei (1973) and Chakraborty (1980) was conducted on populations of *O. niloticus.*

Results

Allelic frequencies at the polymorphic loci, polymorphism and heterozygosity are presented in Table 3. Of 30 loci studied, 17 proved polymorphic among or between

Table 1. List of populations of *Oreochromis* spp. studied. The symbols used to represent populations in the following figure and tables are indicated in parentheses.

Populations	Origin	Sampling area	Number of samples
O. niloticus (BK)	Volta+Nil river basins	Bouake station	30
O. niloticus (KO)	"Bouake" strain	Lake Kossou	30
O. niloticus (BU)	"Bouake" strain	Lake Buyo	14
O. niloticus (DA)	"Bouake" strain	Daloa fish farm	24
O. niloticus (OD)	"Bouake" strain	Odiéné fish farm	15
O. niloticus (KU)	Volta river	Kou valley	20
O. niloticus (NI)	Niger river	Niger (near Niamey)	17
O. aureus (AI)	Israel (via Belgium)	Bouake station	30
O. aureus (AM)	idem (via Bouake)	Mopoyem station	14
O. aureus (AE)	Nile (Lake Manzalla)	Bouake station	30
O. mossambicus (MO)	Mozambique	Bouake station	30
O. urolepis hornorum (HO)	Malaysia	Bouake station	20
O. macrochir (MA)	Zaire	Bouake station	30

populations. Diagnostic loci were shown, mainly in *O. macrochir* as compared to the other species. The Hardy Weinberg proportions were tested in all populations. Three tests out of 67 are significant at the 5% level. The Hardy-Weinberg equilibrium is generally respected and the populations can be considered panmictic.

For each population, rates of P95 and P99 polymorphism are close enough, except in the case of the "Israel-Bouake" strain of *O. aureus* where all polymorphic loci have a majority allele of a frequency higher than 0.95 (except for IDHP-1*).

Heterozygosity varies between 0 (*O. aureus* "Israel-Mopoyem" strain) and 8.4% (*O. niloticus* "Buyo" strain). Heterozygosity in farmed populations of *O. niloticus* is higher than in natural populations.

The genetic distance matrix is given in Table 4 and the resulting dendogram is presented in Fig. 1. Four groups are distinguished: (1) strains of *O. niloticus*, (2) *O. mossambicus* and *O. urolepis hornorum*, (3) *O. aureus* and (4) *O. macrochir*.

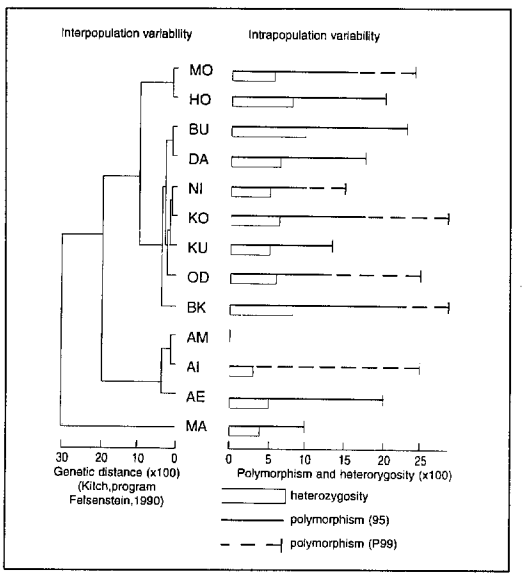

Fig. 1. Genetic structure of populations of *Oreochromis* spp.

Discussion

Comparison of the results from all populations of the five *Oreochromis* species show four different groups: (1) *O. macrochir*, (2) populations of *O. aureus*, (3) populations of *O. niloticus* and (4) *O. mossambicus* and *O. urolepis hornorum*. Genetic distances between species are small, but they are compatible with those calculated by McAndrew and Majumdar (1984) with the exception of *O. mossambicus* plus *O. urolepis hornorum*. The diagnostic loci, identified by Brummett et al. (1988) among American populations of these two species, share common alleles in the two Bouake populations (in the two studies, *O. urolepis hornorum* had the same origin). Apart from

Table 2. List of enzyme systems studied with their location in the tissues, their genetic structures and the migration buffers used. Tissues: L = liver, M = muscle and E = eye.

System	Locus	Tissues	Buffer	Remarks
AAT	1*	L,M,E	MC2	MC2 (Morpholin citrate)
	2*	L,E		citric acid 0.08 M, adjusted to pH 6.2
	3*	M,E		with morpholin
ADH	1*	L	RW	300 V - 110 mA - 3 hours and 30 minutes
AK	1*	M	MC2	gel: dilute buffer to 5%
CK	1*	M,E	RW	-
	2*	E		MC4 (Morpholin citrate buffer)
EST	2*	M,E	RW	citric acid 0.08 M, adjusted to pH 6.6
FBP	1*	M	MC4	with morpholin
	2*	L		200 V - 110 mA - 3 hours and 30 minutes
FH	1*	M	MC2	gel: dilute to 10%
G3PDH	1*	M	MC4	-
	2*	L,M		RW (Ridgway buffer)
GPI	1*	M,E	RW	electrode buffer: LiOH 0.06 M,
	2*	L,E		H3BO3 0.3 M,
IDDH	1*	L	RW	gel buffer: tris 0.03 M, cetric acid
IDHP	1*	L	MC4	0.005 M
	2*	M		350 V - 80 mA - 2 hours and 15 minutes
LDH	1*	M,E	RW	gel: gel buffer + 1% electrode buffer
	2*	L,M,E		-
	3*	E		TEB (Tris-EDTA-Borate)
MDH	1*	M,E	MC2	tris 0.5 M EDTA 0.016 M, H3BO3
	2*	L,M,E		O,24 M
	3*	M		200 V - 100 mA - 3 hours
MEP	1*	M	MC4	gel: dilute to 10%
	2*	L,M		-
MPI	1*	M	TEB	-
PGDH	1*	L,M	MC4	-
PGM	1*	M	TEB	-
SOD	1*	L	RW	-

the action of genetic drift among American populations, in which polymorphism is zero or greatly reduced, such a difference could be due to the introduction of exotic alleles as a result of introgression between them or with the other species present at the Bouake station.

Heterozygosity in the Ivorian farmed populations of *O. niloticus* is higher than in the natural populations of the Niger and Volta rivers and in the natural population of Lake Manzalla (Nile basin) studied by McAndrew and Majumdar (1983). Such a level of variation among these populations that originated from the Bouake strain can be explained by the double origin of the strain, which resulted from a 1971-72 mixing of the station's Volta and Nile populations. In this hypothesis, the polymorphism observed in certain fixed loci of the Volta population (which originates from the same place as the founder population of the "Bouake" strain) could be due to the genetic contribution of the Nile individuals.

The Daloa, Odiéné, Kossou and Buyo populations originated from the "Bouake"

Table 3. Allelic frequencies at polymorphic loci, mean heterozygosity (H) and polymorphism (P at 95 and 99%) for the different populations of *Oreochromis* spp. (see Table 1 for letter codes).

LOCUS	ALLELE	BK	KO	BU	DA	OD	KU	NI
AAT-2*	*117							
	*100	0.600	0.607	0.633	0.813	0.633	0.300	0.500
	*55	0.400	0.393	0.367	0.187	0.367	0.700	0.500
AAT-3*	*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	*78							
ADH*	*-50							
	*-100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-1*	*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	*56							
EST-2*	*105		0.017					
	*100	1.000	0.983	1.000	1.000	1.000	1.000	1.000
FH*	*120	0.500	0.017	0.071		0.133		
	*100	0.500	0.983	0.929	1.000	0.867	1.000	1.000
GPI-2*	*114							
	*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IDDH*	*169	0.100	0.143					
	*100	0.750	0.375	0.643	0.646	0.800	0.684	0.500
	*16	0.150	0.482	0.357	0.354	0.200	0.316	0.500
IDHP-1*	*108							
	*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	*70							
LDH-2*	*100	0.950	0.833	0.750	0.750	0.967	0.800	0.971
	*52	0.050	0.167	0.250	0.250	0.033	0.200	0.029
	*46							
MDH-1*	*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	*42							
MDH-3*	*119	0.017						
	*100	0.983	1.000	1.000	1.000	1.000	1.000	1.000
MEP-1*	*125	0.050	0.083	0.286	0.229	0.100	0.200	0.286
	*100	0.950	0.917	0.714	0.771	0.900	0.800	0.714
MEP-2*	*120	0.183	0.017	0.179		0.033		
	*100	0.817	0.983	0.821	1.000	0.967	1.000	1.000
	*80							
	*70							
MPI*	*107	0.100	0.050	0.250	0.208			
	*100	0.900	0.950	0.750	0.792	0.100	1.000	1.000
	*92							
PGDH*	*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	*69							
SOD*	*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	*19							
H (%)		6.96	5.68	8.40	6.06	4.41	4.97	4.88
P95 (%)		23.33	16.67	23.33	16.67	13.33	13.33	10.00
P99 (%)		26.67	26.67	23.33	16.67	20.00	13.33	13.33

(cont.)

Table 3. *(cont.)*

LOCUS	ALLELE	AI	AM	AE	MO	HO	MA
AAT-2*	*117				0.183		
	*100	0.983	1.000	1.000	0.467	0.450	1.000
	*55	0.017			0.350	0.550	
AAT-3*	*100	0.033			0.983	1.000	1.000
	*78	0.967	1.000	1.000	0.017		
ADH*	*-50	0.967		0.883			
	*-100	0.033	1.000	0.117	1.000	1.000	1.000
CK-1*	*100	1.000	1.000	1.000	1.000	1.000	
	*56						
EST-2*	*105	0.967	1.000	0.987	0.050		1.000
	*100	0.033		0.033	0.950	1.000	
FH*	*120	0.017		0.117			
	*100	0.983	1.000	0.883	1.000	1.000	1.000
GPI-2*	*114				0.950	0.583	
	*100	1.000	1.000	1.000	0.050	0.417	1.000
IDDH*	*169	0.033			0.850	0.575	
	*100	0.967	1.000	1.000	0.150	0.425	0.362
	*16						0.638
IDHP-1*	*108	0.950	1.000	0.950	0.033		
	*100	0.050		0.050	0.967	0.925	1.000
	*70					0.075	
LDH-2*	*100	1.000	1.000	0.630	1.000	1.000	
	*52						
	*46			0.367			1.000
MDH-1*	*100	1.000	1.000	1.000	1.000	1.000	
	*42						1.000
MDH-3*	*119	1.000	1.000	1.000	1.000	1.000	1.000
	*100						
MEP-1*	*125			0.200			
	*100	1.000	1.000	0.800	1.000	1.000	1.000
MEP-2*	*120				0.033		
	*100	1.000	1.000	1.000	0.967	0.750	0.550
	*80					0.250	
	*70						0.450
MPI*	*107						
	*100	1.000	1.000	1.000	1.000	1.000	
	*92						1.000
PGDH*	*100	1.000	1.000	1.000	1.000	1.000	0.900
	*69						0.100
SOD*	*100	0.967	1.000	1.000	0.317	0.250	
	*19	0.033			0.683	0.750	1.000
H (%)		1.60	0.000	4.52	5.55	7.87	3.79
P95 (%)		3.33	0.000	20.00	16.67	20.00	10.00
P99 (%)		26.67	0.000	20.00	26.67	20.00	10.00

Table 4. Matrix of standard genetic distances (Nei 1973) estimated among the different populations studied.

	BK	KO	BU	DA	OD	KU	NI	AI	AM	AE	MO	HO
KO	0.0164											
BU	0.0124	0.0061										
DA	0.0178	0.0052	0.0030									
OD	0.0079	0.0062	0.0067	0.0064								
KU	0.0179	0.0067	0.0072	0.0110	0.0063							
NI	0.0181	0.0034	0.0063	0.0076	0.0056	0.0039						
AI	0.1974	0.1934	0.1987	0.1881	0.1815	0.2010	0.1973					
AM	0.2118	0.2075	0.2126	0.2014	0.1950	0.2151	0.2113	0.0004				
AE	0.2000	0.1984	0.1982	0.1885	0.1861	0.2036	0.2009	0.0066	0.0069			
MO	0.1189	0.1059	0.1204	0.1197	0.1126	0.1162	0.1135	0.2201	0.2374	0.2334		
HO	0.0913	0.0835	0.0930	0.0943	0.0843	0.0862	0.0872	0.1951	0.2121	0.2076	0.0109	
MA	0.3129	0.2884	0.2905	0.2828	0.3030	0.3119	0.3046	0.3355	0.3464	0.3150	0.2761	0.2495

strain. Some differentiation is observed compared to this strain and between the populations, particularly in the number of polymorphic loci. Two hypotheses, not mutually exclusive, can address this. On the one hand, the separation of these stocks took place at different times and therefore at various stages of mixing of the two genetic pools originating from the "Bouake" strain. It will be noted that, although genetic distances are all very small, the farmed population most similar to that of the Volta river comes from Lake Koussou, which was stocked close to the time when genetic mixing began. This may explain the weaker influence of the original Nile population whose presence at the station is limited to few individuals. On the other hand, such differences can be accounted for simply by the effect of genetic drift acting independently on these groups that originate from the same genetic pool.

Other studies on farmed stocks of *O. niloticus* show great variations, with heterozygosity ranging from 0 to 11.5% (Basiao and Taniguchi 1984; Taniguchi et al. 1985; Macaranas et al. 1986; Brummett et al. 1988). The highest rates are obtained in Philippine populations for which genetic introgression by *O. mossambicus* has occurred. In the populations of Bouake and Kossou, a locus (MDH-3* and EST-2*, respectively) possesses an allele (*119 and *105, respectively) that is present at a very low frequency (f=0.017) and that is not found in any other population of *O. niloticus*. This presence could be the sign of an introgression that occurred before the separation of the two populations. In any case, this introgression is probably very weak.

The study of genetic distances and the distribution of genetic diversity in *O. niloticus* (Table 5) shows that most genetic diversity is of intrastock origin (90% of the total gene diversity). This confirms the relative homogeneity among Ivorian farmed stocks and between them and the two natural populations. The differentiation between the two natural populations could be the sign of a certain homogeneity in stock arising from periods of contact between these two basins.

O. macrochir shows a relatively low mean heterozygosity (3.8%), but that is close to

Table 5. Hierarchical breakdown of total genetic diversity ($H_T = H + D$) in *Oreochromis niloticus*: 90% of the genetic diversity is of intrastock origin.

Total gene diversity (H_T)	6.56%
Intrastock gene diversity ($\bar{H}$)	5.90%
Interstock gene diversity (D)	0.66%

the rate found by McAndrew and Majumdar (1983, H=3.1%) in another farmed population. This species has been present at the station since 1958, and comes, via Cameroon, from a stock created in 1945 in Zaire (Thys van den Audenaerde 1988) from 10 pairs. This history suggests a significant risk of bottleneck.

The intrastock variability found in the two stocks of *O. aureus* "Israel" is zero in the Mopoyem stock, and very low in the Bouake stock where the most common alleles at the polymorphic loci show frequencies higher than 0.95 (except for IDHP-1*). This may be due to bottlenecks that have occurred during the successive transfers (Israel→Belgium→Côte d'Ivoire/Bouake→Mopoyem). In this case, the Bouake population could show a residual state of the initial polymorphism of the Israeli strain, a polymorphism that would have been lost during the constitution and maintenance of the Mopoyem stock. It is also possible that the Mopoyem stock represents the initial state of the Israeli strain upon its arrival in Côte d'Ivoire. In this case, variations observed in the Bouake stock could be due to an introgression—particularly by *O. mossambicus*, with which many interspecific hybridizations have been performed and which shows specific alleles of *O. aureus* at frequencies similar to those of the polymorphic loci of *O. aureus* "Bouake". These two hypotheses are not exclusive, and to better assess the respective influence of these two phenomena, it will be necessary:

- to study the genetic diversity of the wild populations of *O. aureus*, particularly to describe the different allelic forms present in this species; and
- to develop other techniques to reveal an introgression, such as mitochondrial DNA analysis.

Conclusion

Based on these preliminary results, and compared to other studies, it seems that farmed populations of *O. niloticus* in Côte d'Ivoire have been constituted, then maintained as aquaculture stocks without loss of variability or evident introgression by the other species.

For populations of *O. aureus* used in experiments on lagoon aquaculture, the existence of a weak introgression is possible. Since this can have significant consequences on culture performance (fertility, growth and survival), the actual state of the populations will have to be monitored and, if necessary, a new strain should be constituted.

Finally, it will be noted that there is a great similarity between the natural *O. niloticus* populations of the Volta and Niger river basins..

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Basis of the Sexual and Territorial Behavior in Males of *Oreochromis niloticus* and *Oreochromis mossambicus*

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ROUGER, Y. 1996. Basis of the sexual and territorial behavior in males of *Oreochromis niloticus* and *Oreochromis mossambicus*, p. 407-414. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

This study compared the social hierarchy of three groups of five males of two tilapia species: *Oreochromis niloticus* and *Oreochromis mossambicus*. Measurements of circulating steroid (testosterone, 11-KT and 17.20P) showed high testosterone levels in dominant males of *O. niloticus*, whereas no significant difference was observed in the levels of testosterone between dominant and dominated males of *O. mossambicus*. In *O. niloticus*, highly aggressive and territorial behaviors are correlated whereas *O. mossambicus* has low endocrine levels and does not have a highly hierarchical social behavior. In *O. niloticus*, the color of the dominant male is testosterone-dependent and has an appeasing function: pre-spawning females adopt this color after a sharp increase in testosterone and are thus able to enter the nest guarded by the male.

The use of different steroid implants confirms the role of testosterone in the aggressive behavior of the tilapia, allowing some fish to rise in the social hierarchy. The effects of 11-KT are more obvious for nest-digging activities than for chasing behavior.

Introduction

Previous studies have shown a correlation between gonadal cycles, circulating steroid levels and sexual behavior in many fish, especially in salmonids (Liley and Stacey 1983).

In cichlids, Aronson (1945, 1951) described the role of external stimuli (visual stimuli, sound emission, contact between the lateral lines and chemical communication) produced by other mates on spawning frequency. In *Oreochromis mossambicus*, Silverman (1978a, 1978b) suggested an effect comparable to pheromones as shown by Solomon (1977) in other species. In *O. mossambicus*, males as well as females can increase spawning frequency.

This study compares the social behavior and the endocrine levels of two species of tilapia: *Oreochromis niloticus* and *O. mossambicus*.

Materials and Methods

Fish

The fish used in the experiments were *O. niloticus* from a laboratory strain originally coming from Bouake (Côte d'Ivoire). All animals came from the same culture batch and were one year old. They measured 14.5±1.29 cm and weighed 96.3±3.5 g (mean body weight). Experiments were performed every year between April and September from 1989 to 1992.

For *O. mossambicus*, the animals came from a strain developed in Louvain-la-Neuve (Belgium) by Ursula Falter. In this experiment, 10 females of *O. mossambicus* and eight females of *O. niloticus* were used as well as 15 males of each species.

Culture Conditions

Outside experimental periods, males and females were separated and placed in tanks containing 0.5 m^3 recirculated water kept at 27°C. These tanks contained only tilapias. During experimental periods, the animals were placed in 300-l glass tanks containing adsorbing sand at the bottom and were transferred during tests to an 800-l tank also containing adsorbing sand at the bottom.

Experimental Protocols and Ethograms

Males were divided into groups of five animals. An ethogram was used to establish the social hierarchy for each of these groups and to visualize the social relationships within the group. For easy identification, the animals were tagged by fixing a nylon string with different colored beads forming a determined pattern on the posterior part of the head. The fish were anesthetized and a blood test was done before and after a series of behavioral tests conducted over a five-day period to evaluate their social hierarchy. These tests were triplicated for each groups.

On the ethogram where the five fish are represented in a circle (see Fig. 1), the dominant male, i.e., that which directs the greatest number of activities towards its conspecifics, is placed at the top. The dominated animal, i.e., that which sustains the greatest number of aggressive actions without reciprocation, is placed at the bottom. Interactions between animals are represented by arrows oriented in the direction of the action. The thicker the line, the higher the number of aggressive actions between fish. The number of fish is indicated in circles.

Social and Sexual Behavior

The different elements of the social and sexual behavior were recorded during a series of 10-minute tests every hour, eight times a day. This behavior was characterized by pursuits, mouth-to-mouth contacts, mouth-to-flank contacts, parallel swimming, erection of the dorsal fin and nest-digging with removal of sand.

Each group studied was composed of five males of similar size and body weight.

Blood Tests

The animals were anesthetized individually with a solution of phenoxyethanol at 2 $ml \cdot l^{-1}$ of water poured in a 10-l water tank. Blood was taken (1-1.5 ml) between 0900 and 1100 hours using a 2-ml heparinized syringe, and preserved in an ice box before centrifugation of all samples in a refrigerated centrifuge (4°C) for 10 minutes at 2,500 revolutions per minute.

Hormone Assay

Hormone concentrations were determined by radio-immunology. Testosterone and 11-KT were measured using the method developed by Fostier et al. (1982), giving a coefficient of variation of 8.12% (18.4 $ng \cdot ml^{-1}$, n=15) for testosterone and 6.37% (17.5 $ng \cdot ml^{-1}$, n=15) for 11-KT. The method described by Fostier et al. (1981) was used to measure 17.2P with a coefficient of variation of 5.74% (9.25 ng, n=15).

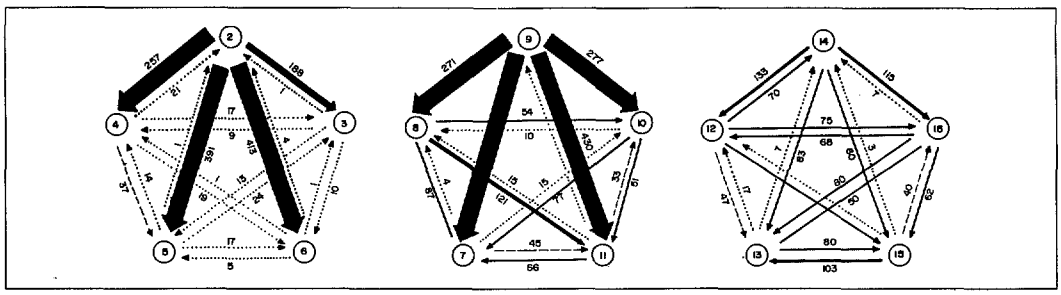

Fig. 1. Ethograms for three groups of five males of *Oreochromis niloticus*. The numbers in circles indicate individual fish. Numbers along arrows indicate the numbers of aggressive actions (see text).

Statistical Analysis

Paired comparison was done after an analysis of variance and Barlett's test. Data were compared using a *t*-test. Unless otherwise indicated, the level of significance was P=0.05.

Results

Ethograms of the Male Groups

The study of the three groups of five males clearly showed the presence of a dominant male in two groups, whereas social relationships in the third group were much more homogeneous although a dominant male could be recognized. Interactions between the fish also showed a second dominant animal. This was confirmed by the withdrawal of the dominant animal. Fig. 1 shows three diagrams summarizing all activities directed to the other fish within the groups: pursuits, mouth-to-flank and mouth-to-mouth contacts, and erection of the dorsal fin when approaching another fish.

Hormone Levels and Social Hierarchy in O. niloticus *and* O. mossambicus

The comparison of social hierarchies observed during pursuits showed clear differences between the two species of *Oreochromis*.

In *O. niloticus*, the social hierarchy is clearly established with a strictly dominant fish, with the greatest number of activities. Only the dominant male has access to the substrate and digs the nest. Its body color pattern is pearl white, with tinges of salmon pink, and the extremity of the fins is black. The other fish, males and females, are consigned to the upper corner of the aquarium. They are stressed and their color pattern ranges from greenish dark gray to black.

In contrast, *O. mossambicus* shows little signs of pursuit and there is no apparent social hierarchy in this species. Interactions between individuals are few and similar in all animals.

The comparison of hormone levels for the three steroids in these two species (Fig. 2) showed a very high level of testosterone in *O. niloticus* (52 $ng \cdot ml^{-1}$) in comparison with *O. mossambicus* (8 $ng \cdot ml^{-1}$). Levels of 11-KT and 17.20P were also higher in *O. niloticus* than in *O. mossambicus*.

Moreover, in *O. niloticus*, plasma levels of testosterone were very different, depending on whether the animals are dominant or dominated, whereas in *O. mossambicus*, no significant difference was observed. The same differences were observed, although somewhat reduced,

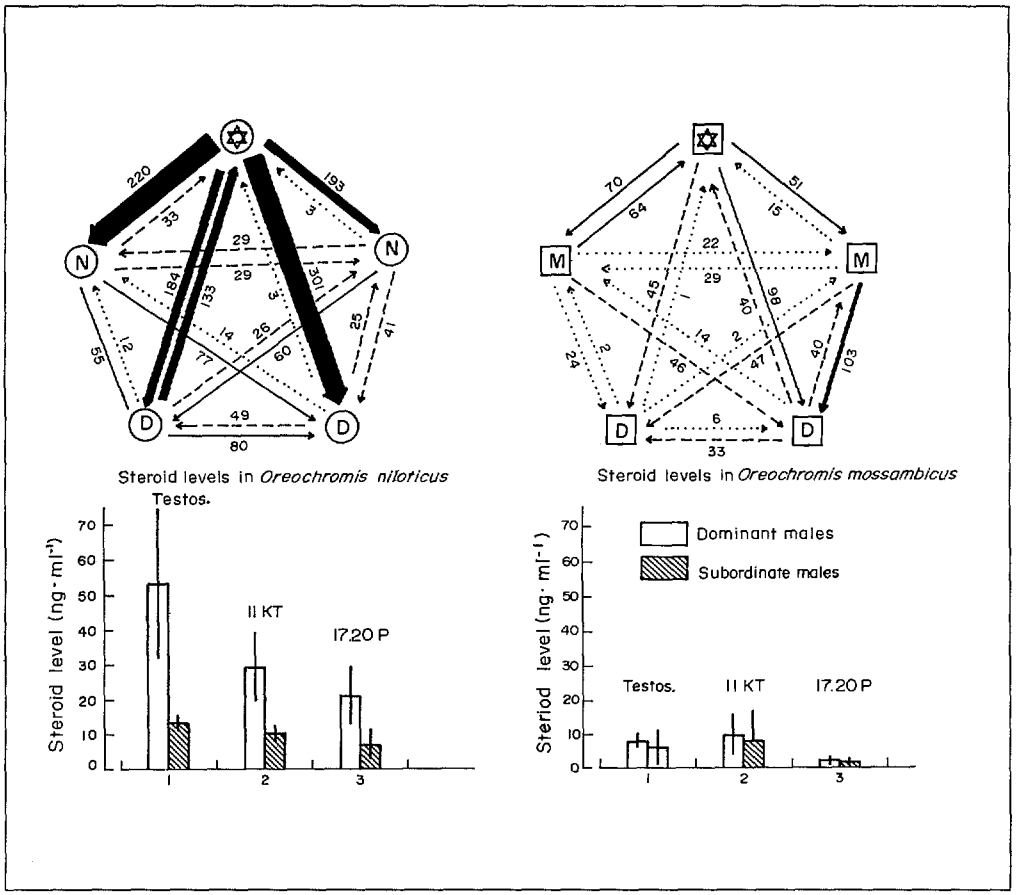

Fig. 2. Steroid hormone levels and social hierarchy in *Oreochromis niloticus* and *O. mossambicus*. On the ethograms, ✡ = dominant, N and M refer to the species used, and D = dominated (see text for discussion of comparisons and significance of differences).

in the levels of the two other steroids (11-KT and 17.20P).

Hormone Levels and Sound Emission in Males

In a collaborative study with U. Falter and Olivier Dufayt from the University of Louvain-la-Neuve (Belgium), we have for the first time established a correlation between sound emissions in males of these two species and other behavior patterns.

The male defending its territory chases the other fish (other males or females that are not ready to spawn) and emits a brief sound for less than half a second. The frequency of this sound emission is high: 400-700 Hz in *O. niloticus* and approximately 250 Hz in *O. mossambicus*.

These emissions which are characteristics in dominant males are, like in the defense of the territory, dependent on plasma testosterone. Only the dominant males can emit these sounds.

Hormone Levels and Acceptance of the Female by Territorial Males

In *O. niloticus*, the color taken by the pre-spawning females (which is identical to that of the dominant male) is related to the high level of aggressive

behavior in this species. This color pattern inhibits the aggressive behavior of the male that accepts the female of the same color. We have observed that some males with strong color patterns are sometimes accepted in the nest of the territorial male. The analysis of the female hormone levels (Fig. 3) showed that the change in color is correlated to an important testosterone level, particularly when the female is ready to spawn.

In *O. mossambicus*, although the same high testosterone level has been observed in the pre-spawning females, changes in the color pattern cannot be perceived by the human eye. The other steroids, circulating at relatively low levels, involve very few changes regardless of the species.

Effect of Steroid Implants on the Social Behavior of O. niloticus

In order to complete our study and to confirm the action of steroids on the social behavior of fish, we used implants with various quantities of several steroids in the fish and studied their effect by observing the corresponding ethogram. Three animals out of a group of five received steroid implants while the two others were used as control.

First, we studied the dynamics of release of pure testosterone from the implants at 0.5 mg·100 mg^{-1} implant 50 ml^{-1} saline solution. Between 6 and 36 hours, the quantity of hormone released was relatively stable for 1.75± 0.25 ng·ml^{-1}. The animals receiving testosterone implants showed increased aggressiveness, thereby rising in the social hierarchy (Fig. 4), the number of pursuits increasing four hours after the implantation of steroids. The nest-digging behavior did not vary significantly in the three days following the implantation (Fig. 5).

The controls' aggressive behavior decreased significantly in terms of pursuits and nest-digging activities. This reduced activity can be explained by increased activity and aggressiveness of the animals with implants living with them (Fig. 6).

There was also a significant increase in nest-digging activities in the animals with implants of 11-KT after implantation (Fig. 7). In contrast, the implantation of 17.20P did not cause any significant change in behavior aside from a general reduced level of activity (Fig. 8).

Conclusion

These results show the significant role of testosterone in the territorial behavior of males and in the species' level of aggressiveness. The sounds emitted by dominant males during pursuits against conspecifics, or to accompany and guide the female ready to spawn to the nest, are testosterone-dependent. This hormone is also responsible for the change in color pattern in the dominant male. This phenomenon is also observed in pre-spawning females which can thus enter the nest. The adoption of this male-specific color by the female has an inhibitory effect on the aggressive behavior of the territorial male.

In *O. mossambicus*, the aggressive behavior is practically nonexistent and endocrine levels are also much lower. The female does not need to take on the male-specific color pattern to be accepted by the male.

Ketotestosterone has a more obvious effect on nest-digging activity. Hence, the various hormones have each a specific action on social and reproductive behaviors. However, the steroid 17.20P does not seem to have any direct action on male behavior when implanted.

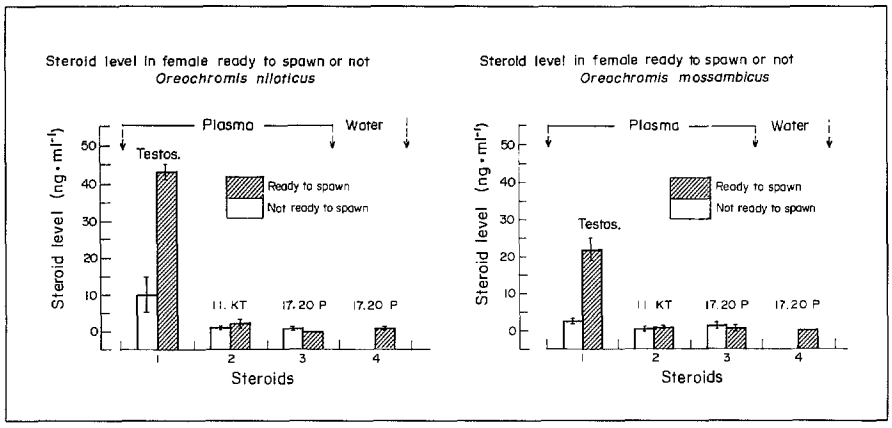

Fig. 3. Comparative study of steroid levels in female of *Oreochromis niloticus* and *O. mossambicus*.

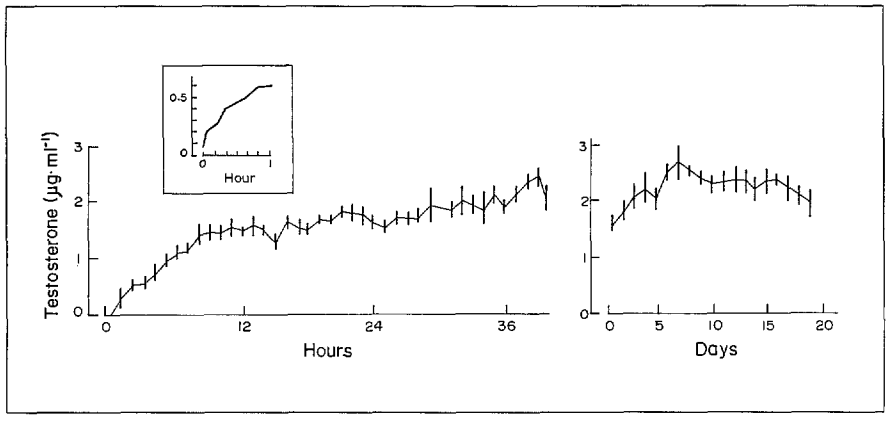

Fig. 4. Dynamics of in vitro release of testosterone implants (0.5 mg T·100 mg⁻¹ implant·50 ml⁻¹ saline solution).

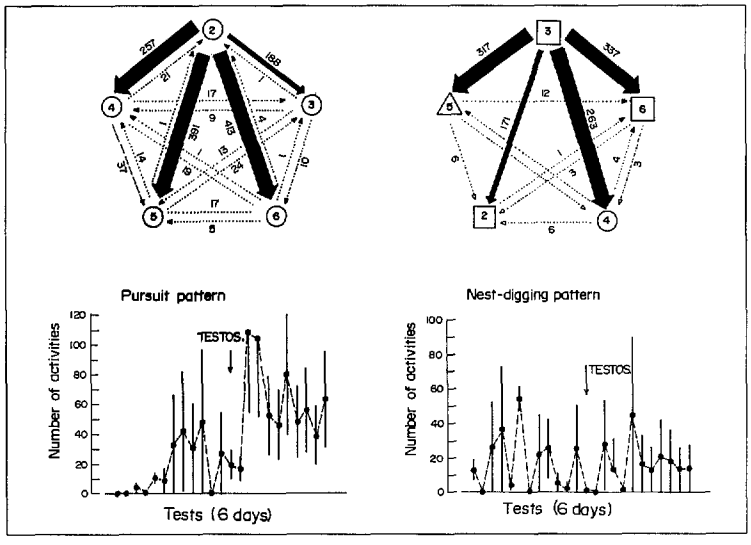

Fig. 5. Effect of implants (testosterone 0.25 mg) on the social behavior of *Oreochromis niloticus*.

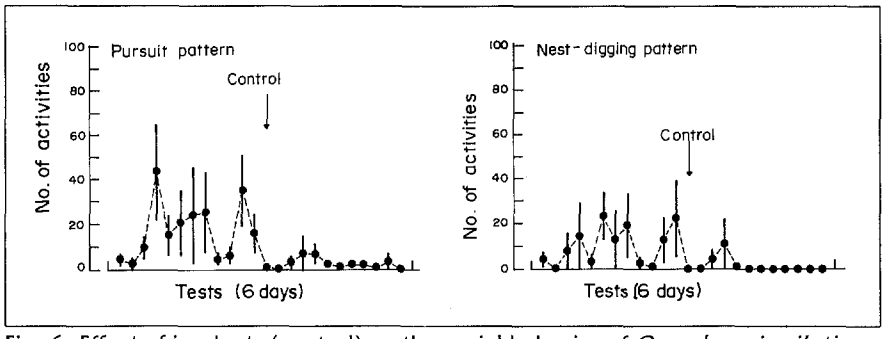

Fig. 6. Effect of implants (control) on the social behavior of *Oreochromis niloticus*.

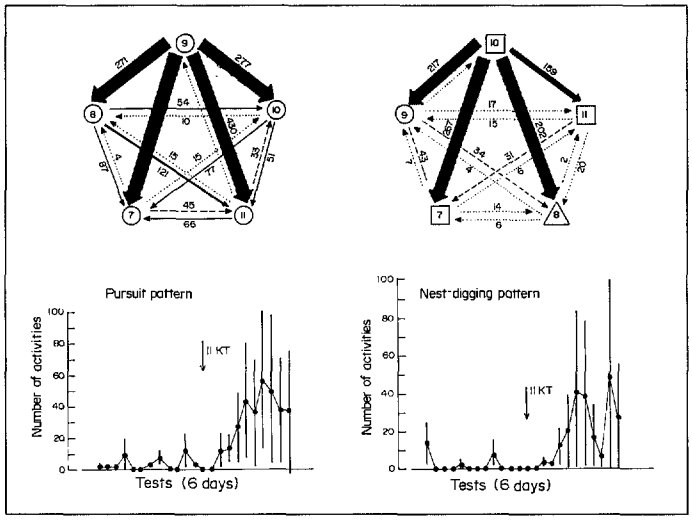

Fig. 7. Effect of implants (11-KT 0.25 mg) on the social behavior of *Oreochromis niloticus*.

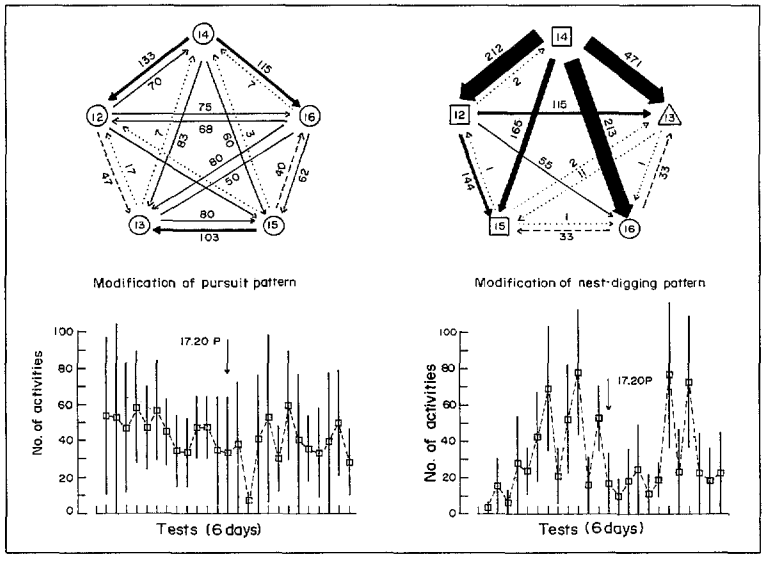

Fig. 8. Effect of implants (17.20P 0.25 mg) on the social behavior of *Oreochromis niloticus*.

Yet, studies by Stacey and Sorensen (1991) have shown that this steroid may act as a pheromone in goldfish (*Carassius auratus*). The different steroids thus appear to have a specific role in the different sequences of behavior involved in the defense of the territory and in the development of the reproductive behavior.

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Truss Morphometric Characterization of Eight Strains of Nile Tilapia (*Oreochromis niloticus*)

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VELASCO, R.R., M.J.R. PANTE, J.M. MACARANAS, C.C. JANAGAP and A.E. EKNATH. 1996. Truss morphometric characterization of eight strains of Nile tilapia (*Oreochromis niloticus*), p. 415-425. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

A study to characterize the eight strains of *Oreochromis niloticus* (Egypt, Ghana, Senegal, Kenya, Israel, Singapore, Taiwan and Thailand) was conducted using truss morphometrics. To remove the effects of size, a standard body size for each sex was established. Principal Component Analysis (PCA) was used to explore intraspecific variation by plotting the principal component (PC) scores. PCA was performed in two stages: (1) using all truss lengths (p=21) for each sex and both sexes combined; and (2) using only selected truss lengths for each sex and both sexes combined. Selection of trusses was by discriminant analysis (selection criterion, Wilk's Lambda=0.05). Analysis of variance (ANOVA) was performed on all PCs to detect strain differences. The PCs which revealed significant strain differences were used for XY plots. To determine error of strain classification, a discriminant analysis was performed using *a priori* strain groupings.

The pattern of variable loadings across the first eight PCs indicated significant differences between males and females of the eight strains. ANOVA on PC7 scores showed significant differences ($P<0.05$) and relatively clear separation of strains. For females, the plot of PC8 against PC7 shows slight separation of Kenya and Thailand strains from the cluster. A similar but weaker separation of strains was observed for males. Overall, the results indicated few truss morphometric differences among the eight strains.

Introduction

Morphometric and anatomical measurements have traditionally been used to differentiate populations in general and stocks of fish in particular (Ihssen et al. 1981). Morphometric characterization of strains within a given species, however, involves detection of subtle differences in shape, independent of size. The truss network method (truss morphometrics) is a powerful method for this (Strauss and Bookstein 1982; Winans 1984). Truss morphometrics overcome the biases of traditional measurements (standard and total lengths, body depth, etc.), namely, dense measurements in some areas of the body and a paucity elsewhere, and biased and uneven coverage of the body. The truss method involves measuring distances between homologous (or landmark) points along the body that form a regular pattern of contiguous

quadrilaterals or cells. These measurements, after appropriate data manipulation, are subjected to multivariate statistical analysis—discriminant analysis or Principal Component Analysis (PCA)—depending on *a priori* recognition of groups. A comprehensive account on the truss network method and its application to carps and tilapias can be found in Brzeski et al. (1989).

Previous studies on truss morphometric characterization of tilapias include those of Brzeski and Doyle (1988) and Pante et al. (1988). The former study reported successful discrimination of sexes long before discernible size differences occur. The study of Pante et al. (1988) suggested successful discrimination between tilapia species, and relatively weaker separation of strains and introgressed hybrids.

This paper describes morphometric characterization of eight diverse strains of Nile tilapia (*Oreochromis niloticus*), based on size and shape, using a total of 21 landmark points. This study builds on that of Pante et al. (1988) and describes several methodological modifications to discriminate strains. It was conducted under the auspices of a collaborative research project on the Genetic Improvement of Farmed Tilapias (GIFT) (Pullin et al. 1991; Eknath et al. 1993).

Materials and Methods

Random samples of 27-30 individuals from a pool of offsprings (Table 1) from single-pair matings (17-25 pairs each) of eight *O. niloticus* strains were used. The four Philippine commercial strains (termed Israel, Singapore, Taiwan and Thailand) and four African strains were collected from Egypt, Senegal, Ghana and Kenya (Eknath et al. 1993). The procedures for collection of truss morphometric data are described by Velasco et al. (1992). A truss network of 21 landmark points (Fig. 1) along the body was measured, using a Computer Aided Monoscopic Analysis (CAMA) program designed to record linear measurements of objects on a photographic image. To remove the effects of size, a standard measure

Table 1. The eight Nile tilapia (*Oreochromis niloticus*) strains and the numbers of males and females used for truss measurements.

Strain	Strain code	Male (M)	Female (F)	Total
African wild strains				
Egypt	E	14	15	29
Ghana	H	15	14	29
Senegal	S	15	15	30
Kenya	K	15	15	30
Subtotal		59	59	118
Philippine farmed strains				
Israel	I	15	15	30
Singapore	G	15	15	30
Taiwan	W	14	15	29
Thailand	T	15	12	27
Subtotal		59	57	116
Total		118	116	234

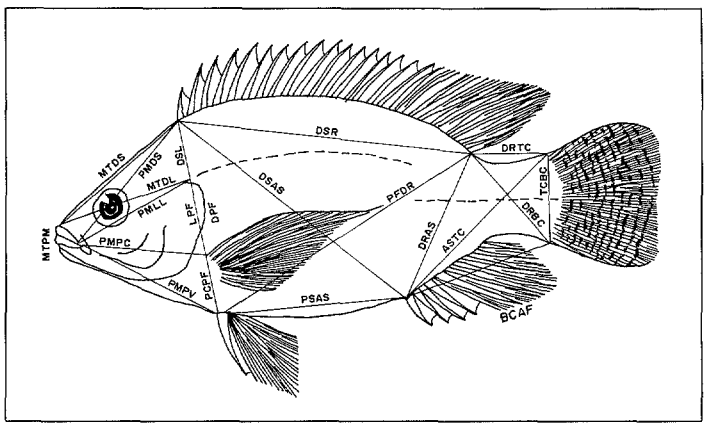

Fig. 1. Truss network of 21 landmark points on the body outline, measured during morphometric characterization of eight test strains of *Oreochromis niloticus*. For explanation of various homologous points, see Table 1.

A. Antero-dorsal measurements		*B. Length and body measurements*		*C. Caudal measurements*	
Mouth tip-1st dorsal-spine	MTDS	Dorsal spine-pelvic fin	DPF	Anal spine-top caudal peduncle	ASTC
Mouth tip-premaxilla	MTPM	Pelvic fin-anal spine	PSAS	Dorsal ray-bottom caudal peduncle	DRBC
Mouth tip-dorsal line	MTDL	Pelvic fin-dorsal ray	PFDR	Dorsal ray-top caudal peduncle	DRTC
Premaxilla-dorsal spine	PMDS	Dorsal spine-anal spine	DSAS	Top caudal-bottom caudal peduncle	TCBC
Premaxilla-lateral line	PMLL	Dorsal spine-dorsal ray	DSR	Bottom caudal peduncle-anal spine	BCAF
Premaxilla-pectoral fin	PMPC	Dorsal ray-anal spine	DRAS		
Premaxilla-pelvic fin	PMPV				
Dorsal spine-lateral line	DSL				
Lateral line-pectoral fin	LPF				
Pectoral fin-pelvic fin	PCPF				

of body size for each sex was estimated as the geometric mean of all truss lengths (Brzeski et al. 1989):

$$X = \sum_{p=1}^{21} \log_p / 21 \qquad \text{...1)}$$

where x_p = measured truss lengths (p=21) and X = standardized measure of body size.

The log truss lengths (x_p) were then regressed on the X values from equation (1), leading to the allometric growth function:

$$\hat{x}_p = ßX^k \qquad \text{...2)}$$

where ß and k are derived from the slope and intercept, respectively, of the linear regression. From this regression, the predicted measure of each truss length ($\hat{x}_p$) was obtained. The corrected truss lengths, expressed as the ratio of ($x_p/\hat{x}_p$) were used in further analysis.

Due to sexual dimorphism (Chervinski 1983) and in the absence of unequivocal techniques to correct for this, males and females were analyzed separately and the data were later pooled for comparison.

PCA was performed in two stages: (1) using all truss lengths (p=21) for each sex and both sexes combined; and (2) using only selected truss lengths for each sex and both sexes combined. Selection was by discriminant analysis (selection criterion, Wilk's Lambda=0.05). Analysis of variance (ANOVA) was performed on all PCs to detect strain differences. The PCs which revealed significant strain differences were used for XY plots.

To test the separation of strains further, discriminant analysis was performed using the *a priori* strain groups and their corresponding selected truss lengths (stage 2, above). The error rate of classification of individuals to their respective *a priori* strain groupings (i.e., their origins) was also estimated.

Results

PCA on All Truss Lengths

The variable loadings of the first eight PCs are presented in Table 2. The total cumulative variation explained by the first eight PCs was about 80%.

The pattern of variable loadings for PC1 was similar for males and females: head measurements loaded negatively whereas body and tail measurements loaded positively. A close similarity of coefficients for PC2 and PC3 for both sexes described body length and diagonals as well as head measurements. PC4 revealed contrasting differences between sexes, the tail region that loaded negatively in females loaded positively in males. PC5 showed similar patterns in the two sexes for the opercular measurements except that no significant coefficients were observed for the tail region in males. For PC6, more variability in head, body and tail measurements was observed in males than in females. PC7 and PC8 exhibited contrasting patterns of coefficients between the sexes. PCA on pooled data of males and females revealed PC patterns either similar to males or females.

ANOVA on PC7 scores showed significant differences ($P<0.05$; Table 2, last row) and relatively clear separation of strains (Duncan's multiple range test). For females, the plot of PC8 against PC7 (Fig. 2) showed slight separation of Kenya and Thailand strains from the cluster. A similar significant but weaker separation of strains was observed for males.

Overall, the differences between sexes were due to differences in head and tail measurements.

PCA on Selected Truss Lengths

The proportions of the total variation in truss lengths (cumulative R^2) explained by the first eight PCs, using the full dataset ($p=21$), were 81%, 80% and 78% for females, males and females+males, respectively (Table 2). These proportions of total variation increased to 97%, 95% and 90%, respectively, when only selected truss lengths were used (Table 3). PC1 revealed a contrasting pattern of coefficients between sexes. For all other PCs, the pattern was similar. PC7 and PC8 differentiated the head and tail regions. The plots of PC3 against PC2 (Figs. 3a and 3b) show slight separation of Egypt and Israel strains among females and Egypt and Senegal strains among males.

The selected truss lengths were further used in discriminant analysis to estimate the error rate of classification of individuals to their respective *a priori* strain groups. Results for males and females are presented in Tables 4 and 5. The strain classification error was high, ranging from 8 to 60% for males and 7 to 47% for females.

Discussion

Overall, the results indicate few significant morphological differences among the eight Nile tilapia strains. Note also the high classification error in discriminant analysis. Data reduction using stepwise discriminant analysis (i.e., by selective removal of redundant and highly correlated truss lengths) improved strain separation, but only marginally. For example, in the case of males, the PC1 using the full dataset ($p=21$) accounted for 17.8% of the total variation, whereas the PC1 of the reduced dataset accounted for 24.5% of the total variation. The plots of PCs (Fig. 3) from the reduced dataset also reveal only weaker separation of strains.

One of the reasons for the poor separation of stocks could be the number of samples used and the number of truss lengths measured. Misra and Ni (1983) suggested that using a large number of characters with limited samples can be inappropriate in discriminant analysis. Harris (1975, cited in Corti et al. 1988), however, suggested that

Table 2. PCA using all 21 truss measurements made on eight strains of Nile tilapia (*Oreochromis niloticus*): Patterns of coefficients for the first eight PCs for males, females and males+females. A '+' or '-' indicates a positive or negative coefficient which absolute value is greater than half the maximum coefficient for the relevant PC. Values for truss lengths which are below the cutoff point are left blank. For explanation of abbreviations, see Fig. 1.

	Females								Males								Both sexes							
Truss length	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Head:																								
MTDS	-						+	+		+				+	+		-		+				+	
MTPM						-	+	-		-	-							-	-			-		
MTDL	-	+					-	+	-			+				-	-	+				-		+
PMDS	-						+		-		+			+			-		+				+	-
PMLL	-						-		-								-	+				-		+
PMPC	-	+			+		-	-	-	+			+		-		-	+			+		-	
PMPV	-						-	-		+	+			-	-		-		+			+	-	-
DSL		-			+					-		-				+		-			+			
LPF			-		+	+							+	-		-			-		+	+		+
PCPF		-	+		-				+				-	-					+		-			
Body:																								
DPF		-					-				+	-		-				-	+	-				+
PSAS	+	+	-	+					+	+	-			+			+	+	-	-				
PFDR	+	+		+					+					+			+	+			+			
DSAS			+	+	-				+	+							+	+		-	-			
DSR	+	+	+				+	-	+	+							+	+						
DRAS	+		+		+				+	-	+						+		+		+			+
Tail:																								
ASTC	+		+	-	+				+	-	+	+					+		+	+				
DRBC				-	-							+			+	+				+	-	+		
DRTC	+			-	-						-	+		-	-				-	+	-			-
TCBC					-	+	+							+	+							+	+	
BCAF	+		+	-	+				+		+	+					+		+	+	+			
Eigen value	3.74	3.08	2.59	2.16	1.73	1.57	1.17	0.89	3.79	2.69	2.37	2.11	1.64	1.61	1.25	1.20	3.64	2.55	2.48	2.21	1.77	1.59	1.21	0.95
R^2 cumulative (%)	17.8	32.5	44.8	55.2	63.4	70.9	76.5	80.8	18.1	30.9	42.2	52.3	60.1	67.8	73.7	79.5	17.3	29.5	41.3	51.9	60.3	67.9	73.7	78.2
ANOVA R^2	15	15	6	16	7	9	23	22	7	34	12	20	22	10	20	6								

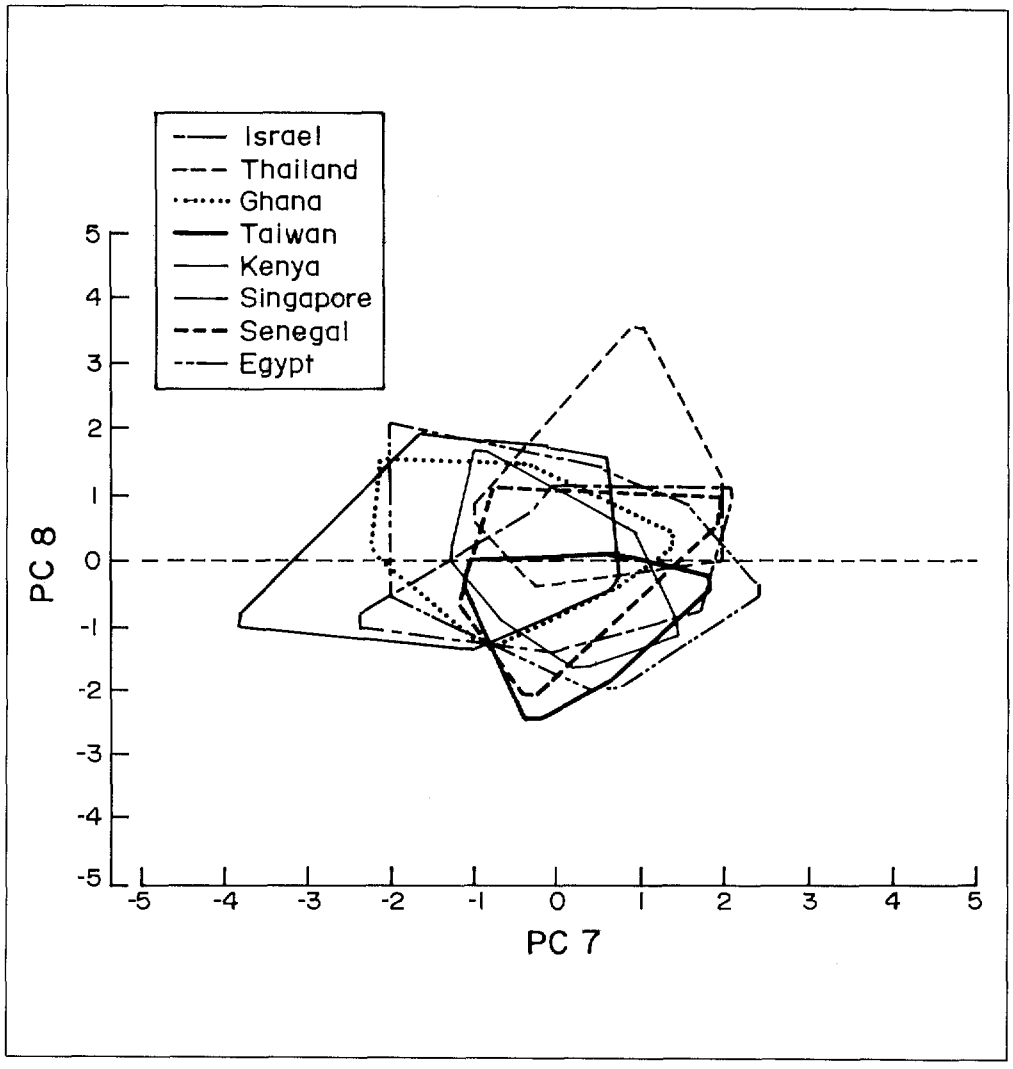

Fig. 2. Plot of PC8 versus PC7 using all truss lengths (p=21) of female for eight strains.

Table 3. PCA using only selected truss measurements made on eight strains of Nile tilapia (*Oreochromis niloticus*): Patterns of coefficients for the first eight PCs for males, females and males+females. A '+' or '-' indicates a positive or negative coefficient which absolute value is greater than half the maximum coefficient for the relevant PC. Values for truss lengths which are below the cutoff point are left blank. Shaded rows represent truss lengths that were dropped from the analysis. For explanation of abbreviations, see Fig. 1.

	Females								Males								Both sexes							
Truss length	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Head:																								
MTDS																								
MTPM																		-	-					
MTDL									+				+	-										
PMDS	-				-		+										+						-	+
PMLL									+								+					+		
PMPC	-		+		+	+	+		+	+						-	+						+	+
PMPV	-	+			+	-				+				+		+	+	+				-		
DSL																								
LPF											-			-										
PCPF																								
Body:																								
DPF																								
PSAS		-						+																
PFDR																								
DSAS									-	+	+			-										
DSR	+		+	+	+	-	+	-	-	+	+							+			+	+		
DRAS	+	+	+					+	-			-		+	+		-	+	-				+	
Tail:																								
ASTC	+	+				+						+					-	+	-		+		+	
DRBC	+		-		+		+			+			+		-				+			-	-	
DRTC										-		+			+				+	-			+	
TCBC			-	+			-													+			+	
BCAF																								
Eigen value	2.20	1.70	1.47	0.99	0.76	0.56	0.53	0.47	2.38	1.86	1.45	1.30	0.88	0.66	0.47	0.42	2.44	1.84	1.59	1.10	0.92	0.82	0.65	0.57
R^2 cumulative	24.5	43.4	59.8	70.9	79.4	85.6	91.5	96.8	23.9	42.6	57.2	70.2	79.1	85.8	90.5	94.8	22.2	39.0	53.5	63.5	71.9	79.3	85.3	90.5
ANOVA-strain R^2	13	20	14	13	12	14	20	23	8	33	27	18	18	11	19	18								

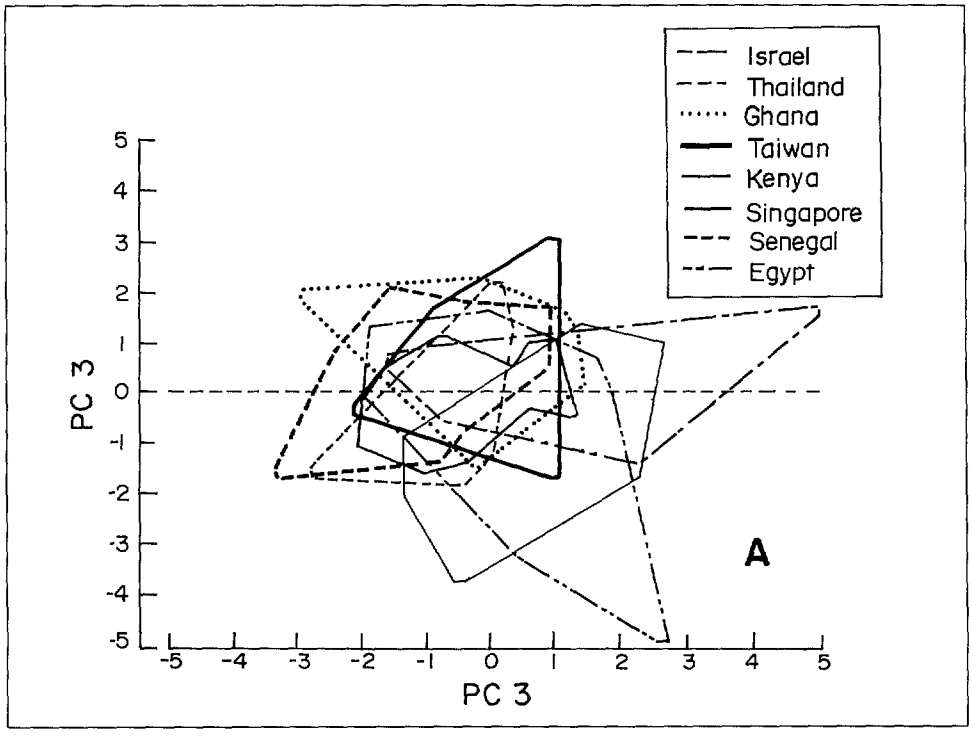

Fig. 3a. Plot of PC3 versus PC2 using selected truss lengths (m=9) of female for eight strains.

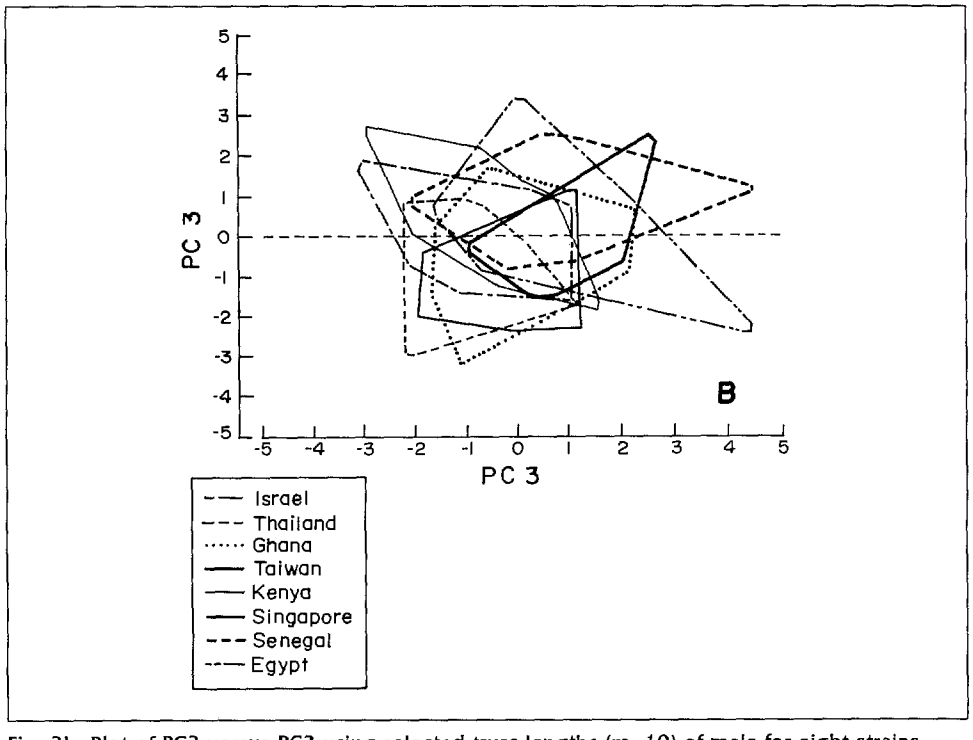

Fig. 3b. Plot of PC3 versus PC2 using selected truss lengths (m=10) of male for eight strains.

Table 4. Females: results of discriminant analysis. The values represent individual Nile tilapia (*Oreochromis niloticus*) as belonging to a given strain. The corresponding classification error is given in parentheses along the diagonal. For explanation of code letters, see Table 1.

Strains	E	G	H	I	K	S	T	W	All
E	6 (60.0)	2	0	1	2	0	1	3	15
G	1	8 (42.90)	0	1	1	0	1	2	14
H	1	0	13 (13.3)	0	0	1	0	0	15
I	0	2	0	11 (26.70)	1	1	0	0	15
K	0	0	0	1	12 (20.0)	0	2	0	15
S	0	0	0	0	0	12 (20.0)	2	1	15
T	0	0	0	0	0	1	11 (8.3)	0	12
W	3	2	0	0	0	0	10	1 (33.3)	15
All	11	14	13	14	16	15	17	16	116

if the number of individuals minus the number of variables measured is greater than 30, then the sample can be considered as large. According to this principle, the sample size adopted in this study is inadequate.

The size correction procedure followed in this study may have also masked strain differences to some extent. Our technique for size correction used a combination of regression coefficients and ratios. There have been serious arguments on the use of ratios because of the statistical and conceptual difficulties that they can pose. Ratios vary with the values of their denominators and

Table 5. Males: results of discriminant analysis. The values represent individual Nile tilapia (*Oreochromis niloticus*) as belonging to a given strain. The corresponding classification error is given in parentheses along the diagonal. For explanation of code letters, see Table 1.

Strains	E	G	H	I	K	S	T	W	All
E	11 (21.4)	0	0	0	1	0	0	2	14
G	1	10 (33.33)	1	1	0	0	1	1	15
H	1	3	8 (46.6)	1	1	0	0	1	15
I	0	0	1	11 (26.70)	0	2	1	0	15
K	1	0	2	0	9 (40.0)	2	0	1	15
S	1	0	1	2	0	9 (40.0)	0	2	15
T	0	0	0	0	1	0	14 (6.67)	0	15
W	1	0	0	1	1	1	1	9 (35.7)	14
All	16	13	13	16	13	14	17	16	118

numerators, and alter the factor structure of the covariance matrix (Thorpe 1976, cited in Corti et al. 1988). Regression coefficients, on the other hand, assume linearity between variables.

In a previous study, the same strains as in the present study were characterized by isozyme electrophoresis, and a dendogram of genetic distances between the strains was constructed (Eknath et al. 1991; Macaranas et al. 1995). The Asian farmed strains were found to be closely related to the wild strains collected from Egypt and Ghana. This is in agreement with the historical documentation records collected by Pullin and Capili (1988). The strain collected from Kenya showed the greatest genetic distance to the other strains, as expected from the recognition by Trewavas (1983) of the Kenya strain as a separate subspecies. As may be seen from Fig. 3, the genetic distances between the strains are not reflected in the morphometric separation of the strains. The strains showing a tendency to separate were Egypt and Israel (among females), and Egypt and Senegal (among males). The Kenya strain was almost completely overlapping with the other strains.

Strain characterization and their evaluation in applied breeding programs will continue through the GIFT project. The emphasis in the future will be on a combination of techniques to characterize strains: electrophoresis, truss morphometrics, and the use of hemoglobin and other blood parameters.

Acknowledgements

This work was undertaken as part of the GIFT project financed by the Asian Development Bank (RETA 5279) and the United Nations Development Program/Division for Global and Interregional Programs (INT/88/019). We are grateful to the GIFT project staff, and to Mr. Ruben Garcia and Ms. Belen O. Acosta for their assistance. The critical comments from Dr. Hans Bentsen (AKVAFORSK) are greatly acknowledged.

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Estimation of Additive and Nonadditive Genetic Parameters in the Growth of Fry of Three Strains of *Oreochromis* spp.

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YAPI-GNAORE, C.V. 1996. Estimation of additive and nonadditive genetic parameters in the growth of fry of three strains of *Oreochromis* spp., p. 426-432. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Heterosis, maternal and additive effects and general combining ability for fry growth during nursing (length and weight at 75 and 120 days post-fertilization) were evaluated in three strains of *Oreochromis* spp. (*O. niloticus* Bouake strain, *O. aureus* Israel strain and *O. aureus* Manzallah strain, Egypt) in a 3x3 diallel system. Mean heterosis for fry production (comparing all crosses within and among species and strains) was 11.2%.

Mean heterosis values were negative for all growth traits studied (-0.13 cm and -0.22 cm for length; -0.10 g and -0.31 g for weight). The absolute values for additive effects were very high in the Israel and Manzallah strains compared to the Bouake strain. The maternal effect in the Bouake strain was higher than in other strains. The general combining ability in the Bouake and Israel strains showed positive values, whereas these values were negative in the Manzallah strain.

Introduction

In farmed animals, the importance of heterosis has been recognized for years. This can be explained genetically by the dominance of favorable alleles; the overdominance or superiority of heterozygotes (intra-allelic interaction); or the epistasy—the interaction of alleles not belonging to the same locus (inter-allelic interaction). Determination of the basis of heterosis is important to maximize heterosis in crossbreeding programs. Estimation of genetic parameters in populations of hybrids and pure breeds can point to the most effective breeding methods.

In aquaculture, and particularly in cultured tilapias, numerous hybridization studies have been conducted (Wohlfarth and Hulata 1983). However, most were performed to produce monosex populations, for the male growth advantage, and to control early and prolific reproduction (Lovshin 1982; Wohlfarth and Hulata 1983). Growth performance was mostly overlooked, as an economically important trait of its own.

Inland fisheries and aquaculture in Côte d'Ivoire are dominated by *Oreochromis niloticus*. Several *Oreochromis* spp. and strains of *O. niloticus* have been introduced and stocked at the Institut des Savanes (IDESSA), Fish Culture Research Station since 1950 (Nugent 1988). These have been used in various studies including those of various production systems, growth comparison, adaptability to the environment, sex ratio in crossbreeding, etc. (IDESSA 1989). Almost none of the previous studies considered growth performance of crossbred individuals. In reorganizing the research activities in 1988, IDESSA launched a large program for the simultaneous comparison and characterization of various species and strains of *Oreochromis*, based on their production, morphometric traits and protein specificity.

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This study was undertaken under this program and its objective was to characterize hybrids among two strains of *O. aureus* and one strain of *O. niloticus*, with respect to additive and nonadditive genetic parameters of the length and weight of fry.

Materials and Methods

Experimental Material

One strain of *O. niloticus*, Bouake (NB) and two of *O. aureus*, Manzallah, Egypt (AM) and Israel (AI) were crossed in a 3x3 diallel system. The Bouake strain of *O. niloticus*, described by Nugent (1988) is the most commonly used for fish culture in Côte d'Ivoire. The strains of *O. aureus* were introduced at the IDESSA in the 1980s; the AI strain came from Israel via Tihange, Belgium in 1981 (Nugent 1988); and the MA strain, from Lake Manzallah, Egypt in 1988 (IDESSA 1989).

The study was conducted at the IDESSA station from April to October 1989. Breeding groups (three females and one male per group) were assembled in 200-l aquaria, with water temperature varying from 20 to 25°C. To reduce injuries on females, the premaxillae of males were cut before their introduction in the aquaria. All broodfish were identified using Floy tags.

These aquaria were observed during the day to identify mouthbrooding females. Once a female was mouthbrooding, the two other females and the male were transferred to storage tanks. After 11 (±2) days post-fertilization (PF), larvae were removed from the mouth of the female, counted and then transferred to mosquito-net cages placed in the aquaria for up to 20-23 days PF. Fry of approximately 23 days PF were transferred from the aquaria into 0.5x0.5x 0.75 m mosquito-net cages in cemented tanks up to 75 days PF, with an initial density of 300-500 fry per cage. They were later transferred to 1x1x1 m cages up to 120 days PF.

The fry were fed with a vitamin-enriched compound feed for salmonid fry, six times a day at a rate ranging from 6 to 20% of the biomass.

Fifty fry per spawning (unless lower numbers prevented it) were weighed (g) and measured (cm total length) at 75 and 120 days PF.

Statistical methods

The statistical models applied to the data collected were those described by Becker (1984) and Bulmer (1985). Two types of analysis were conducted: one on the mean values obtained for each crossbreeding type (Analysis I), the other on the individual data obtained for each fry measured (Analysis II).

ANALYSIS I, MODEL 1

$$E(Y_{ij}) = \mu + G_i + G_j + m_j;\ i \neq j$$

where Y_{ij}, μ, G_i (G_j) and m_j represent the mean value observed in the ij-th crossbreeding type; the general mean value; the general combining ability of the i-th strain (j-th strain); and the maternal effect, respectively. Maternal effect was considered instead of reciprocal effect because there was only one spawning in some crosses and the presence of maternal effect is the most likely biological reason underlying the presence of reciprocal effect.

ANALYSIS I, MODEL 2

$$E(Y_{ii}) = \mu + a_i$$
$$E(Y_{ij}) = \mu + \bar{h} + (\tfrac{1}{2} a_i + h_i) + (\tfrac{1}{2} a_j + h_j);\ i \neq j$$

where $\bar{h}$, h_i (h_j) and a_i (a_j) represent the average heterosis among all strains in all crosses; the heterosis of the i-th (j-th) strain and the additive genetic effect of the i-th

(j-th) strain, respectively. All estimates are measured as deviations from the overall mean.

ANALYSIS II

$$Y_{ijkl} = \mu + c_i + M_{ij} + F_{ijk} + e_{ijkl}$$

where Y_{ijkl}, c_i, M_{ij}, F_{ijk} and e_{ijkl} represent the value observed of the l-th fry of the ijk subclass; the effect of the i-th genetic type; the effect of the j male in the i crossbreeding type; the effect of the female k crossed with the j male in the i crossbreeding type; and the residual error, respectively. This error was used to test the effects of the models of Analysis I. Statistical analysis was done using the least squares method (Harvey 1988), F- and Bonferonni t-tests (Bailey 1977; Gill 1978).

Results

In total, 16 spawnings took place between 12 males and 14 females. The distribution of spawnings for each crossbreeding type is presented in Table 1. In general, crosses between species and between different strains with *O. aureus* produced more than crosses with strains of the same species. However, with so few data, the heterosis value in Table 1 should be treated with caution.

Table 2 shows that the general combining ability had a very significant effect on the traits at 75 days, and less effect ($P<0.01$) at 120 days. The additive effect was high ($P<0.01$) for length at 75 days. The other factors had no significant effects on the length and weight of fry. Of the two random variables, only the dam within sire variable for each genetic type had a significant effect on length at 120 days and weight at both ages.

Genetic parameter estimates are presented in Table 3. For all traits studied, average heterosis estimates were negative. This indicates that the "within" strain fry performed better that the "among" strain fry. The differences were 0.13 and 0.23 cm for total length, and 0.10 and 0.31 g for weight at 75 and 120 days, respectively. Among strains, crosses involving NB and AI contributed positively to the overall performance of the fry. On the other hand, the total length and weight mean values of the fry from crosses involving the AM strain were lower than the overall mean value.

The additive genetic effects among the three strains (Table 3) were significantly different ($P<0.01$). The AI strain showed the highest additive genetic estimate, followed by the NB and AM strains. Significant differences of additive genetic effect were observed only for total length at 75 days.

A similar ranking was observed for general combining ability, with significant difference among strains for both total length and weight. The use of the AI strain increased the length and the weight at 75 days by 2.20 cm and 1.17 g, respectively, compared to the AM strain. The same applied to the NB strain (0.70 cm and 0.20 g). The general combining ability of the strains was less pronounced at 120 days (Table 3). NB and AI strains did not differ with respect to length. For weight, the contribution of the NB strain compared to the AM strain was twice as high as that of the AI strain (1.29 g against 1.51 g).

Table 4 shows the mean values of the least squares for each trait by genetic type. The fry in AI had a higher performance, followed by the fry in NB then by the fry in AM. But at 120 days, there was no difference between NB and AI. Fry from NB♀xAM♂ and AI♀xNB♂ crosses had the highest weights and lengths. Fry from AI♀xAM♂ crosses, as well as those from AM♀xNB♂ crosses, had very poor growth.

It is important to emphasize that the datasets here were very limited and should not be used for firm conclusions. The intention

Table 1. Reproduction and production of fry among different species and strains of *Oreochromis* spp.: AI = *O. aureus* (Israel); AM = *O. aureus* (Manzallah); and NB = *O. niloticus* (Bouake) for each cross.

Cross[a]	Number of spawnings	Mean weight (g) of broodfish		Total number of fry	Number of fry/ weight of female
		Male	Female		
Within species or strains					
NBxNB	5	202.3	137.1	3,122	4.55
AMxAM	1	133.6	126.0	1,082	8.59
AIxAI	1	219.0	120.0	644	5.37
Among species or strains					
NBxAM	3	123.6	83.9	1,365	5.42
AMxNB	2	172.0	126.0	1,148	7.98[b]
NBxAI	1	277.0	187.0	1,050	5.61
AIxNB	1	179.0	121.0	375	3.09
AMxAI	1	183.0	113.0	1,078	9.54
AIxAM	1	133.6	100.0	977	9.77
Mean values					
Within species or strains	7	185.0	127.7	693[c]	6.17
Among species or strains	9	178.0	121.8	731	6.90
Heterosis %	-	-	-	-	11.2

[a]The female parental strain is mentioned first.
[b]The second spawning was not considered; the female rejected more than half of her eggs.
[c]Number of fry per spawning.

Table 2. Analysis of variance for weights and lengths at 75 and 120 (d) days of *Oreochromis* fry obtained from the crosses (in Table 1).

Source of variation	Degree of freedom	Mean squares of deviations			
		Total length (cm)		Weight (g)	
		75 d	120 d	75 d	120 d
Average heterosis	1	0.032	0.091	0.021	0.192
Strain heterosis	2	0.305	0.058	0.101	0.146
Additive effect	2	1.746*	1.085	0.673	1.744
Maternal effect	2	1.488	1.737	0.783	4.259
General combining ability	2	6.549**	2.627*	2.446**	3.863
Genotype	8	26.277	32.125	18.464	85.161
Sire within genotype	4	46.788	154.164	34.972	404.074
Dam within sire/genotype	2	1.302	8.611**	6.031**	96.504**
Error	(a)	0.370	0.541	0.292	1.549

*P<0.01; **P<0.001.
(a) Degree of freedom = 438 and 571 for measures at 75 and 120 days, respectively.

Table 3. Values estimated using the least squares method for genetic parameters of growth traits in hybrid fry of *Oreochromis* spp. (see Table 1).

Parameters		Total length (cm)		Weight (g)	
		75 d	120 d	75 d	120 d
General mean values					
Within species and strains		3.73	5.09	1.08	2.51
Among species and strains		3.60	4.87	0.98	2.20
Average heterosis ($\bar{h}$)		-0.13	-0.22	-0.10	-0.31
Strain heterosis (h/h_i)					
O. niloticus	NB	0.23	0.14	0.24	0.22
O. aureus	AM	-0.55	-0.24	-0.30	0.16
O. aureus	AI	0.32	0.10	0.06	-0.38
Additive effect (a)					
O. niloticus	NB	0.08[b]	0.37	-0.03	0.94
O. aureus	AM	-1.36[c]	-1.18	-0.80	-1.51
O. aureus	AI	1.28[a]	0.81	0.83	0.57
General combining ability (G)					
O. niloticus	NB	0.27[b]	0.33[a]	0.23[b]	0.69
O. aureus	AM	-1.23[c]	-0.83[b]	-0.70[c]	-0.60
O. aureus	AI	0.97[a]	0.50[a]	0.47[a]	-0.09
Maternal effect (m)					
O. niloticus	NB	0.25	0.23	0.21	0.58
O. aureus	AM	-0.26	-0.10	-0.31	-0.53
O. aureus	AI	-0.01	-0.13	0.10	-0.05

Values with different letters in a single column are significantly different ($P<0.001$).

Table 4. Mean values estimated using the least squares method for each hybrid cross among different strains of *Oreochromis* spp.: AI = *O. aureus* (Israel); AM = *O. aureus* (Manzallah); and NB = *O. niloticus* (Bouake).

Cross[a]	Total length (cm)		Weight (g)		Gain ($g \cdot day^{-1}$)[b]
	75 d	120 d	75 d	120 d	
General mean values	3.77	5.01	1.07	2.28	0.027
Within species and strains					
NBxNB	3.89	5.38	1.13	3.22	0.047
AMxAM	2.51	4.05	0.29	1.08	0.018
AIxAI	5.06	5.96	1.94	3.12	0.026
Among species and strains					
NBxAM	4.68	5.85	2.05	4.21	0.048
AMxNB	2.71	4.14	0.34	1.18	0.019
NBxAI	3.89	4.64	1.13	1.48	0.008
AIxNB	4.86	5.71	1.82	2.67	0.019
AMxAI	3.51	5.41	0.58	2.49	0.042
AIxAM	2.83	3.97	0.38	1.06	0.015
Coef. Var. %	15.9	14.5	49.0	48.2	-

[a]The female parental strain is mentioned first.
[b]Differences between the mean values at 75 and 120 days PF.

was to demonstrate the approach and work in progress. For example, although Analysis I above is based on the mean values of each cross, some crosses produced one single spawning only. In such cases, the error term from the analysis of individual observations, expressed as the variance of means, was used to test the significance of the effects in Analysis I.

Discussion

Results of this study give negative heterosis values. However, positive heterosis values for growth have been reported in *Oreochromis* spp. (Jayaprakas et al. 1988; Tave 1988). The study of Jayaprakas et al. (1988) showed that at 60 days, the Egypt strain of *O. niloticus* had a higher growth ($P<0.05$) than the strain of Côte d'Ivoire. The heterosis values for F_1 Côte d'Ivoire x Egypt (CE) and its reciprocal (EC) were 9.5 and 28.3% for length and weight, respectively. For the growth of F_1 hybrids of three *O. niloticus* strains (Côte d'Ivoire, Ghana and Egypt) at 47 days, Tave (1988) found heterosis values between 3.0 and 11.8%.

In the present study, negligible or negative values of heterosis for hybrids of strains of different origins could be attributed to epistasy (Falconer 1981). It is also important to note that in the above studies, the different strains are of the same species whereas in our study the strains are from two species of *Oreochromis*.

The estimated parameters in the AM strain all showed negative values. Similarly, the fry from the AM intra-strain crosses had the lowest growth among the three strains. This poor performance is possibly due to the great number of fry ($8.59 \cdot g^{-1}$) produced by weight of female by this strain. This may imply smaller eggs and smaller fry.

The estimated values of the additive (a) genetic effect in the AI strain were very high compared to those of the nonadditive (G, h and m) parameters. In view of its positive values of G, h and m, the NB strain seems to show an interesting potential for hybridization. It appears to be a good strain for dams. This study has shown that positive additive genetic effects were present in the NB and AI strains. Moreover, maternal effect was important in the NB strain for the growth traits studied (total length and weight). This may offer opportunities for selective breeding and hybridization to improve growth of fry of *Oreochromis* spp. by combining selection and crossbreeding. However, the limited data used in this analysis does not allow for any conclusive results. Further studies would be required.

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SESSION IV. BIOLOGY AND ECOLOGY

A New Method for Comparing the Growth Performance of Fishes, Applied to Wild and Farmed Tilapias[a]

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PAULY, D., J. MOREAU and F. GAYANILO, JR. 1996. A new method for comparing the growth performance of fishes, applied to wild and farmed tilapias, p. 433-441. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

This paper documents a new, computer-based method for identifying via "auximetric grids" (i.e., double log plots of the von Bertalanffy parameters K and W_∞ as ordinates and abscissa, respectively) the range of growth performances likely to occur in a given taxon, of which several populations have been studied in terms of growth. This method is applied to wild tilapia, notably *Oreochromis niloticus* which is defined in terms of the growth parameter "space" it occupies and which is contrasted with that of other species of tilapias and other fishes.

Introduction

Organismic growth, including growth of fishes (e.g., tilapias) is a process through which size (weight or length) changes with time. Thus, any attempt to depict or compare growth must deal with both dimensions. However, comparing growth curves, which link size and time, is not straightforward. Indeed, depending on one's definition of "slow" or "fast" growth, one can get into serious contradictions when growth curves cross one another. Thus, Kinne (1960) writes that "the difference in growth rate established in young fish does not persist throughout life. Initially slow-growing fishes

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may surpass initially fast-growing fishes, and finally reach a greater length-at-age".

Contradiction of this kind, combined with a general preference, by of aquaculturists, for expressions of growth other than full growth curves, e.g., of the von Bertalanffy type (Hopkins 1992) may have prevented intra- and interspecific comparisons of growth in tilapias, and hence appropriate selection of species and/or strains with high growth potential among a wide range of habitats (Pullin 1988).

To address this issue, we combine here two measures of growth performance developed by Pauly (1979) and now referred to as ϕ and ϕ' (Munro and Pauly 1983; Pauly and Munro 1984) which have been widely applied to tilapias (Moreau et al. 1986; Pauly et al. 1988a, 1988b) with the auximetric grid concept of Pauly (1979, 1980). Both rely on the von Bertalanffy growth function (Bertalanffy 1938) or VBGF, of the form,

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right) \qquad \text{...1)}$$

for length and

$$W_t = W_\infty\left(1 - e^{-K(t-t_0)}\right)^b \qquad \text{...2)}$$

for weight, where L_t and W_t are the predicted mean sizes at age t, L_∞ and W_∞ the asymptotic sizes, i.e., the mean size that the fish from a given population would reach if they were to grow indefinitely, K the parameter expressing the curvature of the curve, t_o the "age" at which size = 0, and b the exponent of a length-weight relationship of the form $W = aL^b$ (with b usually close to 3, i.e., proportional to "length cubed", or volume). Given appropriate parameter values, equations (1) and (2) can fit a wide variety of sets of size-at-age data; numerous methods exist to estimate these parameters (Gulland 1983; Pauly 1984).

Important in the context of this contribution is that none of the VBGF parameters has by itself the dimensions of growth (i.e., length *vs* time or weight *vs* time); L_∞ and W_∞ represent size alone, and K and t_o have the dimensions time^{-1} and time, respectively. However, various combinations of these parameters, e.g., $L_\infty \cdot K$ have a suitable dimension (here: l/t), i.e., that of a growth rate (Gallucci and Quinn 1979). Although put on a logarithmic basis, the indices of growth performance:

$$\phi' = \log_{10}K + 2\log_{10}L_\infty \qquad \text{...3)}$$

and

$$\phi = \log_{10}K + (2/3)\log_{10}W_\infty \qquad \text{...4)}$$

also have the correct dimension of a growth rate, and are now widely used to compare the growth performance of different fishes and invertebrates, owing to their being normally (and narrowly) distributed for different populations of the same species (Moreau et al. 1986; Vakily 1992). The latter feature also allows estimation of K from L_∞ or W_∞ when their (mean) ϕ' or ϕ is known from a (number of) population(s).

The slopes of 2 and 2/3 in equations (3) and (4), respectively, which make these indices perform as they do, were estimated by Pauly (1979) from a large dataset including numerous tilapia populations documented in Pauly (1978, 1979). Equation (3) implies that plots of $\log_{10}K$ *vs* $\log_{10}L_\infty$ will have, on average, a slope of 2. Correspondingly, equation (4) implies that plots of $\log_{10}K$ *vs* $\log_{10}W_\infty$ will have, on average, a slope of 2/3.

The auximetric (from the Greek for "growth" and "measure") grid is a double logarithmic plot of the parameter K of the VBGF *vs* asymptotic size (L_∞ or W_∞). Herein, a population with a given set of growth parameters (L_∞, K or W_∞, K) is represented by a single point, and different populations of the same species will tend to form a cluster of points. Since equations (3) and (4) imply that these clusters can be fitted with

regression lines of known slope, the clustering also implies that ellipses can be superimposed on the clusters of points, with long axes parallel to both slopes in (3) or (4), intercepts equal to ϕ' or ϕ, and surface areas related to the variance of the datasets that are represented.

Thus ellipses, with circumference containing the 95% confidence area (S) of a cluster of L_∞, K (or W_∞, K) values, can be readily estimated. Moreover, the value of S expressing the L_∞, K (or W_∞, K) space occupied by a given species can, beyond a critical number of data points ($n \approx 5$-6), be readily made independent of n. A simple resampling method which allows this is presented in Fig. 1.

The auximetric grid approach thus allows quantifying the similarity of growth patterns of fishes through:

- the overlap of ellipses (implemented here through an index of overlap between pairs of ellipses, see below)
- the distance between the center of a larger number of ellipses, and the subsequent construction of a dendrogram.

These approaches were implemented in the form of a computer program called AUXIM, which has major routines, documented in Figs. 1-4, and in the text below.

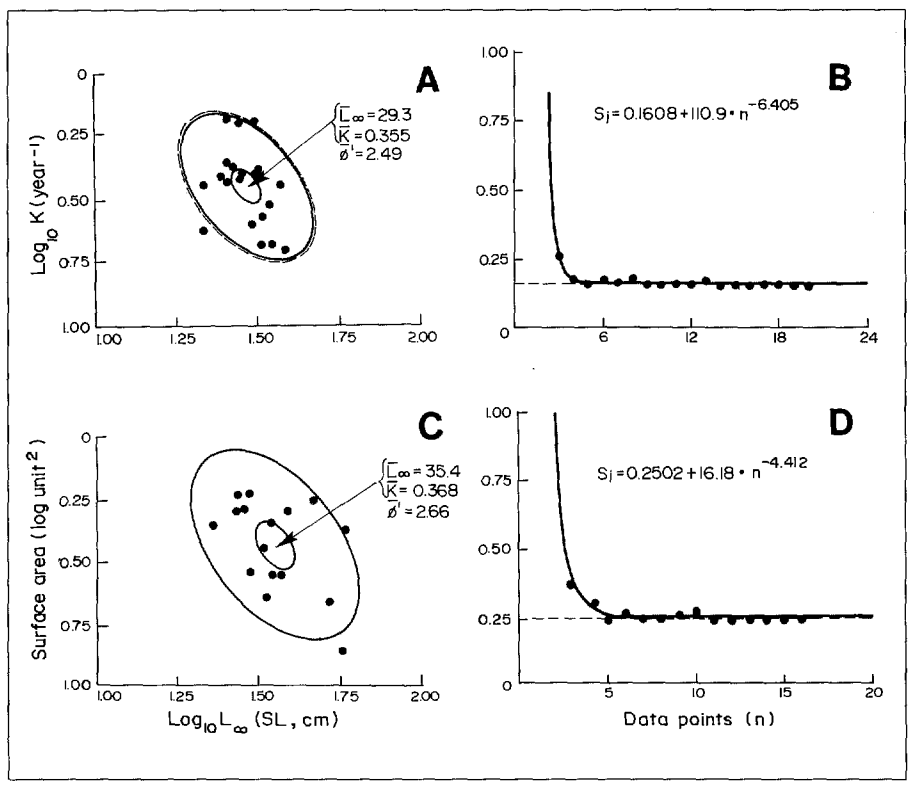

Fig. 1. Confidence ellipses 95% bounding the "growth space" occupied by two species of tilapia, and the plot used to estimates these ellipses:
A = Ellipse for *Oreochromis mossambicus*, based on data in Table 1;
B = Plot based on resampling the data in Table 1 and allowing estimation of S_∞ in *O. mossambicus*; the points shown are n-2 (see also Appendix 2);
C = Ellipse for *O. niloticus*, based on data in Table 2;
D = Same as in B, but for *O. niloticus* and data in Table 2.
Note that for *O. mossambicus*, S_{20} (dotted) is very close to S_∞, while for *O. niloticus*, S_{16} overlaps completely with S_∞.

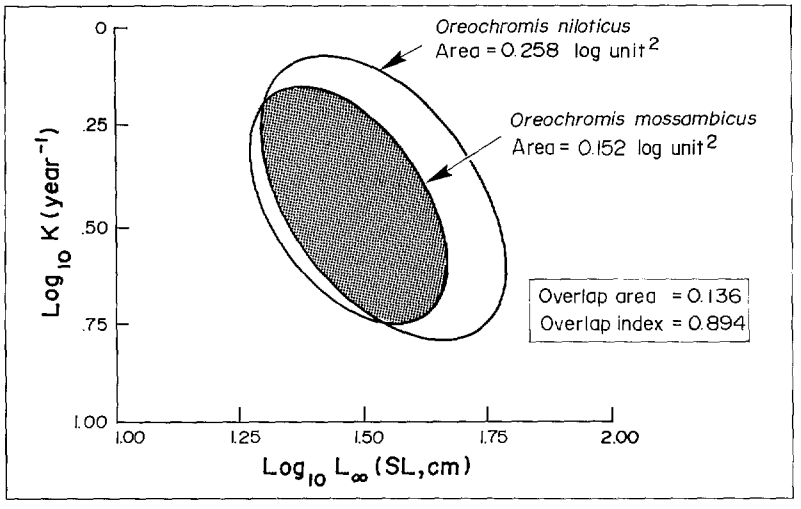

Fig. 2. Overlap between asymptotic ellipses in Figs. 1A and 1C for *Oreochromis mossambicus* and *O. niloticus*. Note high overlap index of 0.9, with non-overlap areas representing high ϕ' values for *O. niloticus* and low ϕ' values for *O. mossambicus*.

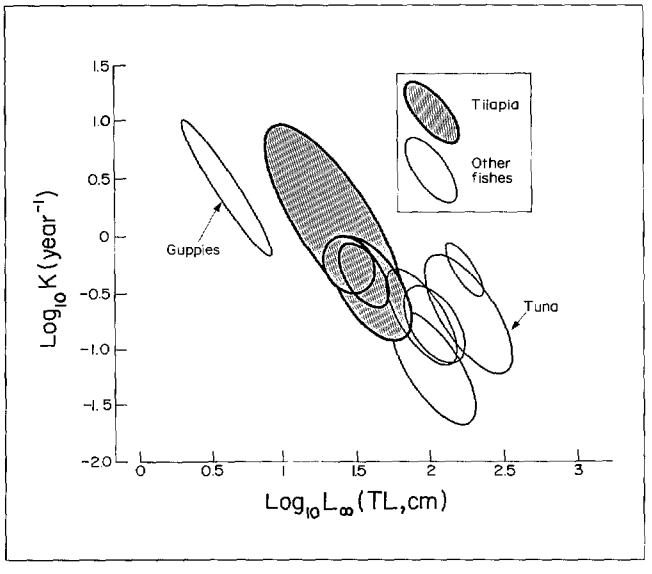

Fig. 3. Auximetric grid based on the file in Table 3, i.e., illustrating the location and the areas in L_∞, K space occupied by 6 species of tilapia, and 6 other species of widely different fishes. Ellipses wholely contained within others are not shown.

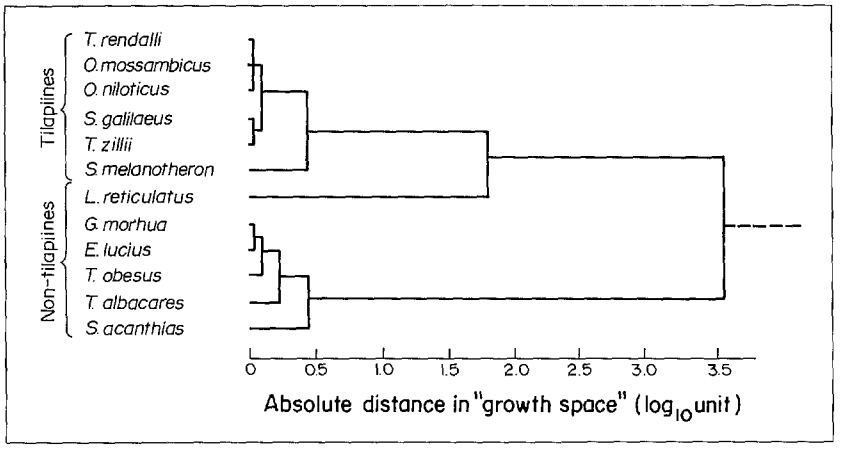

Fig. 4. Dendrogram of the similarities of L_∞ and K in the 12 species of fish in Tables 1-3 and Fig. 3. Note that the *Tilapia* spp., *Oreochromis* spp. and *Sarotherodon galilaeus* form a rather tight cluster, to which *S. melanotheron* can be added (at a lesser level of similarity), to form a well defined cluster for the tilapiines.

Applications to Tilapias

Fig. 1, illustrating the first routine of AUXIM, is a representation, using an auximetric grid, of the data in Tables 1 and 2. As might be seen on Figs. 1A and 1C, ellipses can be readily fitted to the data point, showing their 95% confidence interval.

Figs. 1B and 1D illustrate our method for estimating the asymptotic surface area (S_∞) for the dataset illustrated in Figs. 1A and 1C, respectively. Herein, each point (i) represents the mean of 20 estimates of the surface area (S_i) for a randomly selected set of L_∞, K or W_∞, K data pairs, with i=3, 4... n; the S_i values are then fitted with an equation of the form

$$S_i = S_\infty + a \cdot n^k \qquad ...5)$$

As might be seen, S_i decreases with n; this is because the values of the t-statistic associated with the degrees of freedom (i-1) decline faster than the S_i increase with n (see also Appendix 1).

Figs. 1A and 1C also include the asymptotic ellipses for the data in Tables 1 and 2, respectively. As might be seen, these asymptotic ellipses are close (Fig. 1A) or equal (Fig. 1C) to the ellipses obtained without resampling, due to the n values in these two cases being >>6.

Fig. 2 shows the overlap between two ellipses and hence between two sets of growth parameters (from Tables 1 and 2). In this example, the overlap index is 0.89, a high value indicating a strong similarity in the growth performance of the two species compared here [this index can range from 1 when the smallest of two ellipses is contained completely within the larger one, to 0 when the two ellipses are completely separated].

Figs. 3 and 4 illustrate the third major routine of AUXIM, which enables comparisons of up to 20 files containing up to 200 pairs of estimates of L_∞ K or W_∞K each.

Fig. 3 shows the auximetric grid resulting from the files in Table 3, which enable comparison of various species of tilapias with various "outgroups" included here for illustrative purposes.

As might be seen, the ellipses representing different tilapiine species show strong overlap, indicating similarity in their growth performance. Moreover, their growth performance appears to be well separated from that of other fishes in terms of "the growth parameter space" they occupy on the auximetric grid. The same features are illustrated by the dendrogram of Figure 4, where the tilapiines form a homogeneous group, well separated from other fishes, at least with regard to the species used here for comparison.

Discussion

Obviously, the new methodology presented here will need to be further developed, and applied to numerous other species, before its potential usefulness is fully realized.

Here, we discuss only our two overlapping tilapia species, *Oreochromis niloticus* and *O. mossambicus*. As might be seen on Fig. 2, the overlap between them occurs in conjunction with medium and low values of ϕ' in *O. niloticus*. On the other hand, there is no overlap for the highest values of ϕ' which express the growth of *O. niloticus* in large (natural and artificial) lakes of Africa (Moreau et al. 1986). This then, would define in terms of growth that part of the niche of *O. niloticus* which it does not share with *O. mossambicus*.

Similar consideration may apply when considering several species as illustrated in Fig. 3, and by the dendrogram in Fig. 4 which allows ecological interpretations of differences in species-specific growth performance. For instance, *Tilapia rendalli*, *O. mossambicus* and *O. niloticus* form a cluster in Fig. 4. This is matched in the field by their very similar, predominantly

Table 1. Example of a file such as required by AUXIM: von Bertalanffy growth parameters for 20 populations of *Oreochromis mossambicus* (adapted from Moreau et al. 1986, who give details on sources, locations, methods of parameter estimation, etc.); note that AUXIM requires L_∞ *or* W_∞, not both.

No.	W_∞(g)	L_∞(SL, cm)	K(year^{-1})
1	408	21.6	0.36
2	410	21.7	0.24
3	3,082	38.7	0.20
4	1,725	30.7	0.24
5	655	24.5	0.39
6	1,729	35.1	0.21
7	1,671	34.7	0.30
8	876	28.0	0.38
9	1,450	33.0	0.26
10	897	28.2	0.63
11	1,326	32.0	0.21
12	1,259	32.0	0.41
13	1,082	28.6	0.40
14	770	26.0	0.68
15	719	25.8	0.44
16	737	27.0	0.42
17	675	25.8	0.37
18	1,277	31.2	0.64
19	1,979	37.6	0.36
20	1,132	31.3	0.39

Table 2. Von Bertalanffy growth parameters for 16 populations of *Oreochromis niloticus* (adapted from Moreau et al. 1986, who give details on sources, locations, methods of parameter estimation, etc.).

No.	W_∞(g)	L_∞(SL, cm)	K(year^{-1})
1	1,214	33.4	0.23
2	387	22.8	0.44
3	851	28.6	0.51
4	745	27.2	0.50
5	7,059	57.2	0.14
6	1,579	34.7	0.28
7	1,061	29.4	0.59
8	1,635	34.4	0.45
9	780	27.1	0.58
10	1,013	29.4	0.29
11	1,373	32.6	0.36
12	1,985	36.8	0.28
13	3,504	39.2	0.50
14	7,134	56.3	0.41
15	3,984	46.2	0.55
16	5,663	57.1	0.22

Table 3. Details on growth parameter files used, together with Tables 1 and 2, to create Figures 3 and 4 (adapted from Pauly et al. 1988, Moreau et al. 1986, and Pauly 1978, who give details on sources, locations, methods of parameter estimation, etc.). All L_∞ values are in cm and were converted to TL, all K values are in year^{-1}.

Sarotherodon melanotheron		*Sarotherodon galilaeus*		*Tilapia zillii*		*Tilapia rendalli*		*Squalus acanthias*	
L_∞	K	L_∞	K	L_∞	K	L_∞	K	L_∞	K
19.4	1.73	37.2	0.465	29.8	0.538	27.0	0.667	132.0	0.074
21.8	1.37	34.7	0.490	26.3	0.603	26.2	0.528	102.0	0.071
19.6	1.65	35.9	0.337	32.9	0.632	36.1	0.523	153.0	0.036
20.9	2.19	27.8	0.670	30.6	0.720	29.3	0.324	84.7	0.092
25.9	1.68	29.0	0.976	27.7	0.334	32.6	0.526	125.0	0.031
26.6	0.30	31.9	0.601	20.4	0.660	29.9	0.504	96.1	0.067
25.9	1.24	32.6	0.660	22.7	0.385	34.9	0.745	129.0	0.034
18.2	2.55	36.6	0.530	18.6	0.510	29.2	0.479	79.7	0.210
19.0	2.93	34.2	0.540			47.9	0.128	101.0	0.110
15.2	7.25	24.1	0.530			33.4	0.467		
33.0	0.16	49.2	0.288			31.6	0.456		
		58.1	0.469			48.0	0.138		
						31.8	0.313		
						48.2	0.177		
						49.3	0.157		
						39.7	0.230		

Thunnus[a] *albacares*		*Thunnus*[a] *obesus*		*Gadus morhua*		*Esox lucius*		*Lebistes reticulatus*	
L_∞	K	L_∞	K	L_∞	K	L_∞	K	L_∞	K
189	0.560	206	0.380	132.0	0.200	75.0	0.238	5.0	1.74
178	0.660	260	0.125	154.0	0.069	100.0	0.264	2.4	6.90
190	0.660	214	0.106	120.0	0.125	93.3	0.310	3.0	5.11
214	0.420	244	0.183	111.0	0.333	80.6	0.204	5.0	2.12
186	0.564	186	0.608	99.5	0.200	64.4	0.322	2.3	6.87
210	0.327	244	0.114	106.0	0.177	97.5	0.208	6.1	1.10
209	0.454	216	0.167	102.0	0.234	106.0	0.123	4.4	1.98
				112.0	0.154	141.0	0.097	2.6	6.74
				108.0	0.153			4.9	1.77
				123.0	0.232			5.0	1.56
				122.0	0.247			2.2	5.46
								4.2	2.30

[a]We used for tuna FL x 1.1 = TL, well aware that TL does not mean much in tuna, given the rigidity of their forked tail.

microphagous/omnivorous feeding habits (Costa-Pierce, this vol.). The other cluster includes *Sarotherodon galilaeus* and *T. zillii*, two floodplain species; *S. melanotheron*, a detritivore of West African coastal lagoons (Pauly et al. 1988b) complements the tilapia cluster, which is well separated from the other fish groups.

Conclusion

Our contention is that the concept of "growth space" presented therein, and the use of ellipses for bounding that space, offer considerable scope for advance in studying the ecology of tilapia and other fish species.

We shall support this by sending the AUXIM program to anyone requesting it (c/o ICLARM, MCPO Box 2631, 0718 Makati, Metro Manila, Philippines).

Acknowledgements

Thanks are due to Christian Brière (Department of Quantitative Biology, ENSA, Toulouse) for his kind assistance in implementing a preliminary version of AUXIM, and to Yongshun Xiao, Paul Fanning, Bill Warren and John Hoenig for their suggestions—not all implemented—on drafts of this contribution.

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Appendix 1

Fig. 5 summarizes the key features of the approach used in AUXIM to draw ellipses. (The equations are expressed for growth in weight, but may be easily adapted to growth in length); from (4) above, we have

$$\log_{10}K = \phi - 2/3 \log_{10}W_\infty$$

which is the equation of the major axis of the ellipse, with ϕ as the intercept with the ordinate.

Simultaneously, and because it is perpendicular, the equation for the ellipse's minor axis is

$$\log_{10}K = Y_o + 3/2 \log_{10}W_\infty$$

where Y_o is the ordinate at the intercept with the ordinate axis. The abscissa of the intercept of the minor axis with the abscissa axis is

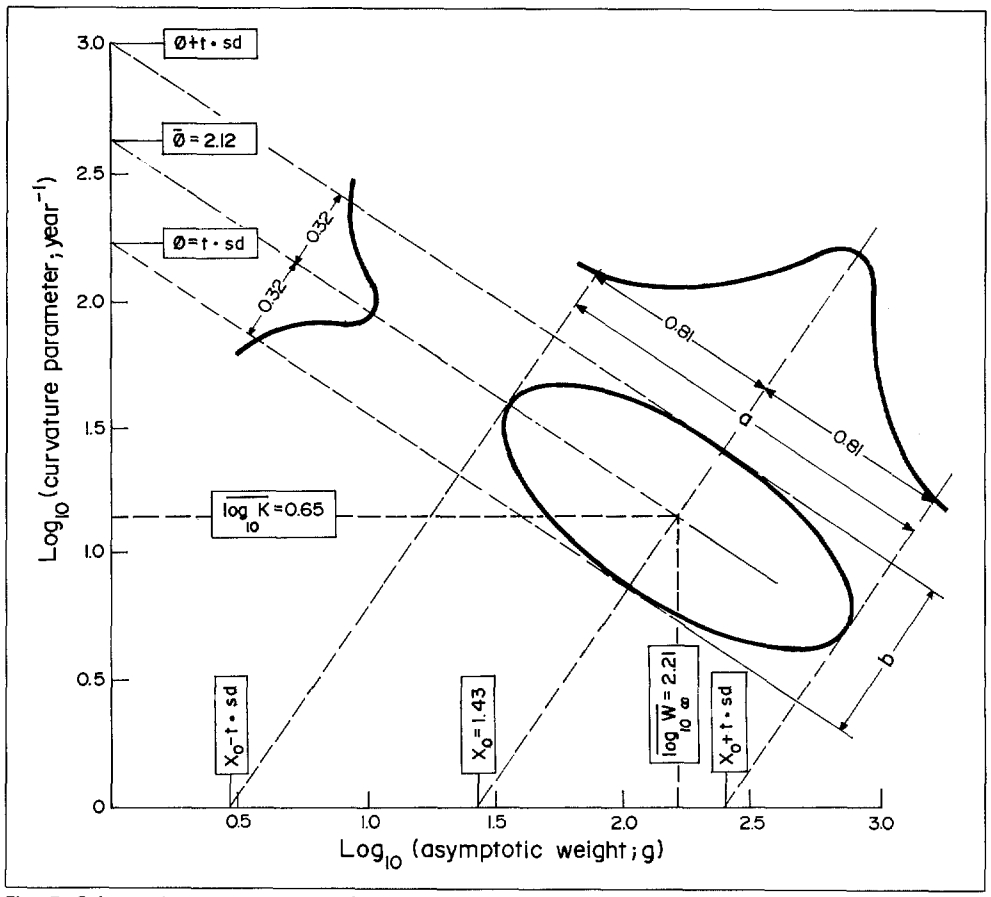

Fig. 5. Schematic representation of method used to derive ellipses from the mean and variance of log K and log W_∞ values (see text).

$$X_o = \log_{10} W_\infty - 2/3 \log_{10} K$$

If an ellipse is to refer to the 95% confidence interval of a cloud of points, the length (2·a) of the major axis must be related to the standard deviation of X_o; at the same time, the length of the minor axis (2·b) must be related to the standard deviation of ϕ, or

$$2 \cdot a = 2 \cdot t \cdot sd(X_o) \cdot 3/2 \cdot (1/((1+(3/2)^2)^{1/2}))$$
$$2 \cdot b = 2 \cdot t \cdot sd(\phi) \cdot 3/2 \cdot (1/((1+(3/2)^2)^{1/2}))$$

where the value of the t-statistic is related to the number of points n, with t=1.96 when n=∞ (Sokal and Rohlf 1981), and where the factor $3/2 \cdot (1/((1+(3/2)^2)^{1/2}))$ takes into account the fact that the axes of the ellipses are not parallel to the axes of the coordinate system.

When the ellipses refer to the standard deviation of the average values of $\log_{10} W_\infty$ and $\log_{10} K$, sd (X_o) and sd (ϕ) are replaced by standard errors, i.e., by $se_{(Xo)}$ and $se_{(\phi)}$, respectively.

Appendix 2

AUXIM estimates the surface area (S_∞) of asymptotic ellipses by random resampling of data points, as follows:

For all values of n, from 4 to the actual number of data points (with n>>4), take, at random, 20 subsamples of (W_∞, K) sets and, for each, compute the ellipses as shown in Appendix 1. Then compute their average values of $\log W_\infty$, logK, ϕ, X_o, 2·a, 2·b and, from these, the area of the mean ellipse corresponding to any value of n. Finally, fit Equation (5) to the series of data points thus obtained (see Fig. 1).

Survival of *Tilapia guineensis* under Conditions of Low Dissolved Oxygen and Low pH

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Abstract

Survival of *Tilapia guineensis* acclimated to a salinity of 10 ppt and a temperature of 29°C was assessed at dissolved oxygen (DO) levels between 5.05 and 0.15 $mg \cdot l^{-1}$, and low pH levels between 3.5 and 2.0. The lethal DO level was 0.18±0.12 $mg \cdot l^{-1}$. The routine metabolic rate of small fish (1.4±0.8 g) was 734 $mg \cdot kg^{-1} \cdot hour^{-1}$ at high ambient oxygen concentrations (6.3±0.79 $mg \cdot l^{-1}$). For medium-sized fish (10.6±3.0 g), the routine metabolic rate was 293 $mg \cdot kg^{-1} \cdot hour^{-1}$ at high ambient oxygen concentrations and 47 $mg \cdot kg^{-1} \cdot hour^{-1}$ at low ambient oxygen concentrations. For pH, the median lethal time (LT_{50}) increased with pH from 1.2 hours at pH 2.0 to 62 hours at pH 3.0. The critical median lethal pH was 3.3.

Introduction

Tilapia guineensis is a euryhaline substrate-spawning tilapia important in the lagoons and estuaries of coastal West Africa (Philippart and Ruwet 1982). *T. guineensis* has continued to attract attention as a possible candidate for aquaculture in this environment (Payne 1983). This has made it imperative to study factors likely to affect its survival under cultivation.

Aquaculture in this coastal and estuarine zone involves making use of the prevalent acid sulfate soils and coping with and controlling water parameters such as pH, dissolved oxygen (DO) and ammonia, all of which affect the health and growth of fish. In addition, traditional aquaculture involves creating and managing algal blooms. This provokes DO fluctuations in the water body (Boyd 1990). Knowledge of lethal DO levels and oxygen consumption of the cultured species helps pond management, but such information is lacking for *T. guineensis*. We therefore studied the pH and DO tolerance of *T. guineensis*, and its rate of oxygen consumption in relation to size and ambient oxygen.

Materials and Methods

A total of 150 fish (size range 0.01-25.0 g), out of a sample of 200, were used for the experiments. All fish were obtained from a pond in the

brackishwater fish farm of the African Regional Aquaculture Centre at Buguma in the Eastern Niger Delta, Nigeria. Prevailing physicochemical parameters in the pond water at the time are presented in Table 1. The fish were kept in creek water in a plastic pool (pH 6.6±0.59, DO 6.8 $mg \cdot l^{-1}$ and salinity 10.2±1.8 ppt; means and standard deviations) for a minimum of 24 hours without feeding, prior to usage.

Low pH Experiments

Two experimental procedures were adopted. In the first, 10 fish of different sizes were abruptly transferred to aerated water at a particular pH, and left there until they died.

For the second procedure, water with a particular pH and DO, held in a raised 90-l reservoir, was made to flow through the chamber containing 10 fish, at 60 ml per minute. At that rate, the reservoir water could sustain the experiment for a day before being recycled.

For both procedures, the desired pH was obtained by mixing small amounts of sulfuric acid into the test medium: read from a LITMAX pH meter model cp-20 and checked with litmus paper. The time to mortality was recorded for each fish. Fish were considered dead when the opercular beat ceased and the fish failed to respond to touch stimuli. The experimental procedure was as described by Alabaster and Lloyd (1980) and Mohamed and Kutty (1987).

Lethal Oxygen Levels

Test fish were placed singly in a sealed respirometer (1,155 ml or 300 ml). The fish reduced the oxygen content of the water until it became asphyxiated (Kutty 1972). The lethal (asphyxial) oxygen concentration was determined directly from the water through a probe connected to an oxygen meter, and confirmed by titration using the unmodified Winkler technique (APHA 1975). The experiment was terminated when all movements of the test fish ceased.

Oxygen Consumption

T. guineensis of sizes ranging from 0.5 to 14.4 g were used. Fish were confined individually in a sealed respirometer and depleted the oxygen in the water. The oxygen content of the medium was monitored every 30 minutes by oxygen probes until the fish became asphyxiated.

Results

Low pH Experiments

The relationship between resistance times and fish mortality can be represented by a series of sigmoidal curves, representing resistance times against fish mortality (Fig. 1). These curves are displaced to the right with increase in the pH of the medium. The upper flexion points on the curves denote the times to death. The times to 50% mortality are indicated by the broken line, and the values plotted are 75 minutes at pH 2.0, 145 minutes at 2.5, 420 minutes at 2.7 and 3,695 minutes at 3.0.

There is, however, a very close relationship between acid pH and LT_{50} (Fig. 2): LT_{50} increases until the pH becomes too high for 50% mortality to occur in the experimental time frame.

Assessment of Lethal DO Concentration

Fig. 3 shows the relationship between asphyxial dissolved oxygen concentration and the weight of the fish. Though smaller fish had a higher asphyxial level

Table 1. Characteristics of the pond water from which test fish (*Tilapia guineensis*) were obtained and of the acclimation media. The values given are means and standard deviations, or ranges.

Parameters	Pond water	Acclimation medium
pH	4.33 - 4.68	6.6 ± 0.6
Temperature (°C)	29.5 ± 2.0	29.3 ± 0.5
Salinity (ppt)	9.4 - 10.1	10.2 ± 1.8
Ammonia ($mg \cdot l^{-1}$)	1.77 - 2.63	0.56 + 0.20
Dissolved oxygen ($mg \cdot l^{-1}$)	3.16 - 4.04	6.80 ± 0.70
Nitrate ($mg \cdot l^{-1}$)	1.52 - 3.76	Same
Phosphate ($mg \cdot l^{-1}$)	1.46 - 2.30	Same
Sulfide ($mg \cdot l^{-1}$)	0.01 ± 0.21	Same

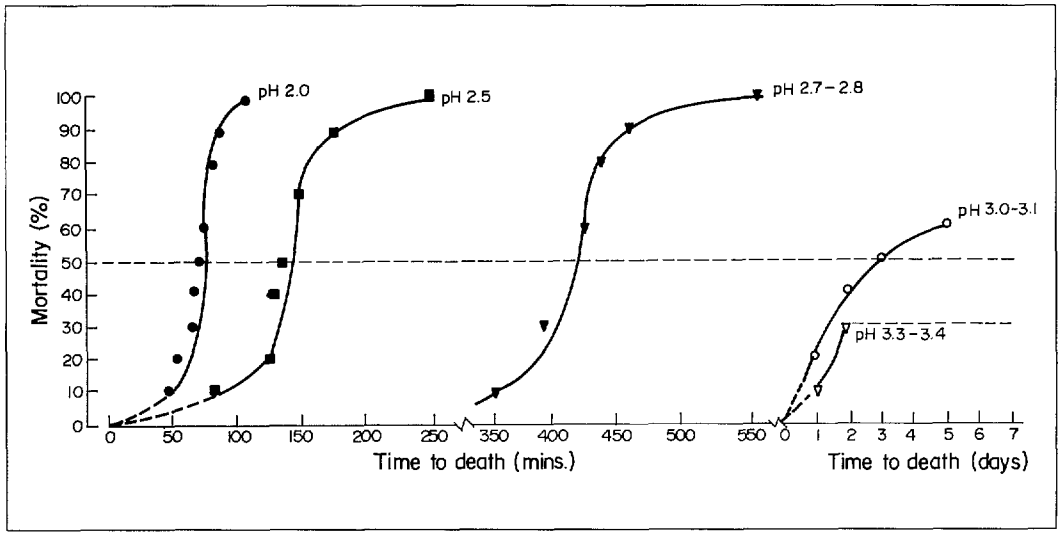

Fig. 1. Time to death of *Tilapia guineensis* juveniles (acclimated to pH 6.6 brackishwater) in various low pH media.

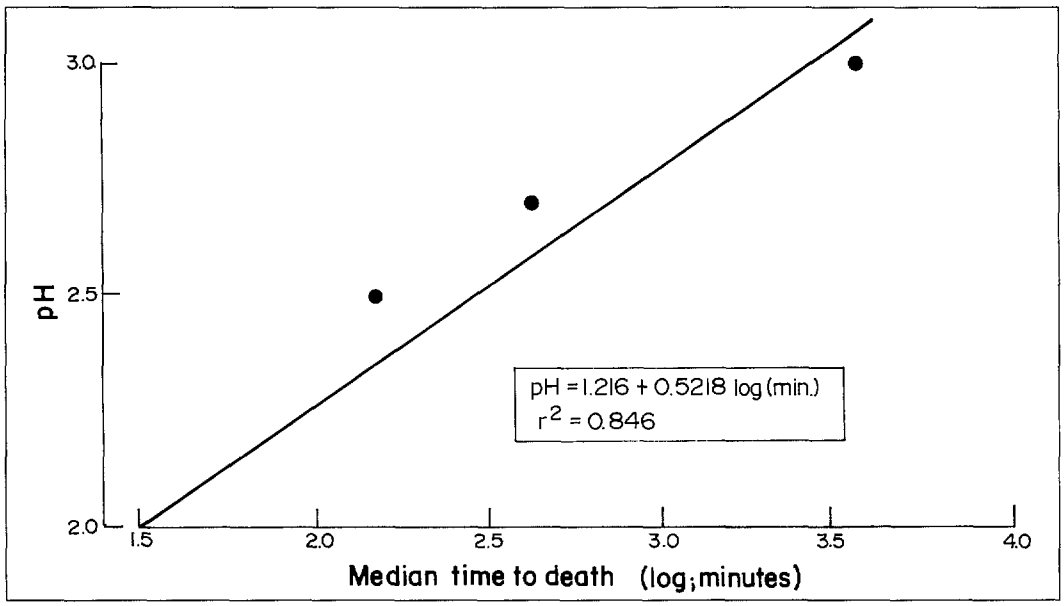

Fig. 2. Relationship between pH and median lethal time (LT_{50}) for juvenile *Tilapia guineensis*.

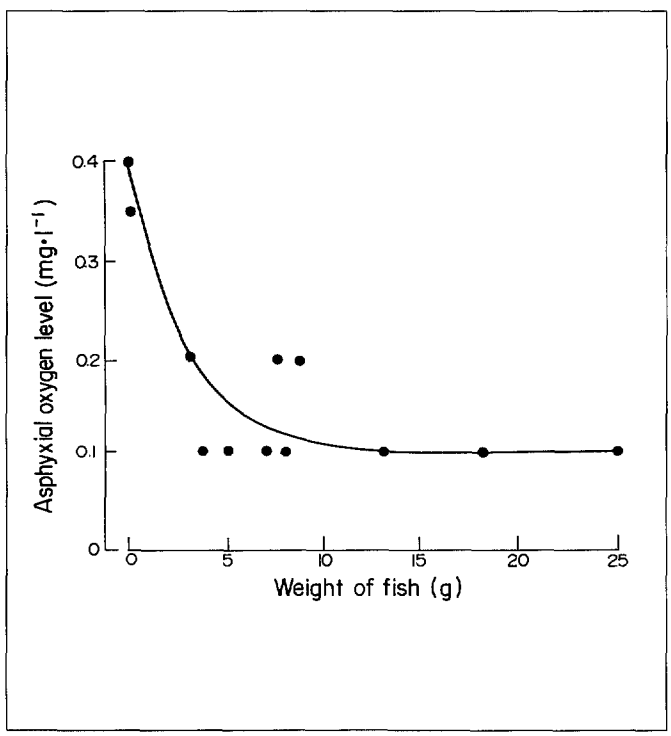

Fig. 3. Asphyxial oxygen concentration in relation to weight for *Tilapia guineensis*. (Each plotted point is a mean of two observations.)

(0.35-0.4 mg·l^{-1}), the mean asphyxial level (±SD) for all fish was 0.18±0.12 mg·l^{-1}.

Oxygen Consumption

Analysis of data from this series of experiments showed that, generally, there was a decrease in oxygen consumption as the ambient oxygen level of the medium fell, up to the point of asphyxiation when the oxygen consumption became inadequate to sustain metabolism.

Fig. 4 shows that the rate of oxygen consumption decreased with the increase in the weight of fish, thus small fish had a higher consumption rate than larger fish. Bigger fish, of course, consume more oxygen per unit time than small fish. Fig. 5 shows the relationships between oxygen consumption and fish weight at normoxic and hypoxic levels.

Discussion

Survival of T. guineensis *at Low pH*

From our results, it is clear that all pH values below 3.0 are lethal to *T. guineensis*, with 100% mortality in all cases. At pH 3.0, however, survival can be as high as 40%, rising to 70% at pH 3.3 and to 100% at pH 3.4. Therefore, pH 3.3 may be regarded as the incipient lethal pH, at which the medium becomes unsuitable for the fish, whereas pH 3.3 to 3.5 correspond to a tolerance zone. Alabaster and Lloyd (1980) believed that it was "unlikely that any fish can survive for more than a few hours in this pH zone" (3.0-3.5). Balarin and Hatton (1979) also gave the lethal pH for tilapia as approximately 3.5. With 100% survival at pH 3.4, *T. guineensis* appears to be quite resistant to low pH, and should cope with any acid conditions that may be generated within ponds. It

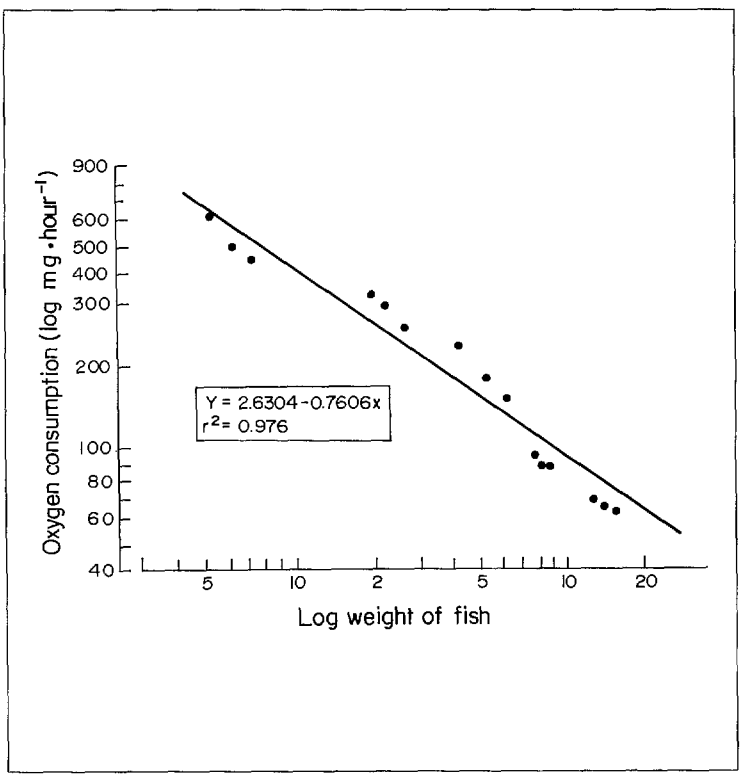

Fig. 4. Log-log relationship between oxygen consumption of *Tilapia guineensis* and fish weight at various ambient oxygen concentrations. (Each plotted point is a mean of two observations.)

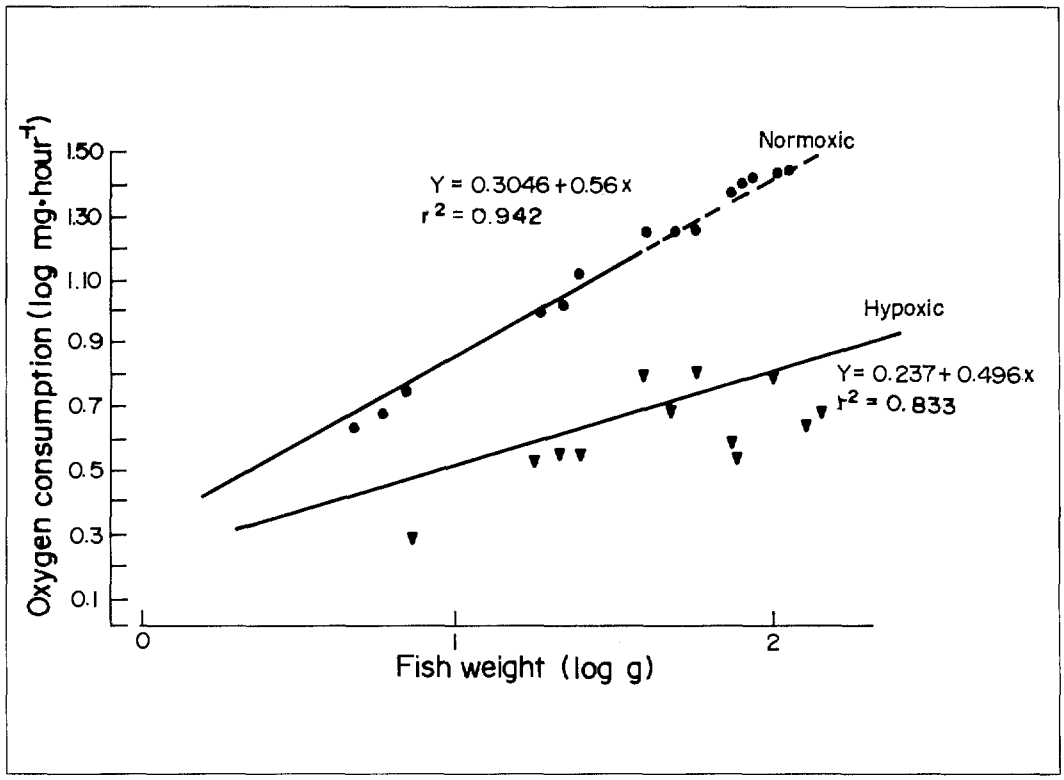

Fig. 5. Log oxygen consumption (mg·hour^{-1}) in relation to log weight of *Tilapia guineensis* under (normoxic and hypoxic) oxygen conditions. (A point represents the mean of two observations.)

is, however, not clear to what extent prior acclimation to the fairly low pH of the ponds (4.33 to 4.68) may have contributed to the good survival of the test fish at low pH. Even so, for 40% of the test fish to have survived at pH 3.0 for one week is remarkable, and marks *T. guineensis* as one of the most resistant tilapia species to low pH.

Lethal (Asphyxial) DO Levels

The asphyxial DO level of 0.18 mg·l^{-1} obtained for *T. guineensis* is well below the 1.0 mg·l^{-1} projected by Magid and Babiker (1975) as critical for most tropical fish, but is within the range found for some other cichlids (Balarin and Hatton 1979; Philippart and Ruwet 1982). The survival of *T. guineensis* at such a low DO level could be because this fish, like another estuarine tilapia, *Oreochromis mossambicus*, is capable of anaerobiosis at very low DOs (Kutty 1972). From the point of view of the tropical aquaculturist, this makes the species very desirable for culture in fertilized ponds where nighttime and early morning DO values can plummet catastrophically (Boyd 1990).

Oxygen Consumption in Relation to Ambient Oxygen Concentration and Size

The sigmoid curves (not shown) obtained for each test fish show that oxygen consumption increases with the level of ambient oxygen concentration up to a point, beyond which it becomes independent of oxygen concentration. Similarly, with reduction of oxygen concentration, oxygen

consumption decreases with lowered activity (Fry 1957, 1971), beyond which anaerobiosis occurs, followed by asphyxia. The lowest metabolic rate for *T. guineensis* from this work was 46.6 $mg \cdot kg^{-1} \cdot hour^{-1} \pm$ 16.5 g (n=8) which, according to Kutty (1987), appears to be the closest value to the standard metabolism of the fish.

From our experiments, the rate of oxygen consumption in *T. guineensis* is negatively correlated with unit weight. For the comparison between oxygen consumption and fish weights under normoxic and hypoxic conditions (Fig. 5), the slopes of the lines (0.56 and 0.48) were not significantly different (ANOVA) and are quite close to the value 0.5 reported by Fry (1971) for other tilapia species.

Acknowledgements

The first author gratefully acknowledges the help of Prof. M.N. Kutty who was his major supervisor on this project, the EEC who gave him a fellowship award, and the Rivers State Government, his employers.

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SESSION V. PHYSIOLOGY

The Role of Prolactin in the Adaptation of Tilapia to Hypo- and Hyperosmotic Environments

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Abstract

Many studies have shown the essential role of prolactin (PRL) in osmoregulation. When tilapia are transferred to hyperosmotic environments, an increase is observed in plasma ion concentrations, in the rate of ion turnover and in gill Na/K-ATPase activity. Plasma PRL concentrations decrease. Hypophysectomy of freshwater fish causes an imbalance in the hydromineral equilibrium leading to the animals' death. Supplements of PRL allow their survival by re-establishing the sodium net flux. In hyperosmotic environments, hypophysectomy does not lead to the animals' death. In this environment, PRL reduces sodium permeability and causes an increase in plasma sodium concentrations. PRL seems to have an effect on chloride cells but does not decrease gill Na/K-ATPase activity. Stress related to anesthesia, to confinement or to the water's physicochemical characteristics causes an increase in plasma PRL concentrations.

Introduction

The fish of the genera *Tilapia*, *Sarotherodon* and *Oreochromis* are characterized by a great tolerance to a wide range of environmental conditions. They tolerate great temperature variations (Chervinski 1982) as well as salinity (Dharmamba and Nishioka 1968 on *O. mossambicus*; Fukusho 1969 on *O. niloticus*) and some species known to be freshwater species are found in estuaries or in the sea (Stickney 1986). The range of tolerance also extends to water quality: anoxia, pH and dissolved nitrogen.

Efforts have been made to develop tilapia culture in marginal areas, such as in brackishwater or in seawater (Payne 1983). However, the intensive development of this farming system presents several difficulties. For example, species with a high culture potential such as *O. niloticus* have low euryhalinity, while species with poor growth such as *O. mossambicus* show adequate euryhalinity. Consequently, Doudet (1986) and Morissens (1987) report high mortalities associated with low growth rates in tilapias that are or could be farmed in brackishwater lagoons. These results, which show the low resistance of tilapias to salinity, contradict the laboratory findings. Environmental fluctuations during culture cycles, which are not reproduced in the laboratory and which the animals must resist for long periods of time (temperature, salinity and quality), could account for the observed differences.

In order to understand these mortalities, it is necessary to analyze the influences of the environmental parameters on fish physiology, and especially on the endocrine control of osmoregulation. Knowledge on the subject is still fragmentary (see review of Prunet and Bornancin 1989).

This paper is limited to the study of prolactin (PRL), and reviews both the major bibliographic data and our specific results about this hormone and its role in osmoregulation.

In tilapia, prolactin is found in two forms (Specker et al. 1985; Rentier-Delrue et al. 1989) which are synthesized by two distinct genes (Rentier-Delrue et al. 1989). The complete sequences of both PRL are well known (Yamaguchi et al. 1988; Rentier-Delrue et al. 1989). The heavier of the two, PRL-I (24 kDa, 188 amino acids) contains 11 more amino acids than the other, PRL-II (20 kDa, 177 amino acids).

Changes in Some Physiological Parameters during the Adaptation of Tilapias to Hyperosmotic Environments

Descriptive Analysis of Some Parameters during the Adaptation to a Hyperosmotic Environment

In tilapias (*O. mossambicus*) adapted to seawater, the plasma Na and plasma Cl concentrations, and the osmotic pressure are slightly higher (5 to 10%) than those of animals kept in freshwater (Table 1) (Dharmamba et al. 1973; Dharmamba et al. 1975; Dangé 1985; Young et al. 1988). In *O. aureus*, the direct transfer from freshwater to brackishwater (14 ppt) results in an increase in plasma chloride levels, followed by a rapid decrease. After three days in brackishwater, the chloremia of the animals transferred is no longer significantly different from that of the animals kept in freshwater (Fig. 1).

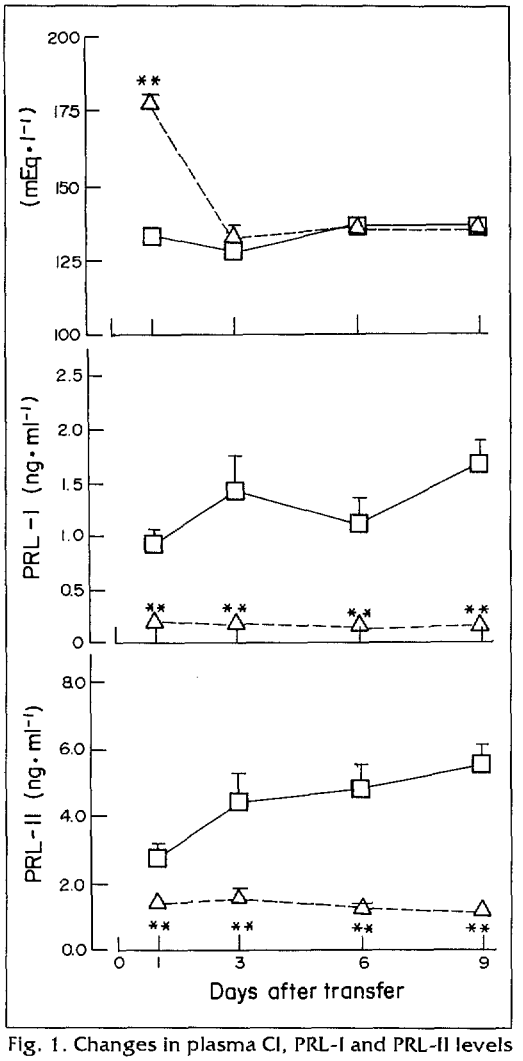

Fig. 1. Changes in plasma Cl, PRL-I and PRL-II levels in the adaptation of *Oreochromis aureus* to a brackish environment (14 ppt). Experiments were conducted on animals of 70 g mean body weight. The samples, taken at different dates after the beginning of treatment, were always taken at the same hour. Each point represents nine animals. □: control animals in freshwater; Δ: animals transferred from freshwater to brackishwater (14 ppt). *: $P<0.05$; ** $P<0.01$.

The adaptation of *O. mossambicus* to brackishwater results in an increase in gill Na/K-ATPase activity as shown either by direct measurement (Table 1)

Table 1. Effect of salinity on plasma Na and Cl concentrations, gill Na/K-ATPase activity, transbranchial potential and Na fluxes (Dharmamba et al. 1973, 1975; Dangé 1985; Young et al. 1988) in *Oreochromis mossambicus*. Values are means ± standard errors with numbers of determination in brackets.

		Environment		
		Freshwater	1/3 seawater	Seawater
Plasma values ($\mu Eq \cdot l^{-1}$)	Na	158.5 ± 2.2(12)	163.2 ± 2.5(9)	163.8 ± 2.0(11)
	Cl	139.0 ± 1.0(12)	149.5 ± 2.2(9)	146.2 ± 1.7(11)
NA/K-ATPase activity of the microsomal fraction of gill epithelium (μmol of phosphate/hour·milligram of proteins)		1.5 ± 0.1(12)	2.8 ± 0.7(9)	3.5 ± 0.8(11)
Transbranchial potential (Int-Ext) mV			14.7 ± 1.8(3)	35.2 ± 3.7(3)
Na flux	inflow	13.6 ± 2.5(8)	116 ± 29 (6)	746 ± 79 (6)
$\mu Eg \cdot 100\ g^{-1} \cdot h^{-1}$	outflow	8.4 ± 1.3(8)	123 ± 56 (5)	1,651 ± 171 (8)

(Dharmamba et al. 1975; Dangé 1985; Young et al. 1988) or by fluorescence measurements on the opercular membranes using fluorescent ouabain (McCormick 1990).

Finally, during adaptation to seawater, the transbranchial potential and the transepithelial potential, which are positive compared to the external environment, increase strongly in *O. mossambicus* (Table 1) (Dharmamba et al. 1975; Young et al. 1988).

Analysis of Ion Movements Involved in Plasma Ion Variations

Experiments were conducted on Na fluxes in order to understand better the variations observed in plasma ion concentrations. In *O. mossambicus* reared in freshwater, the rate of Na turnover is 0.15%·hour^{-1} (Dharmamba et al. 1973) with a positive net flux of sodium (inflow). This Na turnover value increases to 26%·hour^{-1} in animals adapted to seawater (Dharmamba et al. 1973).

This increase in turnover in seawater can be explained by an increase in the sodium (and chloride) outflow at the gill level, which is multiplied 10 times for diluted seawater (1/3 part) and 200 times for pure seawater (Table 1) (Dharmamba et al. 1975). The increase in Cl fluxes at the opercular membrane level is just as strong in seawater (Table 2) (Foskett et al. 1981).

In addition to this modification in ion fluxes, the adaptation of *O. mossambicus* to seawater results in reduced water permeability compared to freshwater fish (Potts et al. 1967).

Concomitance between Ion Movement Variations and Gill Cell Modification during Adaptation to a Hyperosmotic Environment

The increase in chloride excretion described previously, and demonstrated by Foskett et al. (1982), can be correlated to a modification of chloride cells at the gill and opercular membrane levels. These structures are rich in chloride-excreting chloride cells (Foskett and Scheffey 1982). For example, chloride cells are small and poorly developed in freshwater, but in seawater

Table 2. Fluxes of ^{36}Cl measured under short-circuit current at the level of the opercular membranes isolated in *Oreochromis mossambicus* adapted to different environments (Foskett et al. 1981) Values are means ± standard errors with numbers of determination in brackets.

$\mu Eq \cdot cm^{-2} \cdot h^{-1}$	Freshwater	After 10 days adaptation to seawater
Outflow	0.26 ± 0.07(6)	2.60 ± 0.30(5)
Inflow	0.18 ± 0.04(6)	0.50 ± 0.08(5)
Net flux	0.07 ± 0.07(-)	2.10 ± 0.30(-)

they are rich in mitochondria and show a well developed tubular system (seat of the Na/K-ATPase activity) in connection with the basolateral membrane (Foskett and Scheffey 1982; Foskett et al. 1982).

Foskett et al. (1982) describe two periods in the relationship between the increase in chloride excretion and the modifications observed at the chloride cell level. The first period in the increase in chloride excretion, which lasts three days, seems to be related to an increase in the number of chloride cells. The second period in the increase of excretion, noted after three days, seems to be due to an increase in chloride cell diameter and, therefore, to an increase in secretion in each cell.

These electrophysiological and microscopic studies allow us to conclude that the increase in transopercular ion fluxes observed during adaptation to seawater are closely related to an increase in the number of differentiated chloride cells which are responsible for the excretion of chlorides.

Plasma Endocrine Parameters

In endocrine terms, the adaptation of *O. mossambicus* to brackishwater results in a decrease in the circulating levels of prolactin. Such decrease occurs as soon as salinity drops 10% below seawater salinity (Nicoll et al. 1981).

Studies conducted on *O. aureus* transferred to brackishwater (14 ppt) show that the drop in PRL-I and PRL-II circulating levels occurs in 24 hours (Fig. 2) (RIA titration: Aupérin et al. 1994). The evolution of both PRL-I and PRL-II plasma levels follows the same pattern, but the PRL-II concentration is higher than the PRL-I concentration after two weeks adaptation in brackishwater. This seems to indicate a coordinated regulation of the secretion of both PRL forms.

These results concur with the studies conducted in vitro on *O. mossambicus*, which show that the hypophyses of animals reared in freshwater synthesize and secrete more PRL than those of animals reared in seawater (Nagahama et al. 1975; Grau et al. 1981).

Therefore, the adaptation of tilapias to seawater results in a slight increase in plasma ion concentrations, an increase in their turnover rate, an increase of the gill Na/K-ATPase activity and a decrease in plasma PRL concentrations. It also results in an increase in the transmembrane potential which is held responsible for the passive Na outflow.

Effects of Hypophysectomy and Prolactin Supplement in Animals Reared in Different Salinity Environments

The studies of Handin et al. (1964) and Dharmamba et al. (1967) on hypophysectomized tilapias (*O. mossambicus*) have shown that these animals were unable to survive in

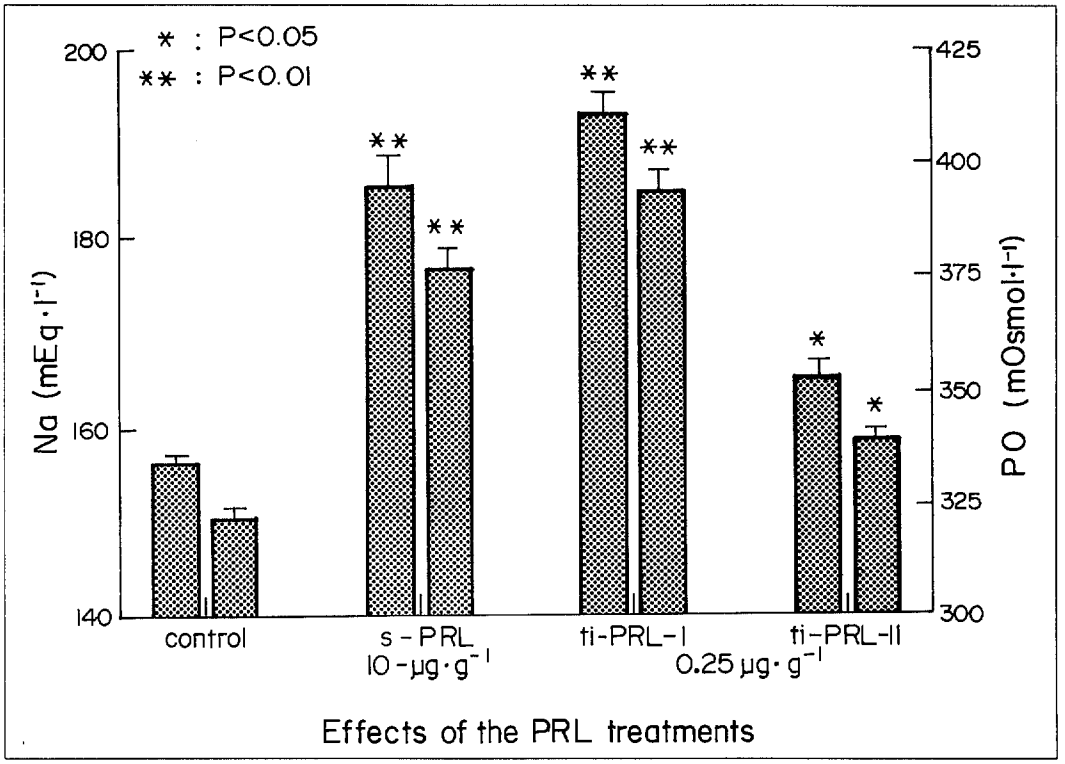

Fig. 2. Effect of sheep (s) or tilapia (ti) PRL treatments on the osmotic pressure (OP) and plasma Na concentrations in Nile tilapia (*Oreochromis niloticus*) adapted for 15 days to brackishwater (20 ppt) at the time of the first injection. PRL was injected every other day, 1 hour after daybreak (12/12) with a total of four injections. Injected doses are: sPRL: 10 $\mu g \cdot g^{-1}$ of wet weight per injection; tiPRL: 0.25 $\mu g \cdot g^{-1}$ of whole weight per injection (ti PRL's I and II were provided by F. Rentier-Delrue).
*: $P<0.05$; **: $P<0.01$.

freshwater, but that treatments using sheep prolactin (sPRL) allow their survival. These first results have led to many other studies showing the role of prolactin in osmoregulation.

Hypophysectomized Animals Reared in Freshwater

The culture of hypophysectomized *O. mossambicus* in a Ringer environment (isotonic environment) allows the survival of the animals. The transfer to freshwater causes 100% mortality in 10 days (Dharmamba et al. 1967; Dharmamba 1970). These mortalities in freshwater are due to an imbalance of the hydromineral equilibrium, which results in a continuous decrease in osmotic pressure (OP) and in Na concentrations as compared to the control animals reared in freshwater or to hypophysectomized animals kept in Ringer (Dharmamba et al. 1967; Dharmamba 1970).

The analysis of Na fluxes shows that the hypophysectomy of tilapia transferred to freshwater results in an inversion of the sodium net flux: from an inflow to an outflow. This inversion is due to a large increase in the passive outflow

and a decrease in the inflow (Table 3) (Dharmamba and Maetz 1972).

These results concur with the decrease in gill Na/K-ATPase activity, and in the decrease in transepithelial potential responsible for the passive Na outflow observed in hypophysectomized animals (Young et al. 1988).

Hypophysectomized Animals Reared In Freshwater and Supplemented with Prolactin

Daily injections of sheep prolactin (10 $\mu g \cdot g^{-1}$ of wet weight) in hypophysectomized *O. mossambicus* allow the survival of these animals in freshwater for several days. The osmotic pressure and the plasma Na concentrations in these animals are, based on the number of days of treatment, slightly lower than or equal to those in operated yet not hypophysectomized control animals (Dharmamba et al. 1967; Dharmamba 1970).

Similar results were obtained with injections of tilapia PRL (PRL-I, PRL-II or a mix of the two forms) before the transfer of hypophysectomized animals to freshwater. Thus these treatments allow the maintenance of plasma Na and Cl concentrations, the OP, and the transepithelial potential in the control animals (Specker et al. 1985; Young et al. 1988). Specker et al. (1985) did not observe any difference between the two forms of PRL in the retention of Na.

The analysis of the Na fluxes in hypophysectomized animals transferred to freshwater shows that injections of sheep PRL in *O. mossambicus* reestablish a net inflow by decreasing the outflow, but do not seem to act on the inflow (Table 3) (Dharmamba and Maetz 1972).

Intact Animals in Hyperosmotic Environments

In tilapias (*O. mossambicus*) adapted to seawater, injections of sheep prolactin over a period of five days produce a 40 to 50% increase in plasma Na and Cl concentrations and an increase in osmotic pressure (Table 4) (Clarke 1973; Dharmamba et al. 1973; Dharmamba and Maetz 1976; Herndon et al. 1991). In *O. niloticus* adapted to the brackishwater (20 ppt), injections of tilapia prolactin (PRL-I or PRL-II) also cause an increase in plasma Na and Cl concentrations and in the OP (Fig. 2).

This increase in plasma Na after the injections of PRL can be explained by a modification in the hydrogen and sodium balance. For example, the results of Wendelaar Bonga and Van Der Meij (1981) on tilapias (*O. mossambicus*) adapted to a calcium-poor, 9 ppt saline environment (isoosmotic) indicate that sheep prolactin reduces the osmotic

Table 3. Na Flux ($\mu Eq \cdot 100\ g^{-1} \cdot h^{-1}$) at the gill level in *Oreochromis mossambicus* after six to seven days in freshwater: Effect of the hypophysectomy and the sheep PRL supplement (10 $\mu g \cdot g^{-1}$, one injection per day for five days). Values are means ± standard errors (Dharmamba and Maetz 1972).

	Inflow	Outflow	Net flux
Whole animals (8)	13.63 ± 2.49	8.43 ± 1.27	+5.19 ± 1.66
Hypophysectomized animals (6)	5.58 ± 2.83	21.56 ± 3.18	-15.98 ± 2.32
Hypophysectomized animals (7) + NaCl (0.9%)	9.42 ± 0.89	14.81 ± 1.35	-5.40 ± 1.40
Hypophysectomized animals (13) + sPRL (10 $\mu g \cdot g^{-1}$)	9.87 ± 1.24	8.74 ± 0.94	+1.13 ± 0.81

Table 4. Osmotic pressure (OP), plasma Na concentration and Na inflow and outflow ($\mu Eq \cdot hour^{-1} \cdot 100\ g^{-1}$) in *Oreochromis mossambicus* adapted to seawater. Effect of the hypophysectomy and the sheep PRL supplement (10 $\mu g \cdot g^{-1}$, one injection per day for five days) (Dharmamba et al. 1973; *: Dharmamba and Maetz 1976; and **: Dharmamba et al. 1975). Values are means ± standard errors with number of determinations in brackets.

	OP ($mOsmol \cdot l^{-1}$)	Plasma Na concentration ($\mu Eq \cdot l^{-1}$)	Inflow ($\mu Eq \cdot h^{-1} \cdot 100g^{-1}$)	Outflow ($\mu Eq \cdot h^{-1} \cdot 100g^{-1}$)
Whole animals in seawater (5)	293 ± 7	165.7 ± 4.5	746 ± 79 (6)**	1,651 ± 17 (8)**
Whole animals in seawater + injections of NaCl (5)	294 ± 9	161.7 ± 2.5	739 ± 141.2	1,275 ± 199.5
Whole animals in seawater + injections of sheep PRL	414 ±10 (7)	230.6 ± 5.2 (7)	191 ± 23.6 (5)	321 ± 61.8(5)
Controls in seawater (7)		153.6 ± 4.8*		
Hypophysectomized animals in seawater (6)		175.7 ± 1.5*		

water inflow and outflow at the gill level. In the same species adapted to seawater, the increase in plasma Na after a sheep prolactin treatment causes 70 to 75% decrease in the Na turnover (Dharmamba et al. 1973). This coincides with the decrease in sodium permeability (Young et al. 1988) which results in a fivefold reduction of the Na inflow, and a fourfold reduction of the outflow (Table 4). Under these conditions, the net outflow is inhibited by 75% (Dharmamba et al. 1975).

These studies support the results of Foskett et al. (1982) which indicate that in tilapias (*O. mossambicus*) adapted to seawater, sheep PRL inhibits, in a dose-dependent manner, the excretion of chloride and conductance at the opercular membrane level. Similar effects are observed during injections of PRL from the rostral *pars distalis* of the PRL-rich adenohypophysis.

Foskett et al. (1982) also suggest that the inhibition of chloride (and consequently Na) secretion after an injection of PRL occurs due to a decline in the population of sodium-excreting chloride cells and/or a decline in the active transport to the remaining cells. In fact, recent studies (Herndon et al. 1991) have shown that a treatment using sheep prolactin inhibits chloride cell differentiation. For example, the number of chloride cells at the opercular membrane level does not vary, but the average size (diameter and depth) decreases. These chloride cells are therefore no longer in contact with both the external and internal environments. The effect of prolactin seems to be to inhibit hypertrophy and the differentiation of new chloride cells (Herndon et al. 1991).

Jointly, these results suggest that sheep prolactin inhibits the gill Na/K-ATPase in animals adapted to seawater (Dharmamba et al. 1973). However, under the conditions used by Young et al. (1988) (two injections to hypophysectomized animals kept in 25%

seawater), the two prolactin isoforms do not change the Na/K-ATPase activity. These results support those of Herndon et al. (1991) (Table 5), who did not observe any variation in the Na/K-ATPase activity after five injections of sheep prolactin.

These two sets of results (decrease in the number of chloride cells and absence of variation in the Na/K-ATPase activity) seem to be contradictory and suggest a complex effect of prolactin on chloride cells.

Hypophysectomized Animals in Hyperosmotic Environments

Hypophysectomy performed on *O. mossambicus* reared in seawater does not significantly modify Na and Cl concentrations as found in whole animals reared in the same environment (Table 4) (Dharmamba and Maetz 1976), but it produces an inhibition of 50% of the Na outflow (Dharmamba and Maetz 1976).

Whatever the environment salinity, sheep PRL or its equivalent leads to an increase in OP and plasma Na and Cl concentrations. But the action of prolactin on sodium movements seems to be different depending on whether animals are kept in fresh- or seawater. In freshwater, prolactin acts only on the outflow, whereas in seawater, it reduces both inflow and outflow.

These results suggest that the decrease in plasma PRL concentrations is a necessary condition to ensure the animals' survival and an optimal salt excretion at the gill level in seawater animals. However, the effect of hypophysectomy in tilapias adapted to seawater, which leads to an inhibition in the Na outflow, suggests that other endocrine mechanisms are involved.

Effects of Stress on Prolactin Levels

Two types of stress must be distinguished: stress related to farming conditions (stocking density, manipulation of the animals, etc.), which causes modifications in plasma PRL concentrations in salmonids (Avella et al. 1991), and stress related to the physicochemical characteristics of the environment (acidic pH, pollution by heavy metals, etc.) which causes an increase in hypophyseal prolactin cell activity in *O. mossambicus*.

Stress Related to Farming Conditions

Two types of experiment have provided the means to trace the plasma PRL-I and PRL-II levels. In *O. niloticus*, an analysis of the following effects was conducted:

Table 5. Number and size of chloride cells at the opercular membrane level and gill Na/K-ATPase activity in *Oreochromis mossambicus* adapted to seawater. Effect of the sheep(s) PRL supplement (10 $\mu g \cdot g^{-1}$, one injection per day for five days) (Herndon et al. 1991). Values are means ± standard errors.

	Animals injected with NaCl	Animals injected with s-PRL
Number of chloride cells ($cells \cdot cm^{-2}$)	6,979 ± 1,825	7,499 ±1,258
Size of chloride cells (μm^2)	325 ± 55	130 ± 17
Gill Na/K-ATPase activity ($mmol\ ADP \cdot mg \cdot prot \cdot hour^{-1}$)	9.9 ± 1.4	11.0 ± 1.8

1) the effect of the sampling method: rapid sampling without anaesthesia upon release from the culture tank. This technique was used as a control. Also a sampling of the animals after anesthesia in 4 min (0.5 ml phenoxyethanol·l^{-1} of water) or in 1 min (1 ml phenoxyethanol·l^{-1} of water); and

2) the effect of stress when animals (five fish in a 10x40x45 cm space) were confined in a reduced volume of water for 1 hour, then anesthetized in 4 min.

PRL circulating levels (Fig. 3) are higher (but not significantly different) in animals anesthetized in 1 min than in control animals. In contrast, stressed animals (showing plasma cortisol concentrations [93.1±6.3 ng·ml^{-1}] significantly higher than those in control animals [2.4±1.5 ng·ml^{-1}] or animals anesthetized in 4 min (cortisol concentrations = 113.8±10.6 ng·ml^{-1}]) show PRL circulating levels significantly higher than those of control animals. However, the increase in PRL circulating levels in stressed animals is not significant when compared to animals anesthetized in 4 min.

Environment-related Stress

The exposure of *O. mossambicus* to acidic pH (pH 3.5) causes severe stress leading to a continuous decline in the plasma osmotic pressure (Wendelaar Bonga et al. 1984). This reduction is accentuated in the first six days after

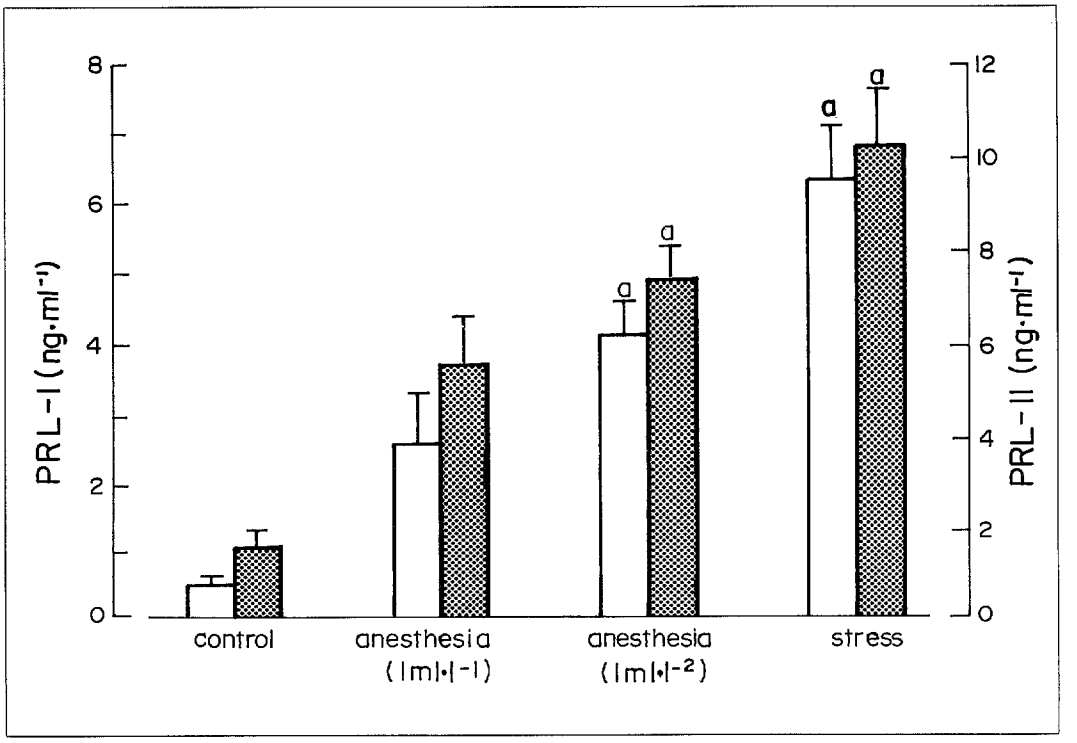

Fig. 3. Effect of anaesthesia and stress on plasma PRL-I (bars with no shading) and PRL-II (shaded bars) concentrations in *Oreochromis niloticus*. Animals were adapted to farming conditions for seven days. Two doses of anesthetic (phenoxyethanol) were used. Stress was produced by confining the animals in a reduced space in the aquarium for periods of 1 hour. a: P<0.05 compared to control animals.

the transfer, then a partial restoration occurs after 14 days. The authors describe a rapid change (in 24 hours after the transfer to an acidic environment) in the ultrastructure of the prolactin cells, and after 14 days, an increase in the size of the rostral *pars distalis*, from 31% to 47% of the total hypophysis volume, suggesting an increase in the synthesis and release of PRL.

Subsequent to a three-month adaptation in acidic pH (4.5), there is no longer any difference between plasma Na concentrations in animals adapted to acidic pH and those in the control animals (Flick et al. 1989).

These authors indicate that at the beginning of the experiment, the sodium inflow is reduced by 55% and the outflow by 70% of the value found in control animals. In contrast, they describe a restoration of the outflow after 10 days. At the end of this period, the net flux reaches zero, while there is an inflow of 93 $nmol \cdot hour^{-1} \cdot g^{-1}$ in animals with pH 7.0.

These results support the observations made previously, i.e., that the increase in prolactin secretion observed in the adaptation to acidic pH seems to be causing the decrease in Na outflow observed in these animals.

The effect of cadmium pollution also causes a temporary decline in plasma electrolytes as well as an increase in prolactin cell activity. But such an increase is only temporary, and it ceases when metallothionines appear in the liver and the gills (Fu et al. 1989; Pratap et al. 1989). These detoxifying proteins apparently protect osmoregulating mechanisms against cadmium.

Conclusion

This study shows that prolactin plays an important role in freshwater tilapias. It is essential to their survival because it reduced the Na outflow. On the other hand, the absence of prolactin in animals adapted to a hyperosmotic environment also seems essential, since this hormone causes a retention of the plasma Na, which leads to their death. In this environment, prolactin acts by reducing Na inflow and outflow.

Incidents of stress (manipulation, confinement, modification of the quality of farming conditions) lead to a significant and rapid increase in plasma PRL levels. If these factors also cause a similar increase in plasma PRL concentrations in brackishwater, it is likely that it will lead to an imbalance of the hydromineral equilibrium in these animals. Such an imbalance, if it persists in a hypertonic environment, could cause death and could account for the unexplained mortalities in some cultures in brackishwater lagoons. The hypothesis of an increase in plasma PRL concentrations following environmental fluctuations during culture cycles should be studied further.

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Physiological Adaptation of *Oreochromis niloticus* and *O. aureus* to Salinity[a]

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AVELLA, M. and T. DOUDET. 1996. Physiological adaptation of *Oreochromis niloticus* and *O. aureus* to salinity, p. 461-470. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Experiments in Ivorian lagoons on the tolerance of tilapias to brackishwater showed significant differences among species and hybrids of *Oreochromis*, particularly in their survival rate. To document these differences, the physiological adaptation to salinity was studied by choosing the species most tolerant to lagoon environment, *O. aureus*, and the least tolerant, *O. niloticus*. This study was conducted on populations from Côte d'Ivoire (field strain: FS) and on a population of *O. niloticus* of the same origin; but cultured in laboratory conditions (laboratory strain: LS). The populations were transferred from freshwater (FW) to brackishwater (BW) both progressively (FW→BW 10 ppt →BW 20 ppt) and rapidly (FW→BW 20 ppt→BW 30 ppt). Four parameters were used to assess the adaptability to saltwater: mortality rate, plasma natremia, gill Na^+/K^+-ATPase activity and the presence of gill interlamellar chloride cells.

Results showed that *O. niloticus* (LS) is the only strain to adapt rapidly to 30 ppt while *O. niloticus* (FS) had difficulties tolerating the progressive transfer to 20 ppt. However, such a transfer was well-tolerated by *O. aureus*. Plasma natremia increased (15%) in each strain only when the maximum level of salt tolerance was approached. Mortalities occurred at 190 µM natremia. Gill Na^+/K^+-ATPase activity was stimulated, but its kinetics was different from that of the natremia. Interlamellar chloride cells, apparently rare in freshwater, occurred after transfer to brackishwater. These cells were found in large numbers in *O. niloticus* (LS) and *O. aureus*.

Based on the results, the following classification was established for progressive adaptation to salinity: *O. niloticus* (FS) < *O. aureus* (FS) < *O. niloticus* (LS). A number of hypotheses are derived from these findings.

[a]A more detailed account of this study, including a description of the gill ultrastructure, has been published by M. Avella, J. Berhaut and M. Bornancin. 1993. Salinity tolerance of two tropical fishes, *Oreochromis aureus* and *O. niloticus*. I. Biochemical and morphological change in the gill epithelium. J. Fish Biol. 42:243-254.

Introduction

Competition with agriculture over land use and water resources, and the will of some African countries like Benin (Morissens et al. 1986) and Côte d'Ivoire (Doudet 1992) to use lagoon waterbodies, have encouraged the development of tilapia culture in brackishwater. In effect, many tilapias show a distinct euryhaline nature (Chervinski 1982; Payne 1983) as also described by many studies on the species of African coastal areas (Daget and Iltis 1965; Payne and Collinson 1983).

However, high tolerance to salinity and high culture potential are not necessarily synonymous, as highly euryhaline species respond generally poorly to profitability criteria in intensive production. This is particularly true of *Oreochromis mossambicus* although the same observation was made in *Sarotherodon melanotheron* and *Tilapia guineensis*, both native species of West African lagoon environments (Legendre et al. 1990). In Côte d'Ivoire, this situation has prompted the development of a comparative program of species and hybrids of the allochtonous genus *Oreochromis* to breed a tilapia for aquaculture in lagoon environments (Doudet 1992). Significant differences among species and hybrids have been observed, particularly in their survival (Table 1).

As these differences could be explained by the species' greater aptitude to osmoregulate in hypertonic environments, a laboratory study on the physiological adaptation to salinity was conducted using *O. aureus* and *O. niloticus*. These two species were selected because of their highly dissimilar survival in lagoon environments.

The study consisted mainly in observing the evolution of plasma sodium and gill Na^+/K^+-ATPase activity as well as the morphological changes occurring in the gill epithelia during transfers from freshwater to saltwater.

Materials and Methods

Biological Material and Adaptation Environments

The study was conducted on populations of *O. aureus* and *O. niloticus* transferred directly from Côte d'Ivoire (field strains: FS) and on a population of *O. niloticus* cultured for several years at the National Institute of Agriculture

Table 1. Survival (%) of species and hybrids of *Oreochromis* observed during tests conducted in lagoon environments in Côte d'Ivoire. Maximum salinity: 15 ppt; mean temperature: 27-30.5°C; and duration of tests: 45-153 days (from Doudet 1992).

Test number	Species or hybrids							
	Oa	On x Oa	Om x Ouh	Om	Ouh	On x Ouh	Om x On	On
1	96^a	92^{ab}	91^{ab}	86^b	-	-	-	57^c
2	-	98^a	85^b	85^b	80^b	80^b	-	67^c
3	-	99^a	84^b	74^c	77^c	78^c	-	58^d
4	85^a	77^a	-	-	-	-	-	55^b
5	91^a	-	-	-	-	-	80^a	-

In a single test, mean values with the same superscript letter do not differ significantly (P>0.05). Oa: *Oreochromis aureus*; On: *O. niloticus*; Om: *O. mossambicus*; and Ouh: *O. urolepis hornorum*.

Research (INRA) laboratory in Rennes, France (originally from Bouake, Côte d'Ivoire, then kept as a laboratory strain: LS). In Côte d'Ivoire, the fish were maintained at a density of 9 $g \cdot l^{-1}$ in 7-m^3 well water-fed PVC tanks. The animals were transferred at different periods of the year to the University of Nice where they were maintained in 27°C recirculated freshwater (FW) with an 8-9 hour photoperiod. Controls were stocked at a density of 3-12 $g \cdot l^{-1}$ in rectangular PVC tanks. Cement tanks (140-l) were used for the adaptation periods at a density of 3 $g \cdot l^{-1}$.

The transfer experiments in brackishwater (BW) were conducted at different intervals after the initial arrival of the fish at the laboratory: 15 days or three months later for *O. niloticus* LS, six months later for *O. niloticus* FS, and three months later for *O. aureus* FS.

The physicochemical composition of the freshwater in Côte d'Ivoire was (mM): $[Na^+]=220$; $[K^+]=30$; and $[Ca^{++}]=125$; and in Nice: $[Na^+]=148$; $[Cl^-]=116$; $[K^+]=14$; $[Ca^{++}]=1,585$; and pH=7.89.

The different brackish environments were prepared using seawater from the Mediterranean (osm: 1,000 m $Osm \cdot l^{-1}$; salinity: 36 ppt) diluted with freshwater.

The study was conducted on animals of approximately 30 g mean body weight. These were transferred to brackishwater either progressively or, whenever possible, rapidly. The transfer protocols and the sampling frequency for analysis are summarized in Fig. 1.

The animals were initially starved and were not fed throughout the experiment.

Plasma Analysis

Blood was taken from the caudal vein and centrifuged. The plasma sodium was measured using an Eppendorf flame photometer.

Gill Na^+/K^+-ATPase Activity

After killing the fish, gills were immediately removed, homogenized and placed in a solution containing 10% sucrose and 1µM dithiothreitol (pH=7.41). Later, the preparation was successively centrifuged at 2,000 G for 10 minutes, 11,500 G for 30 minutes and finally 30,000 G for 30 minutes. The microsomal activity level was determined

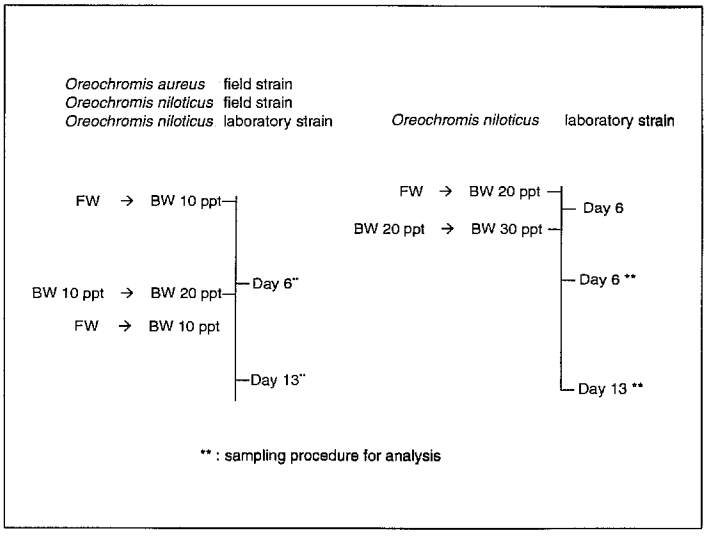

Fig. 1. Protocols for transferring tilapias ls from freshwater (FW) to brackishwater (BW).

by assessing differences between the quantities of inorganic phosphate released by the membrane preparation obtained in the presence or absence of ouabain at 10^{-3} M. These measures were done using a TECHNICON auto-analyzer following the methods described by Bornancin and de Renzis (1976).

Morphological Study

For the light microscopic study, small gill fragments, collected immediately after killing the fish, were fixed in the Champy-Maillet solution (Maillet 1959) for about 10 hours after which they were rinsed and dehydrated. This fixative, which allows an excellent preservation of the tissues, stains chloride cells black by reduction of osmium tetroxide on membrane phospholipids. Since chloride cells possess a highly developed endoplasmic reticulum and a largely invaginated plasma membrane, they are easily stained (Garcia-Romeu and Masoni 1970).

Statistical Analysis

One-way ANOVA was used to interpret the results and mean values were compared in sets of two using the smallest significant difference method.

Results

Tolerance to Salinity

O. niloticus FS showed the poorest adaptability to salinity (Table 2). This strain had difficulties tolerating a progressive transfer in 10 ppt brackishwater (10% mortality) and in 20 ppt (65% final mortality rate), and does not tolerate rapid transfer FW→BW 20 ppt (100% mortality). No mortalities were observed for either *O. aureus* FS and *O. niloticus* LS when transferred progressively. However, *O. aureus* did not adapt when transferred rapidly from FW→BW 20 ppt (100% mortality) while *O. niloticus* LS easily tolerated this transfer and could even tolerate a transfer to water up to 30 ppt salinity (25% mortality).

Plasma Natremia

When the fish were kept in freshwater for a long time, all strains showed constant levels of plasma Na^+, but these levels were always lower in *O. aureus* (144.1±2.1; n=11) than in *O. niloticus* (160.7±2.3; n=19) regardless of the origin of the strain (Fig. 2). For *O. niloticus* LS, the rapid increase of external salinity to 20 ppt did not modify the natremia which increased significantly

Table 2. Mortality of *Oreochromis aureus* and *O. niloticus* during transfers from freshwater (FW) to brackishwater (BW). FS = field strains; LS = donating strain.

Mode of transfer	*O. aureus* FS	*O. niloticus* FS	*O. niloticus* LS
FW→BW 10 ppt→20 ppt (6 days) (7 days)	0 (n=16)	65 (n=14)	0 (n=15)
FW→BW 20 ppt→30 ppt (2 days) (4 days)	100 (n=11)	100 (n=10)	25 (n=20)

n: initial number of fish studied.

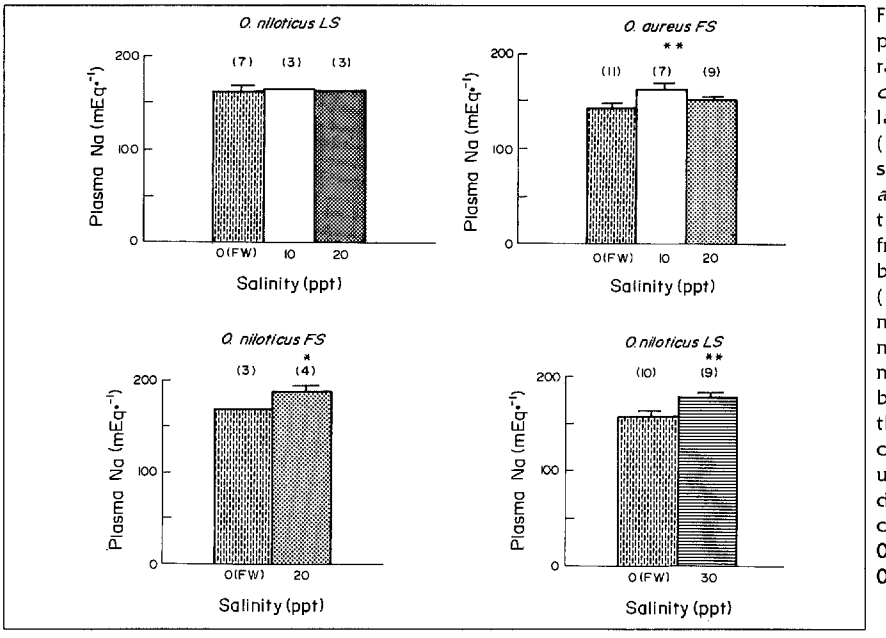

Fig. 2. Changes in physiological parameters in *Oreochromis niloticus* laboratory strain (LS) and field strain (FS), and *O. aureus* FS during transfers from freshwater (FW) to brackishwater (BW): plasma natremia. The number of experiments is indicated between parentheses. Statistical comparisons: values significantly different from controls * for $P < 0.05$ and ** for $P < 0.001$.

(+13.5%) only after a transfer to 30 ppt. For *O. aureus* FS, the progressive increase in salinity produced a temporary rise (+12.5%) in plasma Na^+ at 10 ppt, but these levels returned to normal during transfer to 20 ppt. Finally, in progressive transfer to 20 ppt, natremia levels in *O. niloticus* FS were sharply increased (+18.8%).

Gill Na^+/K^+-ATPase Activity

Enzyme activity, measured only in *O. niloticus* LS and *O. aureus* FS, appeared twice as low in freshwater in the latter species (Fig. 3). The effects of progressive transfer from FW→BW 10 ppt→BW 20 ppt on enzyme activity were similar in these two strains. Enzyme activity was stimulated significantly only during transfer from BW 10 ppt→BW 20 ppt by a factor 1.6 for *O. niloticus* and by a factor 3 for *O. aureus*. The rapid transfer from FW→BW 20 ppt→BW 30 ppt, possible only for *O. niloticus* LS, had a temporary stimulating effect (by a factor 2 after four days) on gill enzyme activity, which returns to normal after a week.

Gill Morphology

In freshwater, regardless of the species or strain studied, lamellar chloride cells (CC) are absent (secondary lamella) and interlamellar CCs were rare (primary lamella) (Fig. 4A). During transfer to brackishwater, the following was observed in *O. niloticus* LS:

– a significant occurrence of interlamellar CCs at 10 ppt (Fig. 4B);

– an increase in the number of interlamellar CCs at 20 ppt (Fig. 4C); and

– a stable number of interlamellar CCs at 30 ppt (Fig. 4D).

The same morphological development was observed in *O. aureus* FS, but the increase in the number of CCs seemed lower than in *O. niloticus* LS when the external salinity was 20 ppt. Finally, a small number of CCs was observed in *O. niloticus* FS at the same salinity.

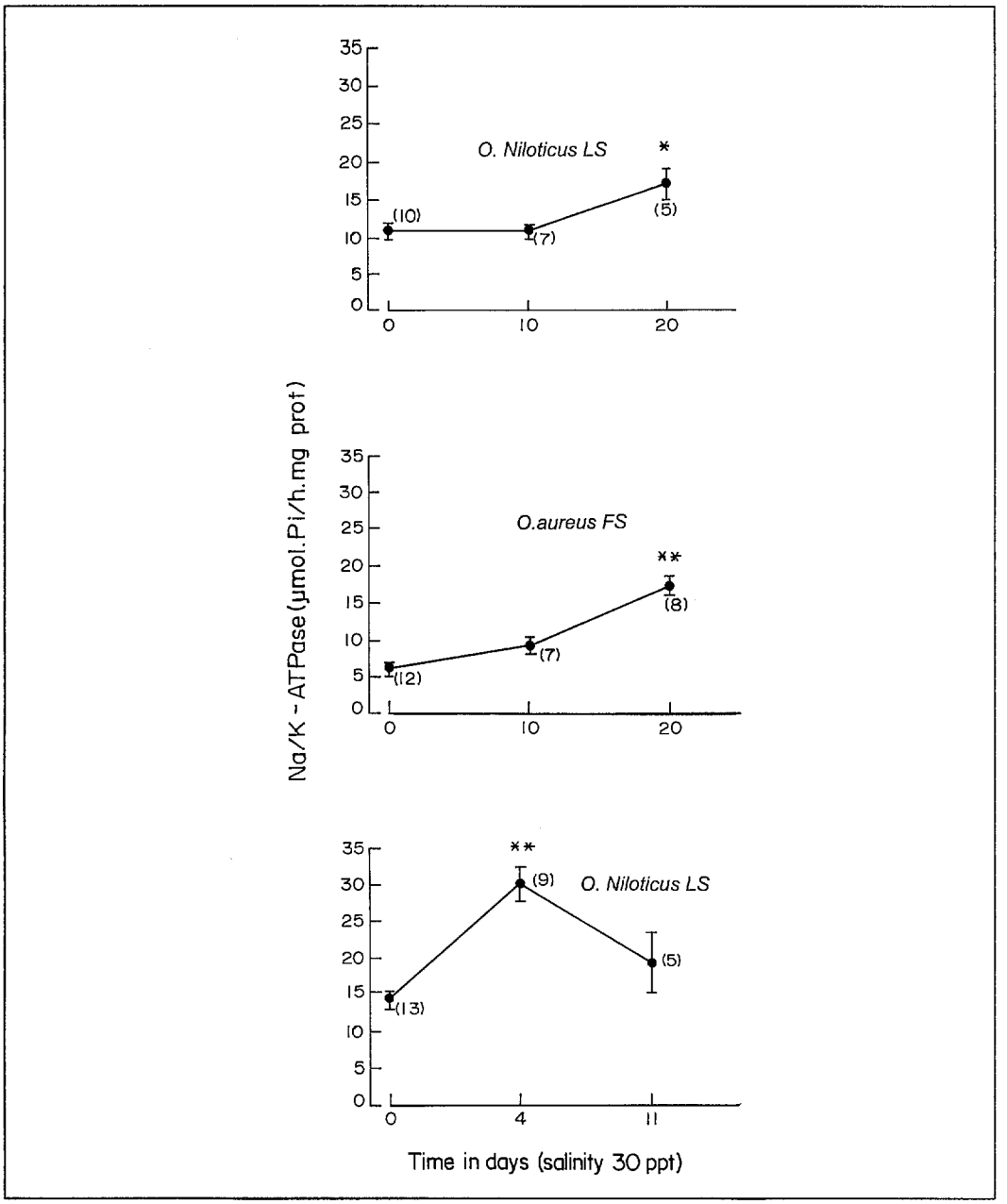

Fig. 3. Changes in physiological parameters in *Oreochromis niloticus* laboratory strain (LS) and *O. aureus* FS during transfer from freshwater (FW) to brackishwater (BW): Na^+/KT^+-ATPase activity. The number of experiments is indicated between parentheses. Statistical comparisons: values significantly different from controls * for $P<0.05$ and ** for $P<0.001$.

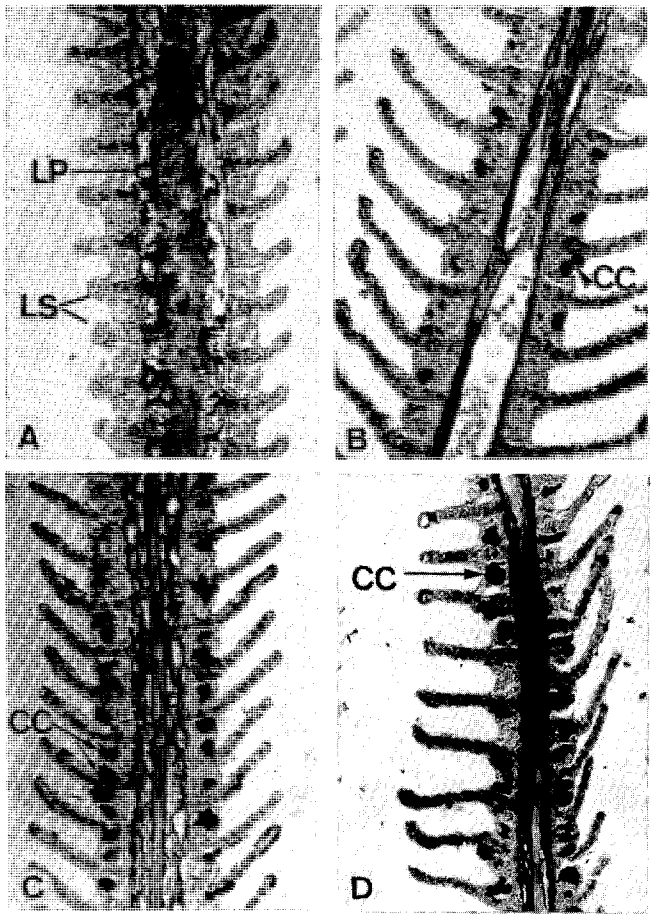

Fig. 4. Morphology of the gill epithelium of *Oreochromis niloticus* laboratory strain (LS) during transfer from freshwater (FW) to brackishwater (BW). Observations made by light microscopy (x330). A: freshwater; B: brackishwater at 10 ppt; C: brackishwater at 20 ppt; D: brackishwater at 30 ppt after four days of adaptation. PL: primary lamella; SL: secondary lamella; and CC: chloride cell.

Discussion

Mortality rates observed here during the progressive transfer from FW→BW 10 ppt →BW 20 ppt clearly indicate a much higher tolerance to salinity in *O. aureus* than in *O. niloticus* for the two strains transferred directly from Côte d'Ivoire. These findings support the results of studies conducted in lagoon environments (Doudet 1992), as well as in laboratory conditions (Watanabe et al. 1985) and in natural environments (Payne and Collinson 1983). None of these two strains survived a direct transfer to 20 ppt without a preacclimation at 10 ppt. This step is therefore necessary to reduce osmotic stress.

The gill morphology of freshwater tilapias resembles that of other freshwater stenohaline fishes. However, transfer to brackishwater caused a proliferation of interlamellar CCs as salinity increased. This morphological change is similar to that observed in euryhaline fishes transferred to seawater (Maetz and Bornancin 1975) *a priori* pointing to a real potential for adaptation to external salinity increases since interlamellar CCs are involved in the excretion of seawater salt (Maetz and Bornancin 1975). Moreover, Foskett et al. (1981) observed the development of typical CCs on *O. mossambicus'* opercular membranes during acclimation to seawater. These authors first noticed an increase in the number of CCs during the first three days following transfer in seawater, then they observed an hypertrophy of these cells.

If the increase in the number of CCs is associated with the adaptability to brackishwater, our results show a greater cell development in the species or strains which best tolerate the increase in salinity, i.e., *O. niloticus* and LS and, to a lesser extent, *O. aureus* FS.

In *O. niloticus* and *O. aureus*, the increase in salinity, particularly the transfer to 20 ppt, is accompanied by a stimulation of gill enzyme activity. This supports the results of studies on other euryhaline fishes (de Renzis and Bornancin 1984), especially *O. mossambicus* (Dange 1985). As Na^+/K^+-ATPase is responsible for the excretion of seawater salt (Foskett et al. 1981), an increase in its activity occurs with the increased number of CCs. This response to osmotic shock thus contributes to osmoregulation. The gill structures observed in freshwater prove therefore to be functional in brackishwater. The values of the enzyme activity measured during this study (between 5 and 34 µmol Pi/h.mg of proteins) are within the range of available data on tilapias, i.e., 1.5 (Dharmamba et al. 1975), 14.4 (Ho and Chan 1980) and 66 µmol Pi/h.mg of proteins (Balm et al. 1988). The differences observed could result either from the sample preparation method or pertain to the species studied, the values reported in freshwater during this experiment being for example twice as high in *O. niloticus* than in *O. aureus*.

Foskett et al. (1981) saw a strong correlation between the increase in the excretion of salt and the proliferation of CCs. This can be explained by the presence of a larger number of enzyme sites (Thompson and Sargent 1977) located at the level of the interlamellar CCs baso-lateral membranes (Karnaky 1980).

During the progressive transfer to 10 and then to 20 ppt, the increase in enzyme activity was accompanied, for the two strains that were perfectly acclimatized, either by a stable or a temporarily fluctuating natremia. However, following direct transfer to 30 ppt, the increase in natremia in *O. niloticus* LS was associated with a very important increase (by a factor 3) in the Na^+/K^+-ATPase activity. Dharmamba et al. (1975) showed an interesting parallel between

the evolution of the gill Na^+ flows and the Na^+/K^+-ATPase activity in *O. mossambicus*. For euryhaline species, it would appear that it is the rate of plasma Na^+ which stimulates enzyme synthesis (Valverde et al. 1982). For *O. niloticus* FS, a strain adapting particularly poorly to salinity, transfer to brackishwater was accompanied by a marked increase in natremia (190 µM) which was certainly responsible for the high mortality observed.

The differences observed between these two sources of *O. niloticus* were unexpected, with regard to survival and the physiological or morphological changes. Several hypotheses can be proposed to explain this situation. Breeding numerous generations from an initially limited number of animals in laboratory conditions can lead to genetic drift. Intraspecific variability has been reported in the adaptability to salinity in *Oncorhynchus mykiss* (Leary et al. 1981), and Pora (1960) and Kiener (1978) have shown in *Leander squilla* and *Aphanus fasciatus*, respectively, that many populations are adapted to biotopes characterized by waters with different ion concentrations. Moreover, the aptitude of animals to adapt to brackishwater might depend upon the quality of their original freshwater (in spite of the fact that the fish were kept in the laboratory for several weeks to several months). Studies by Avella et al. (1987) on *Oncorhynchus mykiss* showed that the term "freshwater" in fact covers different situations in terms of pH, Na^+, Cl^- or Ca^{++} for the adaptation of the fish. When fish are exposed to freshwater with different ion concentrations for long periods of time, changes in the gill tissue morphology as well as in the epithelium physiology occur, which could condition the subsequent adaptability to brackishwater.

A further hypothesis could be that an animal, cultured in Côte d'Ivoire in conditions close to its original natural environment (*O. niloticus* FS), could hardly tolerate transfer to and prolonged captivity in laboratory conditions (physical, chemical, chronic stress, different feeding habits, etc.) and would therefore be weakened, which could explain a decrease in its adaptability to salinity.

In practice, a phase of preacclimation to 10 ppt salinity seems to be necessary for freshwater tilapia strains used in Côte d'Ivoire before transfer to a higher salinity. Moreover, changes in morphological and physiological parameters show that these parameters can be used as indicators of potential adaptation to brackishwater by observing the gill structure, the plasma sodium or the gill Na^+/K^+-ATPase activity (Avella et al. 1993).

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SESSION VI. ECONOMICS AND SOCIOECONOMICS

Economics of Tilapia Aquaculture in Small Waterbodies in Bangladesh[a]

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Abstract

This paper describes the technical and economic efficiency of tilapia aquaculture in small ponds and ditches in Trishal, Fulbaria and Mymensingh thanas (village units), Bangladesh. Sample surveys of 113 and 46 farmers were used in estimating linear and log-linear (Cobb-Douglas) production functions, respectively. Results from the linear model which gave the better fit showed that the rate of fingerling stocking and the use of rice bran have significant effects on tilapia production. This model also indicated that there were different productivities across locations and pond sizes. Estimates also indicated that the current rate of stocking, inorganic fertilizer application and feeding of rice bran were below optimum levels. Increased use of commercial inputs, as suggested by the economic efficiency criteria, may not be realistic given the subsistence nature of these tilapia farms. Assured availability of commercial inputs and cash are prerequisites to exploit further production and income from tilapia aquaculture by these small-scale farmers.

Introduction

Fish is an important source of dietary animal protein for the rural farming population of Bangladesh. The bulk of fish at present comes from capture of wild fish from vast floodlands and rivers. Culture of fish in the sense of regular stocking and harvesting for consumption and sale is not a very common practice among farm households, although the country has 1.3 million ponds of about 147,000 ha that are potentially available for such practices. Only 46% of the ponds or about 52% of the area are currently stocked with fingerlings. Eighty percent of these ponds receive no preparation prior to stocking of fingerlings; 92% are never fertilized (World Bank 1991). Polyculture, using Indian major carps together with recently introduced Chinese and common carps, has been the major aquaculture technology proven suitable only in ponds which are relatively large in size and have perennial water supply.

Research and extension currently emphasize improving culture practices in ponds that are suitable for polyculture, but around 14% of the ponds in the country are small and have seasonal water supplies. There are thousands of ditches, roadside canals and borrow pits which could be used for culture of fish with a short growing cycle, such as the Nile tilapia (*Oreochromis niloticus*) (Gupta et al. 1992).

[a]ICLARM Contribution No. 967.

On-station and on-farm farmer participatory research have produced a simple technology for the culture of Nile tilapia in seasonal ponds and ditches of the type to which millions of rural households have access. With collaboration of NGOs, the technology was transferred to several thanas (village units) of Mymensingh, Tangail and Norshingdi Districts (Gupta et al. 1992). Simple and inexpensive management practices requiring minimal inputs for pond preparation and poststocking feeding and fertilization were easily adopted by the farmers.

Aside from stocking materials (fingerlings), other production inputs that are presently used by the operating farmers include organic manure (e.g., cattle manure), inorganic fertilizers (e.g., urea and triple superphosphate) and rice bran. Positive benefits of the technology on the farm households in terms of income and nutrition have also been reported: an average of 343% return on investment after four to five months rearing of fish in seasonal ponds and ditches (Gupta et al. 1992).

The objective of this paper is to examine the technical and economic efficiency of current use of inputs for tilapia culture in small ponds and ditches operated by rural households.

Materials and Methods

Cross-sectional input-output data on pond culture of *O. niloticus* in Trishal, Fulbaria and Mymensingh thanas in Bangladesh were used. The survey interviews were conducted as part of the ICLARM-assisted Bangladesh Agricultural Research Project funded by the US Agency for International Development. The data, collected in July 1990, covered the past year's production period. More details on the selection of the thanas, pond characteristics, management practices, income from tilapia culture, and the assessment and attitudes of farmers to the technology can be found in Gupta et al. (1992).

The hypothesized production function model was specified as:

$$Y=f(X_1...X_8)$$

where:

Y=yield of tilapia ($kg \cdot m^{-2}$);
X_1=pond age (years);
X_2=no. of fingerlings stocked per m^2;
X_3=quantity of inorganic fertilizer (urea and triple superphosphate [TSP]) applied ($g \cdot m^{-2}$);
X_4=quantity of organic manure (cattle manure) applied ($g \cdot m^{-2}$);
X_5=quantity of feed (rice bran) applied ($g \cdot m^{-2}$);
X_6=production cycle (days);
X_7=pond size (m^2); and
X_8=water retention ($days \cdot year^{-1}$).

Two algebraic forms of the production function were estimated to determine the influence of inputs and/or factors on fish yield from small waterbodies utilized for tilapia aquaculture. These are:

(1) Linear

$$Y=\alpha + \beta_1 X_1 + ... + \beta_8 X_8 \qquad ...1)$$

(2) Cobb-Douglas

$$Y=\alpha X_1 \beta_1 ... X_8 \beta_8 \qquad ...2)$$

where α, β_1, ... β_8 are the production coefficients that were estimated.

To reflect the influence of locations of ponds and water quality on tilapia yields, dummy variables (D_1, D_2 and D_3) were also included, such that:

D_1= 1 if the ponds are located in Trishal; zero, otherwise;

D_2= 1 if the ponds are located in Fulbaria; zero, otherwise; and

D_3= 1 if the pond water is turbid; zero, if the pond water is green/brown.

In the linear formulation (1) the ß coefficient represents constant marginal productivities of individual factors or inputs.

In the Cobb-Douglas formulation (2), the sum of the coefficients will indicate returns to scale. Constant returns to scale ($\Sigma ß=1$) means that doubling all inputs will result in doubling the output. A value of $\Sigma ß>1$ implies that doubling all inputs would result in more than doubling the output, and conversely for $\Sigma ß<1$.

Economic Efficiency of Production Inputs

The economic efficiencies of production inputs were determined by equating the marginal physical products of the inputs with their respective input-output price ratios. Tilapia producers are said to be economically efficient when the value of the marginal physical product of an input is equal to the input-output price ratio. Algebraically,

$$MPPx_i=Px_i/Pyor \quad ...3)$$
$$(MPPx_i)\ (Py)=Px_i \text{ or} \quad ...4)$$
$$VMPx_i=Px_i \quad ...5)$$

where:

$MPPx_i$=marginal physical product of the input i;

Px_i=price of input i;

Py=price of output or tilapia; and

$VMPx_i$=value of marginal product of input i.

If the $MPPx_i$ is greater (less) than the price ratio, the use of the input should be increased (decreased) (equation [3]). Alternatively, the economic efficiency of an input can be examined by comparing the $VMPx_i$ with Px_i. If the $VMPx_i$ is greater (less) than Px_i, profit could be increased by increasing (decreasing) the use of that input (equation [5]).

Results and Discussion

Linear and log-linear (Cobb-Douglas) production functions were estimated for tilapia culture in ponds and small ditches (Table 1). In both models, 11 explanatory variables could explain 41% of the variations in tilapia output. However, in the linear model, five variables were statistically significant whereas in the Cobb-Douglas model, there were only two significant variables.

The linear model revealed that stocking density, quantity of rice bran used as supplementary feed, pond size and location significantly influenced tilapia production. Assuming all other factors are held constant, tilapia output is increased by 0.03% for every 1% increase in stocking density. Likewise, a 1% increase in rate of rice bran use would correspond to a 0.07% increase in tilapia output. A negative relationship existed between pond size and tilapia output; i.e., for every 1% increase in pond area, there is a 0.00028% decrease in tilapia output. Dummy variables representing location of farms were significant at 5% level of confidence which implies that there are differences in tilapia productivities among the three thanas. The coefficient of inorganic fertilizer rates resulted in the expected economic relationship but was not statistically significant: a 0.00034% increase in tilapia output will be realized when application rates of urea and TSP are increased by 1%. The inorganic fertilizer application rates, currently averaging 80 $kg \cdot ha^{-1}$, are very low.

The Cobb-Douglas production function, which employed the same explanatory variables in the linear form revealed that stocking density and pond size are the factors that significantly influenced tilapia output.

Table 1. Estimated production functions and output (per hectare) of tilapia farms in Trishal, Fulbaria and Mymensingh thanas, Bangladesh, 1989-90. For explanation of variables, see text.

Characteristics		
Functional form	Linear	Log-linear (Cobb-Douglas)
Model specification	$Y = 0.0675 - 0.00079 X_1 + 0.03055 X_2$ (1.13) (-1.26) (2.88)**** $+ 0.00034 X_3 - 0.07300 X_4 + 0.06660 X_5$ (0.50) (-0.14) (1.56)* $+ 0.00009 X_6 - 0.00028 X_7 + 0.00160 X_8$ (0.61) (-3.13)**** (0.38) $+ 0.04203 D_1 + 0.85300 D_2 - 0.01110 D_3$ (1.65)*** (2.85)**** (-0.72)	$\log Y = \log 182.47 - 0.01572 \log X_1 + 0.66145 \log X_2$ (219)** (-2.50) (2.84)**** $- 0.15530 \log X_3 + 0.08162 \log X_4 - 0.03580 \log X_5$ (-1.26) (0.93) (-0.25) $+ 0.18187 \log X_6 - 0.52934 \log X_7 + 0.09413 \log X_8$ (0.89) (-2.12)** (0.27) $+ 0.15592 D_1 + 0.21865 D_2 - 0.05396 D_3$ (1.16) (1.28) (-0.94)
R^2	0.46	0.56
R^2 (adjusted)	0.41	0.41
F-value	7.97****	3.88****
n	113	46
Estimated output at $\overline{X}$	1.890 $t \cdot ha^{-1}$	1.460 $t \cdot ha^{-1}$

Note: Significant levels: ****=1%; ***=5%; **=10%; and *=15%.

Table 2. Marginal physical product, input price and price efficiency of tilapia farms in Trishal, Fulbaria and Mymensingh thanas, Bangladesh, 1989-90[a]. (Using linear model; price of output = Tk 27.09·kg^{-1}; Tk 36=US$1 in 1989.)

	Fingerlings	Rice bran	Inorganic fertilizer
$MPPx_i$	0.0306	0.0670	0.0003
$VMPx_i$	0.8290	1.8150	0.0081
Px_i	0.1900	1.1700	0.0052
$VMPx_i/Px_i$	4.36	1.55	1.56
Input use	increase	increase	increase

[a]$MPPx_i$ = marginal physical product of input i; $VMPx_i$ = value of marginal product of input i or ($MPPx_i$ x price of output); and Px_i = price of input i.

A 1% increase in stocking density would increase tilapia output by 0.66%, assuming all other factors are held constant. Likewise, a 1% increase in pond size would bring about a decline in tilapia output by 0.53%. The sum of all partial output ($ß_i$) elasticities was 0.60 which indicates diminishing returns to scale.

Using the linear model, price efficiencies were calculated for three main inputs (Table 2). The estimates showed that fingerlings, rice bran or inorganic fertilizers were used inefficiently. The values of the marginal physical products of these inputs were greater than their respective prices. This implies that profit from tilapia culture can still be increased by increasing either the stocking density, the application rates of rice bran or the inorganic fertilizers.

There is scope for further increasing the production of tilapia in small waterbodies in Bangladesh. Survey results suggest that given the present prices of inputs and output, increasing the stocking density, the use of rice bran and the rates of inorganic fertilizer application will increase the economic efficiency of tilapia production operations. However, with the subsistence nature of the tilapia farms, increased use of commercial inputs may not be affordable by poor farmers. Input-support and credit facilities may enable farmers to exploit further the production and income from tilapia aquaculture.

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Regional Trends in Tilapia Production and Prices in the Philippines[a]

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Abstract

The tremendous growth in tilapia (*Oreochromis* spp.) production in the Philippines during the 1980s has been attributed to the increasing profitability of tilapia as a cultured fish for producers and its acceptability as a table fish for consumers. Tilapia production from aquaculture registered a 7% annual growth from 1983 to 1990, whereas supply from small-scale fisheries catches declined at 5%. This paper investigates the development prospects of tilapia aquaculture in the twelve regions of the country. The demand for tilapia was analyzed in terms of its own price movements and in relation to price movements of competing fish at the regional markets. The strengths and constraints of promoting tilapia aquaculture in each region of the country have also been assessed.

Introduction

Oreochromis niloticus, introduced in the early 1970s, is the main cultured tilapia species in the Philippines. It has replaced *O. mossambicus* which has been cultured since its introduction in the 1950s. *O. mossambicus* was perceived by producers and consumers as an inferior species (Guerrero 1985). With the farming of *O. niloticus*, tilapia has become a popular species, particularly in freshwater ponds, pens and cages, and there has been tremendous growth in the industry (Bimbao and Ahmed 1990). Tilapia production grew annually at 7% from 62,179 t in 1983 to 97,424 t in 1990 (Table 1). A great percentage of this increase came from aquaculture: its contribution to total tilapia production increased from 49% to 78% during this period. While tilapia production from small-scale inland fisheries declined at 5% from 31,407 t in 1983 to 21,282 t in 1990, aquaculture production increased at 14% from 30,722 t to 76,142 t. The highest production came from freshwater ponds and, in 1990, accounted for 36% of total tilapia production. Tilapia is the main species farmed in freshwater ponds and cages and accounts for 91% and 95%, respectively, of total fish production from these systems. The contribution of tilapia to national aquaculture and total fish production increased from 5% to 11% and from 3% to 6%, respectively, from 1983 to 1990.

This paper examines the trends in tilapia production in different regions in the Philippines. The aquatic resources of the twelve regions are presented and discussed in terms of their development and potential for tilapia culture. Regional trends in prices of tilapia and prices of other alternative fish are also examined.

[a]ICLARM Contribution No. 977.

Table 1. Tilapia production and value by culture system and capture fisheries and its contribution to total fish production and value, Philippines, 1983-1990. (Source: BAS 1991b).

	1983	1984	1985	1986	1987	1988	1989	1990	Growth rate (%)
Tilapia									
Quantity (t)	62,179	54,297	61,836	79,517	93,593	95,006	100,747	97,424	7
Aquaculture	30,772	32,002	43,780	55,836	75,769	75,046	81,675	76,142	14
Freshwater ponds	11,315	11,602	13,874	14,064	26,751	30,149	30,900	35,169	18
Freshwater cages	4,664	7,070	7,187	8,921	16,823	17,249	18,449	18,220	21
Freshwater pens			9,387	16,313	14,264	9,470	9,246	3,948	-16
Brackishwater ponds	14,793	13,330	13,332	16,538	17,931	18,178	23,080	18,805	3
Capture fisheries	31,407	22,295	18,056	23,681	17,824	19,960	19,072	21,282	-5
Value (million pesos)[a]	506	670	1,195	1,904	1,801	2,086	2,462	2,925	28
Aquaculture	250	394	844	1,337	1,459	1,648	1,995	2,327	38
Freshwater ponds	106	146	253	337	542	691	780	1,316	43
Freshwater cages	37	86	131	214	341	395	456	515	46
Freshwater pens			217	391	289	217	233	102	-15
Brackishwater ponds	107	162	243	396	287	345	526	393	20
Capture fisheries	256	275	351	567	342	438	467	598	13
Total fish production quantity ('000 t)	2,110	2,080	2,052	2,089	2,213	2,270	2,371	2,504	2
Aquaculture	445	478	495	471	561	600	629	671	6
Freshwater ponds	12	13	16	16	30	33	34	36	17
Freshwater cages	5	7	7	9	18	18	20	21	24
Freshwater pens	82	82	52	41	36	24	24	24	-16
Brackishwater ponds	184	199	198	207	234	240	254	268	6
Value (million pesos)									
Aquaculture	4,799	7,266	8,724	10,832	11,421	15,213	15,673	20,466	23
Total fish	18,982	25,650	31,297	37,331	37,350	42,118	45,094	52,177	16
% of tilapia production in									Average
Aquaculture	6.9	6.7	8.8	11.8	13.5	12.5	13.0	11.4	10.6
Freshwater ponds	93.9	88.5	87.4	90.7	88.7	91.6	90.2	98.2	91.1
Freshwater cages	100.0	100.0	96.6	95.2	94.6	94.5	94.6	87.1	95.3
Freshwater pens			18.1	39.5	40.1	39.8	38.3	16.2	32.0
Brackishwater ponds	8.1	6.7	6.7	8.0	7.6	7.6	9.1	7.0	7.6
Total fish	3.0	2.6	3.0	3.8	4.2	4.2	4.2	3.9	3.6
% of tilapia value in									
Aquaculture	5.2	5.4	9.7	12.3	12.8	10.8	12.7	11.4	10.0
Total fish	2.7	2.6	3.8	5.1	4.8	5.0	5.5	5.6	4.4

[a]US$1 = P14.002 (1983); P28.000 (1990)

The Philippine Fisheries Profile

The Philippines is an archipelago of 7,101 islands with a land area of 30 million ha. It is divided geographically into three major areas: Luzon, the Visayas and Mindanao, which are further divided into five, three and four regions, respectively (Fig. 1). The aquatic resources of the country comprise marine waters (220 million ha), brackish-waters (swamplands: 232,000 ha; fishponds: 210,319 ha) and freshwaters (lakes: 22 million ha, rivers: 31,000 ha; reservoirs: 19,000 ha; swamplands: 106,238 ha; fishponds: 13,398 ha) (BAR 1989). Marine waters account for 75% of total fish production; compared to freshwater, 15% and brackishwater, 10% (Table 2).

Freshwater aquaculture production is highest in the Southern Tagalog Region which produces 62% of the total regional production followed by Central Luzon at 21% (Table 2). Tilapia is a major species cultured in freshwater ponds in all regions except in the Eastern Visayas. Brackishwater aquaculture production is highest in Central Luzon and the Western Visayas which account for 32% and 30%, respectively, of total regional production. Western Mindanao accounts for almost 77% of regional mariculture production.

Regional Production Trends

Tilapia aquaculture production in all regions posted positive growth rates from 1983 to 1990 although production in some regions fluctuated in some years (Table 3). The bulk of tilapia production came from Central Luzon and the Southern Tagalog Region, accounting for an average of 43% and 26% of total aquaculture production, respectively. Production from the Central Visayas, Eastern Visayas, Northern Mindanao and Central Mindanao each accounted for less then one per cent of the national aquaculture production. These regions, except Central Mindanao, have extensive marine resources which implies that there is little incentive to culture tilapia as a good supply of marine fish exists. On the other hand, although Central Mindanao has one of the most extensive freshwater resources among all regions, tilapia production is insignificant because the region has a relatively small area (115 ha) devoted to fishponds and these are also utilized for common carp (*Cyprinus carpio*) and catfish (*Clarias* spp.) culture.

Luzon, which has the largest developed areas of freshwater and brackishwater ponds, tops all regions in the Visayas and Mindanao in tilapia production. Freshwater ponds in Central Luzon account for the highest contribution (78%) to total production, followed by Ilocos (9%) and Cagayan Valley (6%) (Fig. 1). Tilapia production from cages is highest in the Southern Tagalog Region which contributes 81% of total production. Unlike the other culture systems for which tilapia production is concentrated in Luzon, production from brackishwater ponds is considerable in some regions in the Visayas (Western Visayas, 10%) and Mindanao (Southern Mindanao, 6%; Western Mindanao, 4%), although the highest production still comes from Central Luzon (44%).

Regional Price Trends

Retail Prices in Current and Real Terms

Nationwide, tilapia retail prices in current (nominal) terms increased at an annual rate of 18% from P10.53/kg in 1982 to P25.96/kg in 1988 (Table 4). However, in real terms (deflated or at constant prices; i.e., after adjusting for inflation) they increased by only 2% for the said period. Increases in tilapia retail prices in current and real terms were greater in Mindanao, although

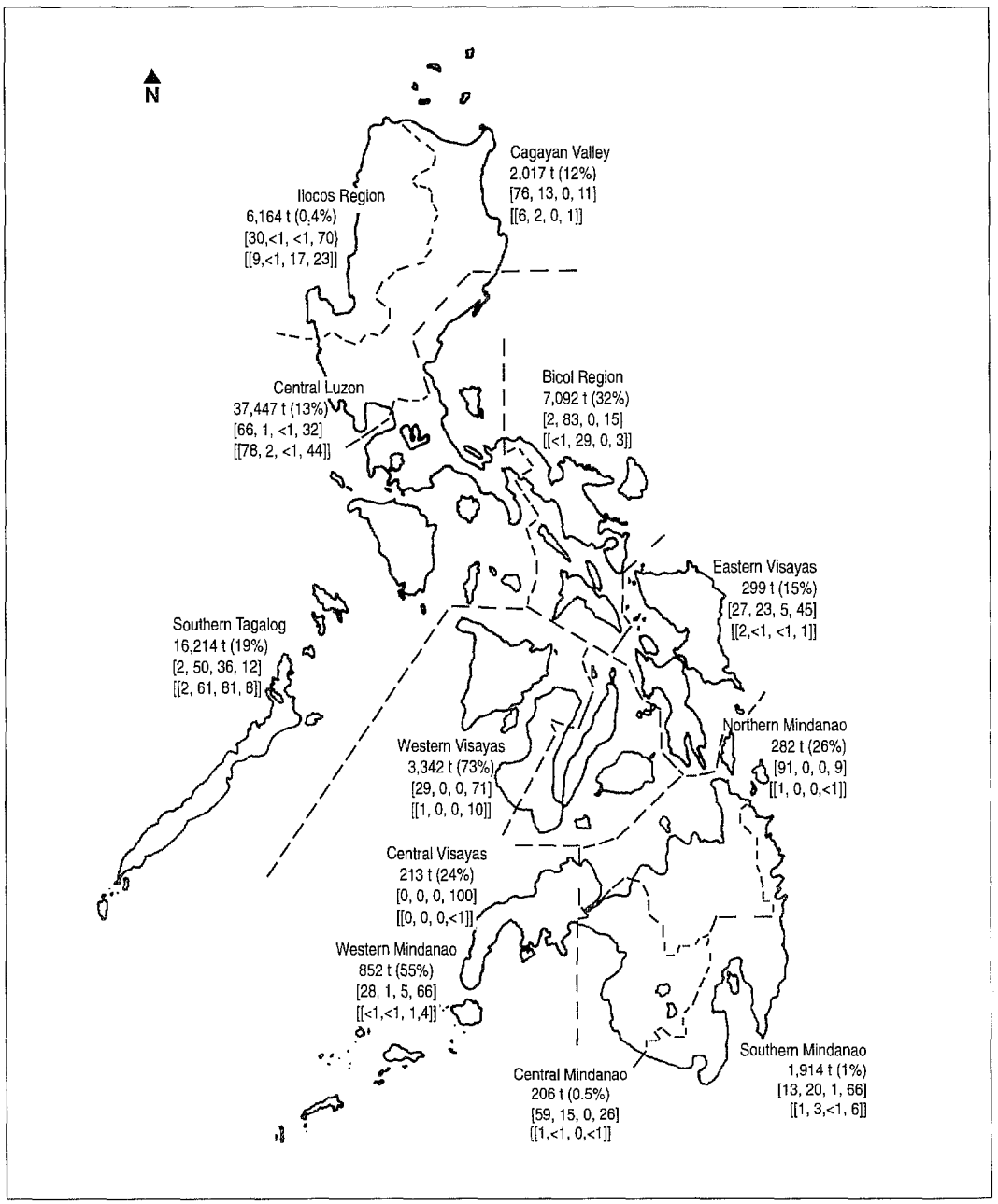

Fig. 1. Map of the Philippines showing 1990 tilapia aquaculture production in t and per cent growth rates (parentheses) for the period 1983 to 1990; and per cent production from freshwater ponds, freshwater cages, freshwater pens and brackishwater ponds to total production [within regions] and [[across regions]]. (Source: BAS 1991b).

Table 2. Regional distribution of aquatic and fisheries resources, Philippines. (Source: BAR 1989).

Region		Freshwater				Brackishwater			Marine	
		Fishponds								
		Developed (ha)	Major species cultured	Communal freshwater (ha)[a]	Production (t)	Developed (ha)	Major species cultured	Production (t)	Major species caught	Production (t)
I.	Ilocos	1,453	tilapia, carp, catfish	24	627 (capture) 1,787 (culture)	16,631	shrimp, milkfish, siganids	25,145 (culture)	tuna and tuna-like species; demersals	22,429 (capture) 9,531 (culture)
II.	Cagayan Valley	1,237	tilapia	2,423	2,825 (capture) 1,793 (culture)	1,469	shrimp, milkfish, crabs	993 (culture)	small- and medium-size pelagics; demersals	13,119 (capture) 8 (culture)
III.	Central Luzon	9,105	tilapia	37,365	5,714 (capture) 12,632 (culture)	5,342	shrimp, milkfish	69,319 (culture)	tuna, flying fish; demersals	28,886 (capture) 514 (culture)
IV.	Southern Tagalog	372	tilapia, carp		205,789 (capture) 37,991 (culture)	23,984	shrimp, milkfish	17,979 (culture)	anchovy; tuna; demersals	384,741 (capture) 18,759 (culture)
V.	Bicol	131	tilapia		3,161 (capture) 4,005 (culture)	11,028	shrimp, milkfish, crabs	7,791 (culture)	small pelagics, tuna; demersals	118,364 (capture) 245 (culture)
VI.	Western Visayas	118	tilapia		2,270 (capture) 685 (culture)	5,894	shrimp, milkfish, crabs	64,952 (culture)	small pelagics; demersals	263,136 (capture) 2,644 (culture)
VII.	Central Visayas		tilapia, carp	99	21 (capture)	7,110	shrimp, milkfish	7,675 (culture)	small pelagics; demersals	51,986 (capture) 10,691 (culture)

[a]Include major river systems, lakes/dams, reservoirs and swamplands/mashes.

continued

Table 2. (continued)

Region		Freshwater				Brackishwater			Marine	
		Fishponds								
		Developed (ha)	Major species cultured	Communal freshwater (ha)[a]	Production (t)	Developed (ha)	Major species cultured	Production (t)	Major species caught	Production (t)
VIII.	Eastern Visayas	93			558 (capture) 253 (culture)	5,740	shrimp, milkfish	2,200 (culture)	small pelagics; coralline species	48,247 (capture) 6,723 (culture)
IX.	Western Mindanao	148	tilapia		1,167 (capture) 374 (culture)	16,375	shrimp, milkfish	9,181 (culture)	tuna, sardines; demersals	262,049 (capture) 170,076 (culture)
X.	Northern Mindanao	385	tilapia	561	2,550 (capture) 739 (culture)	4,289	shrimp, milkfish	2,370 (culture)	small pelagics, tuna; elasmobranchs, coralline species	81,543 (capture)
XI.	Southern Mindanao	241	tilapia	9,284	722 (capture) 573 (culture)	7,241	shrimp, milkfish	6,065 (culture)	tuna, small pelagics; demersals	92,152 (capture) 315 (culture)
XII.	Central Mindanao	114	tilapia, carp, catfish		28,621 (capture) 123 (culture)	3,986	shrimp, milkfish	3,446 (culture)	tuna, small pelagics; demersals	1,749 (capture)

[a]Include major river systems, lakes/dams, reservoirs and swamplands/mashes.

Table 3. Tilapia production from aquaculture and its percentage to total tilapia production by region, Philippines, 1983-1990. (Source: BAS 1991b).

	1983	1984	1985	1986	1987	1988	1989	1990	Growth rate (%)
Philippines	30,772	32,002	43,780	55,836	75,769	75,046	81,675	76,142	13
NCR/MM[a]	2	3	2,082	3,092	3,117	1,800	2,014	1,069	145
I. Ilocos	5,979	4,487	4,894	5,243	5,690	5,828	6,414	6,164	0.4
II. Cagayan Valley	902	1,111	1,308	1,636	1,690	1,625	1,793	2,017	12
III. Central Luzon	15,667	13,318	15,309	18,131	31,453	34,784	35,723	37,447	13
IV. Southern Tagalog	4,926	6,248	14,173	18,991	23,469	19,851	20,821	15,145	19
V. Bicol	1,011	2,418	1,881	4,351	5,881	6,060	6,419	7,092	32
VI. Western Visayas	72	2,026	1,821	1,993	1,726	858	3,792	3,342	73
VII. Central Visayas	46		3	16	13	14		213	24
VIII. Eastern Visayas	110	218	87	152	171	199	261	299	15
IX. Western Mindanao	40	75	821	903	1,196	1,688	1,766	852	55
X. Northern Mindanao	77	246	324	322	331	348	397	382	26
XI. Southern Mindanao	1,741	1,671	862	842	869	1,837	2,093	1,914	1
XII. Central Mindanao	199	181	215	164	163	154	182	206	0.5
% to total tilapia production									Average
NCR/MM[a]	0.01	0.01	4.76	5.54	4.11	2.40	2.47	1.40	2.59
I. Ilocos	19.43	14.02	11.18	9.39	7.51	7.77	7.85	8.10	10.66
II. Cagayan Valley	2.93	3.47	2.99	2.93	2.23	2.17	2.20	2.65	2.70
III. Central Luzon	50.91	41.62	34.97	32.47	41.51	46.35	43.74	49.18	42.59
IV. Southern Tagalog	16.01	19.52	32.37	34.01	30.97	26.45	25.49	19.89	25.59
V. Bicol	3.29	7.56	4.30	7.79	7.76	8.08	7.86	9.31	6.99
VI. Western Visayas	0.23	6.33	4.16	3.57	2.28	1.14	4.64	4.39	3.34
VII. Central Visayas	0.15	0.00	0.01	0.03	0.02	0.02	0.00	0.28	0.06
VIII. Eastern Visayas	0.36	0.68	0.20	0.27	0.23	0.27	0.32	0.39	0.34
IX. Western Mindanao	0.13	0.23	1.88	1.62	1.58	2.25	2.16	1.12	1.37
X. Northern Mindanao	0.25	0.77	0.74	0.58	0.44	0.46	0.49	0.50	0.53
XI. Southern Mindanao	5.66	5.22	1.97	1.51	1.15	2.45	2.56	2.51	2.88
XII. Central Mindanao	0.65	0.57	0.49	0.29	0.22	0.21	0.22	0.27	0.36
Luzon (I-V and NCR/MM)	92.57	86.19	85.80	86.60	89.99	90.81	87.14	89.13	88.53
Visayas (VI-VIII)	0.74	7.01	4.37	3.87	2.52	1.43	4.96	5.06	3.75
Mindanao (IX-XII)	6.68	6.79	5.08	4.00	3.38	5.37	5.43	4.40	5.14

[a]National Capital Region/Metropolitan Manila

price levels in these regions were lower than in Luzon.

With reference to Metropolitan Manila, considered here as the central market, retail prices in some regions in Luzon were generally above Metropolitan Manila prices (Table 4). From 1983 to 1989, Metropolitan Manila retail prices were lower by 73% (current) and 63% (real) compared with prices in Ilocos; 77% (current) and 79% (real) in Cagayan Valley; and 90% (current) and 76% (real) in Central Luzon. Tilapia retail prices in the other regions were lower compared to Metropolitan Manila prices with Central Visayas registering the lowest prices, representing only 47% of the prices in Metropolitan Manila.

Wholesale Prices In Current and Real Terms

Tilapia wholesale prices in current and real terms increased at 20% and 6%, respectively, from 1982 to 1989, the same growth registered for retail prices (Table 4). Tilapia wholesale prices in current terms in Luzon posted an average growth of 18%, in Visayas, at 19%, and in Mindanao, at 36%. However, in real terms, the Southern Tagalog Region and Central Visayas registered negative growth rates - which means the increases in wholesale tilapia prices were lower than the general increase in prices in these regions.

Table 4. Tilapia retail and wholesale prices and price margins (Pesos[1]/kg) in current and real terms by region, Philippines, 1982-1988. Retail price data for Eastern Visayas and wholesale price data for the National Capital Region/Metropolitan Manila (NCR/MM) are not available. (Source: BAS 1982-1988, unpublished data).

	1982	1983	1984	1985	1986	1987	1988	Average annual growth rate (%)	1982	1983	1984	1985	1986	1987	1988	Average annual growth rate (%)
	Retail price in current terms								Deflated retail price in real terms							
Philippines	10.53	11.25	17.85	21.94	23.57	23.50	25.96	18	19.91	20.42	21.85	21.94	23.29	22.82	22.89	2
NCR/MM	21.95	11.57	18.14	21.63	22.60	24.54	32.87	13	39.20	20.23	21.19	21.63	21.36	22.29	25.07	-4
Ilocos	15.68	15.84	23.50	27.94	27.97	31.78	36.76	18	31.05	29.72	30.56	27.94	27.83	31.34	33.88	2
Cagayan Valley	14.67	14.61	22.23	28.79	28.88	29.69	35.94	18	23.55	23.15	28.32	28.79	24.92	25.01	28.55	4
Central Luzon	12.47	13.18	21.74	25.31	25.73	25.39	30.02	18	26.65	26.20	27.52	25.31	27.64	26.70	29.72	2
Southern Tagalog	10.56	11.04	17.46	19.28	18.16	18.57	23.34	16	19.45	19.33	21.29	19.28	18.25	18.30	20.93	2
Bicol	9.75	10.31	14.42	17.73	19.13	18.79	20.58	14	17.73	19.06	18.09	17.73	18.83	18.14	17.74	<1
Western Visayas	6.95	7.68	13.91	17.52	21.22	16.74	18.66	22	14.10	14.46	18.16	17.52	14.12	16.32	16.78	-4
Central Visayas	5.68	5.19	9.23	9.03	11.39	12.84	18.17	25	9.36	7.97	10.27	9.03	11.56	12.37	14.89	10
Western Mindanao	6.25	7.32	14.63	15.79	16.84	18.02	17.45	23	12.11	13.71	18.33	15.79	17.06	18.99	17.68	8
Northern Mindanao	4.44	7.71	18.60	14.33	19.74	15.15	21.16	41	8.38	14.44	22.85	14.33	19.15	14.54	21.61	25
Southern Mindanao	7.39	7.97	12.69	18.97	20.05	20.91	21.99	22	14.72	15.30	15.76	18.97	19.45	19.49	18.70	4
Central Mindanao	4.44	7.76	12.75	21.07	21.24	20.29	20.38	34	8.06	13.52	15.42	21.07	20.58	19.18	18.07	17
	Wholesale price in current terms								Deflated wholesale price in real terms							
Philippines	5.88	9.86	10.81	16.31	18.12	17.00	14.61	20	11.12	17.89	13.23	16.31	17.91	16.50	12.88	6
Ilocos	8.52	11.55	20.10	23.90	23.93	24.99	18.75	18	16.87	21.67	26.14	23.90	23.81	24.64	17.28	14
Cagayan Valley	8.82	11.96	18.20	23.54	23.61	27.04	21.21	18	14.16	18.95	23.18	23.54	20.37	22.78	16.85	5
Central Luzon	6.78	6.54	15.06	12.78	24.19	23.14	17.10	26	14.49	13.00	19.06	23.78	25.98	24.38	16.93	6
Southern Tagalog	7.30	10.57	12.35	14.99	15.00	14.17	11.86	10	13.44	18.51	15.06	14.99	15.08	13.96	10.64	-2
Bicol	5.71	7.46	10.76	12.92	15.07	14.62	14.31	18	10.38	13.79	13.50	12.92	14.83	14.11	12.34	4
Western Visayas	4.12	5.35	8.73	11.84	14.21	12.63	12.77	23	8.36	10.08	11.40	11.84	9.45	12.31	11.48	7
Central Visayas	5.33	4.87	8.66	8.47	9.55	8.77	6.60	8	8.78	7.48	9.63	8.47	9.70	8.45	5.41	-5
Eastern Visayas	5.34	9.13	4.70	11.46	11.84	12.22	9.51	25	10.68	17.49	5.47	11.46	13.05	13.49	9.83	16
Western Mindanao	5.76	6.75	5.61	13.00	14.33	15.68	12.42	44	11.16	12.64	7.03	13.00	14.52	16.52	12.58	9
Northern Mindanao	3.38	5.87	6.12	8.77	10.70	12.62	13.36	28	6.38	10.99	7.52	8.77	10.38	12.11	13.65	18
Southern Mindanao	5.14	5.54	6.42	8.76	12.63	16.50	14.57	21	10.24	10.63	7.98	8.76	12.25	15.38	12.39	6
Central Mindanao	3.02	5.84	2.38	4.11	11.66	14.56	13.96	52	5.48	10.17	2.88	4.11	11.30	13.76	12.38	41
	Price margin in current terms								Deflated price margin in real terms							
Philippines	4.65	1.39	7.04	5.63	5.45	6.50	11.35	68	8.79	2.52	8.62	5.63	5.39	6.31	10.01	35
Ilocos	7.16	4.29	3.40	4.04	4.04	6.79	18.01	38	11.48	8.05	4.42	4.04	4.02	6.70	16.60	20
Cagayan Valley	5.85	2.65	4.03	5.25	5.27	2.65	14.73	17	9.39	4.20	5.13	5.25	4.55	2.23	11.70	55
Central Luzon	5.69	6.64	6.68	1.53	1.54	2.25	12.92	77	12.16	13.20	8.46	1.53	1.65	2.37	12.79	64
Southern Tagalog	3.26	0.47	5.11	4.29	3.16	4.40	11.48	176	6.00	0.82	6.23	4.29	3.18	4.33	10.30	115
Bicol	4.04	2.85	3.66	4.81	4.06	4.17	6.27	11	7.35	5.27	4.59	4.81	4.00	4.03	5.41	-3
Western Visayas	2.83	2.33	5.18	5.68	7.01	4.11	5.89	23	5.74	4.39	6.76	5.68	4.66	4.01	5.30	2
Central Visayas	0.35	0.32	0.57	0.56	1.84	4.07	11.57	100	0.58	0.49	0.63	0.56	1.87	3.92	9.48	59
Western Mindanao	0.49	0.57	9.02	2.79	2.51	2.34	5.03	255	0.95	1.07	11.03	2.79	2.54	2.47	5.10	161
Northern Mindanao	1.06	1.84	12.48	5.56	9.04	2.53	7.80	132	2.00	3.45	15.33	5.56	8.77	2.43	7.97	94
Southern Mindanao	2.25	2.43	6.27	10.21	7.42	4.41	7.42	38	4.48	4.66	7.79	10.21	7.20	4.11	6.31	19
Central Mindanao	1.42	1.92	10.37	16.96	9.58	5.73	6.42	78	2.58	3.34	12.54	16.96	9.28	5.42	5.69	43

Tilapia wholesale prices in current and real terms in all regions were also compared to prices in Central Luzon, considered here as the central point of production (Table 4). Compared with prices in Central Luzon, the prices in Ilocos were higher at an average of 21% (current) and 16% (real) and in Cagayan Valley by 24% (current) and 6% (real). Tilapia wholesale prices in the other regions were lower than the prices in Central Luzon: Central Visayas registered the lowest where prices, on average, were 39% (current) and 56% (real) lower than prices in Central Luzon.

Price Margins in Current and Real Terms

Price margins, computed here as the difference between retail and wholesale prices, either indicate the profits or the extent of marketing activities from the two points of sale. Tilapia price margins in current terms averaged P5.57/kg in Mindanao and P5.36/kg in Luzon for the period 1982 to 1988 (Table 4). The price spread in the Visayas is lower at P3.74/kg.

Across regions, tilapia price margins in current terms ranged from P2.75/kg in Central Visayas to P7.49/kg in Central Mindanao (Table 4). However, in real terms, price margins were highest in Ilocos at P8.29/kg and lowest again in Central Visayas at P2.50/kg.

Comparative Price Analysis with Other Fishes

Price Trends of Tilapia and Competing Fish

Price trends of four competing fish were compared with tilapia prices. The criterion for competition is that fish is readily available at regional markets. The preference of consumers was stratified by income level. Tilapia is a second class fish and was compared to two other fish on the same group: fusilier (*Caesio* spp.; locally called *dalagang bukid*) and threadfin bream (*Nemipterus* spp.; *bisugo*). Tilapia was also compared to milkfish (*Chanos chanos*; *bangus*) and roundscad (*Decapterus macrosoma*; *galung-gong*), considered first and third class fish, respectively.

In most regions in Luzon, tilapia prices from 1982 to 1989 moved closely with fusilier, threadfin bream and milkfish prices (BAS 1982-1989). However, in the Visayas and Mindanao (except for Central and Eastern Visayas), tilapia prices moved closely with roundscad prices. This implies that tilapia is less attractive in these regions than in Luzon. This can be attributed to the wide range of alternative marine fish in the Visayas and Mindanao. In terms of yearly fluctuations, tilapia prices exhibited a similar behavior to other fish except for Northern Mindanao where fluctuations in tilapia prices were more pronounced compared to other fish prices.

Price Ratios of Tilapia and Competing Fish

Generally, fusilier retail prices were higher than tilapia prices (Table 5). However, the trend from 1982 to 1989 showed that tilapia retail prices have increased relative to fusilier prices. In 1989, fusilier/ tilapia price ratios dropped to less than 100% in four regions in Luzon which means that tilapia prices are higher in these regions. On the other hand, although fusilier/tilapia price ratios were declining since 1982, fusilier retail prices were still higher than tilapia prices in the Western Visayas and the regions in Mindanao. These price changes indicate an increase in tilapia demand relative to fusilier.

Threadfin bream/tilapia retail price ratios also showed a declining trend from 1982 to 1989 (Table 5). But in 1989, threadfin bream retail prices were still greater than tilapia prices, except in the Cagayan Val-

Table 5. Deflated retail price ratios of tilapia [*Oreochromis* spp.] with competing species (fusilier [*Caesio* spp.], threadfin bream [*Nemipterus* spp.], milkfish [*Chanos chanos*] and roundscad [*Decapterus macrosoma*]) in regional markets, Philippines, 1988-1989. (BAS 1982-1989)

	1982	1983	1984	1985	1986	1987	1988	1989	1982	1983	1984	1985	1986	1987	1988	1989
	Fusilier/tilapia deflated retail price ratio								Threadfin bream/tilapia deflated price ratio							
Philippines	118	124	116	111	110	117	113	84	130	143	139	129	122	123	113	106
Ilocos	103	106	98	98	113	104	99	85	114	114	114	114	128	116	108	106
Cagayan Valley	98	108	112	101	107	108	101	90	104	116	113	110	111	106	97	92
Central Luzon	105	101	106	103	106	108	105	104	155	148	139	131	132	132	114	117
Southern Tagalog	118	109	123	127	137	139	112	88	143	146	153	158	151	145	123	119
Bicol	116	108	108	100	90	105	113	87	110	104	109	108	99	108	112	114
Western Visayas	172	178	125	130	121	135	124	98	177	162	155	148	130	142	136	128
Central Visayas	207	207	200	218	203	186	150	157	198	208	195	217	175	164	130	190
Western Mindanao	175	140	113	123	99	104	129	107	180	150	124	133	121	118	135	131
Northern Mindanao	274	157	102	155	124	178	138	129	278	166	112	165	138	178	134	167
Southern Mindanao	201	193	170	138	136	163	167	125	227	207	169	138	143	142	141	148
Central Mindanao	331	186	168	113	122	125	152	135	316	183	178	125	127	147	161	135
	Milkfish/tilapia deflated retail price ratio								Roundscad/tilapia deflated retail price ratio							
Philippines	121	112	120	122	125	127	126	131	84	88	99	94	88	85	88	79
Ilocos	104	93	106	119	130	117	115	127	67	66	84	82	78	74	78	71
Cagayan Valley	105	101	111	115	114	121	112	116	68	67	81	82	80	80	76	74
Central Luzon	115	108	113	114	123	125	118	131	54	60	41	34	70	85	80	80
Southern Tagalog	130	120	130	140	167	154	135	129	95	88	111	115	116	105	88	85
Bicol	143	130	141	100	136	143	142	157	96	105	113	110	109	107	107	100
Western Visayas	157	149	138	140	137	153	154	165	124	111	118	105	98	97	103	99
Central Visayas	177	193	178	207	183	167	131	192	170	181	176	194	173	141	121	145
Western Mindanao	170	144	122	130	124	123	138	145	115	100	96	94	82	71	89	80
Northern Mindanao	274	160	104	152	125	172	140	158	231	126	91	120	96	134	108	101
Southern Mindanao	177	167	167	139	137	136	136	142	119	118	114	94	94	96	100	88
Central Mindanao	295	174	161	114	123	140	159	173	227	139	136	99	101	94	121	125

ley where threadfin bream prices were lower by 8.4%. Regions in the Visayas and Mindanao have higher threadfin bream/tilapia retail price ratios compared to other regions.

Milkfish/tilapia retail price ratios exhibited a fluctuating but increasing trend from 1982 to 1989 except in Southern Tagalog and the regions in Mindanao (Table 5). On average, milkfish retail prices were higher than tilapia prices by 24% in Luzon, 64% in the Visayas and 49% in Mindanao.

Roundscad retail prices were generally below tilapia prices in Ilocos, Cagayan Valley and Central Luzon; were higher in the Central Visayas and Central Mindanao, but had mixed movements in other regions (Table 5). Price ratios of greater than 100% for the Central Visayas and Central Mindanao indicate that roundscad is preferred to tilapia in these regions.

Discussion

Growth in tilapia production in the past decade has come from aquaculture. This is expected to continue as catches from inland fisheries decline. Tilapia is the main species in freshwater ponds, cages and pens throughout the country except for the Eastern Visayas. Production of tilapia is greatest in Luzon particularly in Central Luzon which is predominantly a landlocked region, and Southern Tagalog which is endowed with extensive inland fisheries resources. In the Visayas and Mindanao, tilapia production is low and its culture is not very popular. These regions have extensive marine resources which offer little incentive to producers to engage in tilapia culture and consumers have a wide range of choices of marine fish.

Generally, tilapia prices have moved closely with prices of roundscad, fusilier and threadfin bream but regional differences exist: tilapia retail prices in Luzon have been distinctly higher than roundscad prices. However, tilapia retail prices have been close to roundscad prices in Mindanao. This implies that tilapia is less preferred by consumers in Mindanao, where it is still considered a third class fish. Retail price trends from 1982 to 1989 showed that increases in tilapia prices were greater than the price increases of fusilier and threadfin bream, although tilapia prices continued to be lower. However, in 1989, tilapia prices in Luzon were already above fusilier prices. This may imply that the demand for tilapia is increasing relative to fusilier and threadfin bream. Price trends with milkfish showed that milkfish prices increased more than tilapia prices and tilapia prices were far below milkfish prices. Although this may give an impression that milkfish has not been influenced by tilapia, on the contrary, the decline in milkfish production from pens in 1985 was attributed to the shift from milkfish to tilapia and carp by the fishpen operators in Laguna de Bay, a 90,000-ha freshwater lake in Southern Tagalog, Luzon (BAS 1991a).

Differences in tilapia production and prices signify that the demand for tilapia varies across the twelve regions of the country. Generally, production and prices are higher in Luzon compared to the Visayas and Mindanao. Although a suitable resource base for tilapia culture is abundant throughout the country, the growth of the industry has been slow in the Visayas and Mindanao, largely because of the wide range of marine fish available in these regions. In Luzon, tilapia has a significant market because of the relatively large demand for fish in relation to supply from marine waters. An analysis of the price trends of tilapia with other fish species showed that tilapia can compete in the fish markets. Generally, increases in tilapia prices were greater relative to the increases in some alternative fish and this is a promising signal of increasing demand for tilapia.

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Tilapia Culture in the Senegal River Basin and the Causes of its Failure

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Abstract

Although tilapia culture in the Senegal river basin was promising at its beginnings, it now faces major difficulties (production < 50 tonnes). In spite of the many positive elements such as a vast water potential, abundance of by-products from agricultural and animal farming, and a deficit of about 21,000 t in fish supply from the river watershed, tilapia culture has not been successful. Its development has been affected by many constraints: (1) environmental—irregularity in the natural water system, poor mineral content in the rivers, high turbidity during the rainy season and low water temperatures from November to March; (2) technical—poor choice of sites, insufficient depth of the fishponds and inadequate dike construction, poor water quality control and poor fry quality; (3) administrative—poor definition of responsibilities, insufficient management support services and constant confusion in the experimental and extension objectives; and (4) socioeconomic—farmers' perception of fish farming as a secondary activity, high costs of construction and management, and competition with the fisheries sector. However, the construction of the Diama and Manantali dams seems to offer better prospects.

Introduction

After nearly 50 years of experiment, fish culture in many African countries has yet to realize its potential (Lazard et al. 1990). The production from fish farming for the African continent (62,000 t·year^{-1}) and particularly, for subSaharan Africa (10,000 t·year^{-1}) is low compared to the world production of 13 million t·year^{-1} (FAO 1989).

Although fish culture in Senegal gave early promising results, annual production does not exceed 100 t. The effort to develop tilapia culture in the Senegal river basin was encouraged by several factors. In the past two decades, the Senegal river basin had insufficient rainfalls and the land and water management strategies that were implemented drastically reduced flooded areas (Lazard 1981; Denneville and Jamet 1982; Diouf and Bousso 1988). From 400,000 ha in 1969, today these areas cover less than 100,000 ha (OMVS 1986). The reduction of flooded areas resulted in diminishing fish catches. From approximately 20,000 t in 1969 (Fall 1980), catches dropped to 8,000 t in 1988 (Diouf et al. 1991). However, the demand in fish continued to increase due to population growth. At present, the demand in fish for this region of Senegal is estimated at 21,000 t·year^{-1} (Diouf et al. 1991) considering the ideal consumption at 36.5 kg·head·year^{-1} (Lazard 1981). Imports of marine fish from other regions of Senegal hardly make up a third of this deficit (Diouf et al. 1991).

Although the distribution channels for marine fish have become more efficient over the last few years, fish supply is still irregular in the landlocked areas

such as the Bakel region (Fig. 1), especially during the rainy season, because of processing and transportation problems. In these regions, marine fish is expensive (Table 1) and often of poor quality (Diouf et al. 1991). In these areas, freshwater fish, particularly tilapia (from capture fisheries and fish farming) is preferred.

The average price of meat in the river basin is relatively high, especially in urban areas where it reaches approximately 800 F CFA·kg^{-1} (US$2.7). In rural areas, meat consumption is low because of social traditions. In this context, fish culture is needed.

Tilapia (essentially, *Oreochromis niloticus*) was chosen by developers because it is appreciated by consumers: it is a resistant species and farming techniques are relatively well-understood.

This article describes briefly the Senegal river basin environment, reviews its history of tilapia culture and assesses strengths and weaknesses.

The Environment

The Senegal river, approximately 1,800 km in length, is the seventh largest river in Africa and the second largest in West Africa. Its watershed covers an area of 340,000 km^2.

There are significant variations in the rainfall pattern across the basin: from 2,000 mm in the southern parts to approximately 300 mm at its northern limit.

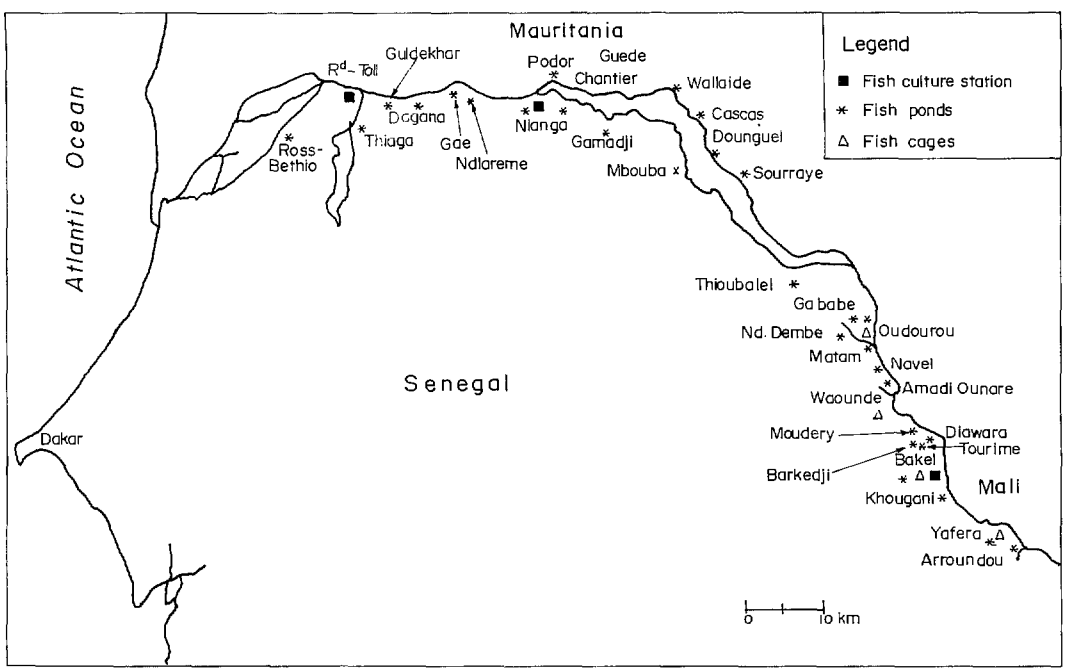

Fig. 1. Location of fish culture stations, ponds and fish cages along the Senegal River.

Table 1. Average retail price (F CFA)/kg of some marine fishes in Dakar (for comparison), Podor, Matam and Bakel between 1986 and 1987. (Source: Chaboud and Kébé 1990).

Species	Scientific names	Dakar	Podor	Matam	Bakel
Bonga	*Ethmalosa fimbriata*	96	138	227	400
Round sardinella	*Sardinella aurita*	107	176	232	295
Flat sardinella	*Sardinella maderensis*	77	188	231	296
Barracuda	*Sphyraena* spp.	426	600	481	589
Mullet	*Liza* spp. and *Mugil* spp.	280	383	425	540
White grouper	*Epinephelus aeneus*	554	711	752	603
Silver mullet	*Pomadasys jubelini* and *P. peroteti*	276	376	591	573
Cassava croaker	*Pseudotolithus senegalensis*	233	500	484	550
Meagre	*Argyrosomus regius*	435	699	689	576

US$1.00=300 F CFA at the time of the study.

In this region, annual rainfall patterns are highly irregular, particularly in the north.

The climatic history of this basin is characterized by a succession of dry and humid periods (Olivry 1982; Sow 1984; Kane 1985). At present, there is a persistent pattern of low rainfall.

Annual water evaporation is high. Data recorded with a Piche evaporometer over several decades showed mean values of 2,950 mm at Saint-Louis where the air moisture is relatively high, 3,220 mm at Matam and 3,550 mm at Rosso (Platon 1981).

The Senegal River system, classified as tropical by Frecault (1982), Gac and Kane (1985), and Kane (1985), is characterized by extreme year-to-year irregularity. The dams at Diama (Fig. 1) and at Manantali (Mali) will greatly modify this natural system. The objective of the Organization for the Development of the Senegal River (OMVS) is to substitute flood-recession farming by irrigated farming. However, to avoid drastic changes which would cause important socioeconomic problems, it was decided during the first years of operation that specific discharges (artificial flooding) would be allowed at Manantali to create and to maintain the necessary conditions for the pursuit of flood-recession cultures.

With regard to salinity, before the construction of the dams, freshwater covered the entire Senegal river basin in times of floods. But, from the second half of October, saltwater started to penetrate.

From the construction of the Diama dam, the tail-bay has operated as an evaporation basin and salinity has progressively increased up to 40-45 ppt. Upstream, freshwater is found as long as the dam remains closed.

The Senegal River water temperature shows seasonal variations due to two existing air temperature systems: a warm season (June-November) with water temperatures ranging from 30-33°C and a cool season (December-April) with temperatures ranging approximately from 16-26°C. Maxima of approximately

45°C and minima of approximately 12°C are recorded in ponds and shallow areas.

The valley and delta waters show different chemistry profiles. In the valley where there is freshwater all-year round, the water is tetraionic, poor in chloride, sulfate and minerals (Reizer 1974). The river productivity is low as a result. During floods, the delta waters show the same characteristics. In contrast, the seawaters in the delta during flood recession are hexaionic and rich in minerals. There is a progressive decrease in mineral content from downstream to upstream waters.

History of Tilapia Culture in the Senegal River Basin

Pond Culture (USAID and Catholic Relief Service)

PHASE I (1979-1981)

The history of tilapia culture started in the Senegal river basin with the signing in August 1979 of an agreement between USAID and the Senegalese Government for the funding of the "Intensive Fish Culture Accelerated Impact Project." The first phase of this project (December 1979 to December 1981), called "pilot" phase, involved major partners such as USAID, the Department of Forestry and Water Resources, the US Peace Corps and village-based cooperatives. In 1980, the Richard-Toll station was established with two 2,500-m^2 and four 500-m^2 ponds stocked with broodfish of *O. niloticus* from Côte d'Ivoire (FAO Project, Bouake) which in 1981 produced 50,000 fry to be used in demonstration ponds.

Also in 1980, 30,000-40,000-m^2 fishponds were constructed in the villages of Gaya, Ndiareme, Nianga, Guede, Gamadji and Mboumba (Fig. 1) stocked in October-November with fry from the Richard-Toll station at a density of 1.25-1.7 fry·m^{-2}.

In March 1981, the project was evaluated before the ponds were harvested for the first time. According to the conclusions of this evaluation, the project was working well and chances of success were high.

After four-and-a-half months, the fish weighed between 90 and 125 g. Results of the first year of experiment were encouraging with mean yields of 1.2 t·ha^{-1} (Freudenberger 1988).

As reports on this period are not available, it is impossible to reconstruct the details concerning the management of these ponds. However, our inquiries show that feeding and fertilization were essentially based on rice bran and organic fertilizers.

PHASE II (1982-1984)

At the end of the first phase, the USAID funded a second two-year phase under the Bakel Irrigated Areas Project. During this period, the participation of the Senegalese Government was entrusted to the Société pour l'aménagement et l'exploitation des terres du Delta (SAED).

During this period, two new fish farming stations were constructed: Bakel (1982-1983) and Nianga (1983-1984). Forty additional demonstration ponds were constructed, but most of them had to be abandoned because of their disappointing results.

To reduce competition with marine fish, fish culture initiatives were moved to the eastern and central areas of the Senegal river basin.

The Bakel station possessed two 3,500-m^2 and one 7,500-m^2 ponds. It produced only 15,000 fry for the ponds of Arroundou, Koungani, Yafera and Wallalde (Fig. 1).

The Bakel station experienced serious difficulties due to water infiltration and management problems. The ponds

were managed by a fisheries cooperative with the assistance of a volunteer worker from the US Peace Corps. Some members of the group, relying on their own experience in fisheries and "knowledge of fish," refused to follow the recommendations of the volunteer worker. Following poor harvests in 1984, the Bakel station was abandoned.

During this second period, the quality of the fry produced at the Richard-Toll station decreased due to the high stocking densities of the broodfish.

Paradoxically, the number of demonstration ponds increased dramatically in the villages while harvests remained less than adequate.

Although information on feeding during this period is insufficient, Lazard (1984) indicated that a mix containing 90% rice bran and 10% fish meal was used at the Richard-Toll station.

PHASE III (1985-1988)

The ponds of the Nianga station were stocked at the end of 1984 and beginning 1985 with *O. niloticus* coming from Richard-Toll, Lake Guiers, the canals around Podor and the Guidekhar pond.

The station produced 20,000 fry during the first year of operation. One of the breeding ponds was stocked during the first season with *Sarotherodon galilaeus*. No data are available on the results of this operation.

During Phase III, the station supplied fry to the neighboring ponds and cages, although insufficiently.

At Nianga, as in the village demonstration ponds, the feed contained 80% rice bran and 20% fish meal. Other mixes were used (blood meal, peanut cake, etc.) without much success.

Experiments conducted at Nianga showed that a density of 2 fry·m^{-2} gave better yields than 1 fry·m^{-2}. Yields obtained with the former density were 1.66 times higher than with the latter (2.427 t·ha^{-1}·year^{-1} against 1.462 t·ha^{-1}·year^{-1}). In contrast, the density of 2 fry·m^{-2} was 1.4 times less profitable because of the added feeding and fertilizer loads (Freudenberger 1988).

Moreover, small ponds (2,750 m^2) were more productive (1.462 t·ha^{-1}·year^{-1}) than large (33,000 m^2) ponds (0.850 t·ha^{-1}·year^{-1}).

In 1985, four years after the beginning of the project, a second evaluation revealed that results were much poorer than expected.

In March 1985, the USAID stopped funding the project. This period coincided with vast social and management problems. Funding by the Catholic Relief Services (CRS) resumed in March 1985.

Based on a new evaluation (Freudenberger 1988), CRS also decided to stop funding the project. The conclusions of the evaluation were as follows:

> "The fish farming project has largely missed its primary objective which was to show concrete proof of its feasibility in the form of successful demonstration ponds, and to show such proof to the different farmers by creating an efficient demonstration program. Neither did this project reach its objectives to help the local farmers increase the quantity and the availability of proteins in their diet, nor did it succeed in increasing their individual income."

Cage Culture

Due to the difficulties encountered in facing the costs of water and pond construction, cage culture experiments were started in 1984 in Bakel and Waounde. Cages were constructed using PVC pipes and wire netting coated with a rust-proof plastic material. Because

of strong currents, the cages did not last for more than a month. Research studies were done to find a solution to this problem and new techniques were tested in June 1985, leading to the creation of new cages made of nylon nets and iron bars.

The fish feed was essentially composed of a mix of 80% rice bran and 20% fish meal. Results were not encouraging.

Extensive Fish Culture

Trials in extensive fish farming were started in the Guia flood plain near the Nianga station. On 2 October 1986, the area was stocked with 2,000 fry of *O. niloticus*. The fish were exclusively fed with rice bran. At the end of June 1987, 895 fish or 24.6 kg were harvested, which discouraged farmers.

Rice-fish Culture

Attempts to develop rice-fish culture were made by volunteers of the US Peace Corps at Ndiareme (Dagana). The project was funded by the USAID and was supported by various public and private organizations such as SAED, the West Africa Rice Development Association (WARDA), the Senegalese Institute for Agricultural Research (ISRA), the Department of Forestry and Water Resources, the Senegalese Sugar Company (CSS), FAO, the US Peace Corps and USAID.

The project used *O. niloticus* and a cold-tolerant, usually high-yielding rice variety (KN-1H-350) from Indonesia.

According to Chopak (1983) and Bloom (1986), all objectives were achieved. Unfortunately, after two seasons of experiment (1982-1983), the USAID stopped funding the project despite encouraging results (Tables 2 and 3).

The Matam III Project

Under the Water and Agriculture Development Project of the district of Matam, Phase III, an aquaculture project was developed. This project, Matam III, started in September 1986 and was funded by the Caisse centrale de coopération économique, and developed by SAED and the Association française des volontaires du progrès (AFVP). The Department of Forestry and Water Resources was responsible for the execution and the administrative support of the project. Four fish farms were created. Construction works were of poor quality: pond depth was insufficient, dikes were prone to erosion and monk drains were poorly built.

In October 1990, only two complete production cycles of marketable fish had been achieved and two other cycles were ongoing. The evaluation of October 1990 (Parrel 1990) revealed that:

- out of the 10 fish farms previously expected, only four were constructed due to the difficulties in finding available and competent firms to undertake such constructions;
- none of the farms was fully operational and infrastructures were of relatively poor quality;
- the support services encountered real difficulties in efficiently mobilizing the fish farmers whose training was insufficient;
- yields were disappointing; and

Table 2. Comparative yields from rice culture, fish culture and rice-fish culture in Senegal. Source: Chopak (1983).

Type of culture	Yield ($t \cdot ha^{-1}$) Fish	Rice
Rice culture	-	4.800
Fish culture	2.134	-
Rice-fish culture	2.098	4.500

Table 3. Costs and returns (for a one-hectare pond, ricefield and rice-fish culture field). All costs are in CFA francs. US$1.00=300 F CFA at the time of the study. Source: Chopak (1983).

Item	Rice culture	Fish culture	Rice-fish culture
I. Equipment			
- Machine rental and labor	90,000	188,000	218,800
- Material	20,000	58,200	49,200
Total	110,000	246,200	268,000
II. Operational costs (per season)			
- Water	25,000	30,000	40,000
- Fry	-	20,000	20,000
- Fertilizer	37,700	87,000	107,200
- Feed (rice bran)	-	60,000	60,000
- Rice seed	9,900	-	9,900
- Machinery	70,306	-	70,306
- Marketing	5,000	7,500	15,000
- Labor	160,000	162,000	190,000
- Rental	-	-	-
Total	307,906	366,500	512,406
III. Returns			
- Fish sales (150 CFA·kg^{-1})	-	320,100	314,700
- Rice sales (66.6 CFA·kg^{-1})	319,680	-	299,700
Total	319,680	320,100	614,400

– the project team lacked "professionalism."

According to Parrel (1990):

> "The current failure of this project is therefore not that of fish culture, inasmuch as aquaculture is successfully developed in Niger (Lazard et al. 1990) in similar climate and physical conditions, and that the production techniques of *O. niloticus* are now well-understood for this type of culture. This failure is therefore more the failure of a particular operation and to accept failure, after that of the Peace Corps project, means to condemn the development of fish farming in Matam for many years."

In an attempt to give the development of fish farming another chance, Parrel (1990) proposed an extension of the Matam III fish culture project until 31 December 1992 provided that a new approach was taken with necessary changes.

Strengths and Weaknesses of Tilapia Culture In the Senegal River Basin

Strengths

The Senegal river basin holds a considerable water potential. In addition to the 1,800 km-long main watercourse, the river basin includes lakes, the most important of which, from the viewpoint of Senegalese aquaculture, are the Lake

Guiers and the hill lakes. In addition to these water bodies, there are many ponds (DEFC 1988). Moreover, agriculture and animal farming are highly developed in this region and the by-products necessary to fish farming are therefore available. However, the competition for food between cattle and farmed fish may occasionally create some problems. In the Senegal river basin, the shortage in fish is great and there is therefore a potential market for cultured fish.

The construction of the Diama and Manantali dams constitutes a positive factor for tilapia culture for several reasons:

- water supply becomes regular and cheap;
- agriculture development is possible and consequently by-products used for tilapia culture increase; and
- the likely increase in revenues for the farmers should improve their purchasing power.

Finally, one of the major strengths of fish culture is the will of the State to develop this sector of activity.

Weaknesses

ENVIRONMENTAL CONSTRAINTS

The great irregularity of the natural water system in the Senegal River constitutes a disadvantage for tilapia culture. High variations hinder efficient water management and threaten fish farming in certain areas.

In addition to this constraint, high evaporation in the major part of the watershed and permeable soils in some areas (Bakel) require the regular recreation of pond water levels.

Moreover, the low level of minerals in the waters of the Senegal River (Reizer 1974; Diouf et al. 1991) constitutes a negative factor for tilapia culture. To improve growth, fish farmers are constrained to put additional nutrients in the ponds. These extra expenses increase the already high costs of production.

During the rainy season, the waters of the Senegal River are generally muddy, increasing turbidity in the ponds. In turn, water turbidity greatly affects the production of phytoplankton, reducing the trophic resources of the ponds. This phenomenon is particularly pronounced in Matam.

From November to March, water temperatures are relatively low (down to 16°C), resulting in a decreased or stunted growth in the pond fish.

The flat lowland terrain often causes problems in the construction of ponds and additional costs for filling and drainage.

ADMINISTRATIVE AND TECHNICAL CONSTRAINTS

The history of the Senegalese fish culture reveals that the choice of sites (Bakel and Navel, for example) has not always been wise. The location of the ponds is extremely important for the technical and economic success of fish farming.

Ponds are not always constructed according to the technical standards required for sound management (lack of depth and poorly constructed dikes).

In addition, the large size of the ponds is often responsible for poor water management.

Defective pond construction is related, on the one hand, to the lack of heavy machinery and to the lack of experienced fishpond technicians, on the other hand.

A major obstacle to the development of fish farming has been the near constant confusion between the objectives of research and extension. Very often, fish culture techniques have been extended before being fully mastered. This has resulted in failures that have strongly shaken the enthusiasm of farmers, even

though efforts in this direction are evident at the stations of Ndouloumadji Dembe and Nianga.

With regard to water quality, the lack of routine control of physico-chemical parameters is to be deplored, even though efforts in this direction are evident at the stations of Ndouloumadji, Dembe and Nianga.

The availability of fry, both in qualitative and quantitative terms, has often been a major constraint. Freudenberger (1988) says of the Richard-Toll station:

> "It is difficult to remember a single moment where fry produced at Richard-Toll fully satisfied the needs of the village fishponds. During the first year of operation, the fishes provided were larger than fry, and during the following years, problems occurred in the breeding ponds which resulted in overstocking and stunting. In fact, the largest part of the fish provided to the village ponds during this period may have been stunted animals rather than real fry. When they were introduced in the ponds, they bred immediately becoming once more too many for the ponds, greatly reducing the yields."

The insufficient and inexperienced management staff (Shelton 1985; Freudenberger 1988) played an important role in the failure of fish farming. This was aggravated by a lack of coordination and poor relationships among the various organizations involved in fish culture in the Senegal river basin.

Also, poor project management and administration seem to have contributed to the failure of fish farming in the Senegal river basin. At least, this is what transpired from the interviews conducted with a number of persons involved in fish culture.

SOCIOECONOMIC CONSTRAINTS

One of the major obstacles to the profitability of fish farming in the Senegal river basin is undoubtedly the high costs of construction. For example, construction costs (main works) for one hectare have been estimated at approximately 8 million F CFA or US$26,700 (Corlay and Seck 1988), which farmers cannot afford.

At this point, it is legitimate to wonder whether fish culture in the Senegal river basin should or should not be subsidized.

The principle of funding aquaculture is widely accepted, particularly in France where up to 50% of total investments can be subsidized (Corlay 1989). It should be pointed out that in Senegal, rice culture, a financially deficient activity, is subsidized for nearly half its price to consumers [against 160 F CFA/kg (US$0.50); 70 F CFA (US$0.20) is subsidized]. Similarly, in the fisheries sector, fuel and fishing gears are subsidized. However, under the Senegalese current economic policy, subsidies to fish farming are difficult to imagine.

With the improvement of distribution channels, marine fish compete seriously for markets with cultured fish (Chaboud and Kébé 1990). In particular, the increase in the number of refrigerated trucks for the transportation of fisheries products has considerably increased the fishmongers' scope of activity.

Fish farming also competes with other activities such as agriculture, animal farming and capture fisheries which are traditional activities that are well integrated into the social life of the people, and take precedence over fish culture. This explains certain attitudes towards fish farming. For example, a number of these farmers are skeptical about investing their physical or financial resources in fish

farming activities, thinking that these resources would be more profitable if invested elsewhere (agriculture, animal farming or capture fisheries), particularly when fish farming has yet to prove successful.

Moreover, cooperatives have been an obstacle to the development of fish culture. Ponds run by a cooperative generally face enormous management problems which threaten the success of this activity and local politics often aggravates the situation.

Discussion

After a little over 10 years of efforts in developing tilapia culture in the Senegal river basin, successes are extremely rare, even nonexistent. We should therefore admit that tilapia culture has failed in this region.

This failure is related to several environmental, technical, administrative and socioeconomic factors which should be analyzed.

Concerning environmental factors, the extreme irregularity in the Senegal river basin water system has long been a major constraint. However, the Diama and Manantali dams can solve this problem. In contrast, the problems of high evaporation, unsuitable terrain, poor water mineralization and turbidity are not likely to be economically soluble.

The choice of sites for fishponds has not always been sensible, particularly in Bakel and some areas of Matam III. A brief study of soils and a summary analysis of the socioeconomic environment would have prevented errors that have greatly contributed to the failure of fish farming in the Senegal river basin.

Defective pond construction has also acted against fish culture. The solution to this problem involves the improved training of pond workers and the hiring of consultants knowledgeable about African and tropical aquaculture. The choice of consultants requires particular attention. Some consultants have set high professional standards and given excellent results, but there are also agencies and independent consultants whose competence and even integrity are questioned.

The absence of routine control of the physicochemical parameters and of a collection of economic and financial data on fish culture, although two distinct problems, greatly affect the success of fish farming operations. It is clear that under such conditions, the chances of success of fish culture are limited.

Regarding administrative factors, fish farming has suffered from its own management system. It is time for the financial and technical management of fish culture projects to be decentralized. Funds should be allocated to project directors. This decentralization will have to be accompanied with regular technical, administrative and financial evaluations. Stringent management practices will also be required from project directors.

All these constraints bring forth the basic issue of the relevance of new perspectives and efforts in fish farming.

In the Senegal river basin, the production from inland fisheries has strongly declined and no longer satisfies the protein requirements of the local populations (Diouf et al. 1991). A solution to this problem would be to improve the distribution channels of marine fish by reinforcing the road infrastructure and the processing and storage facilities for fisheries products. However, this solution may not be achievable in time. For example, the Senegalese population is continuously growing, and although resources are currently well-managed, they will not be able to respond to a considerable increase in population and may collapse as a result

(Bakhayokho et al. 1985). It is therefore to be expected that in some years, marine fisheries will no longer be able to satisfy the demand in fish. The price of marine fish is likely to increase, thereby increasing the competitiveness of cultured fish on the markets of the Senegal river basin.

The price of meat, which could replace fish, is relatively high and out of reach for most rural people.

This new scenario seems to encourage the development of fish culture, which should be further improved by a sufficient and regular supply of water from the dams.

It would be therefore sensible to prepare the grounds for such development by establishing experimental and research structures that will help develop efficient fish farming methods. This period, likely to last five to 10 years, will determine the future success of fish farming.

This approach will prevent the basic error that has been committed from the beginning of fish culture development in the Senegal river basin, namely, to give production precedence over research. This attitude was dictated by the conviction of fish culture developers that techniques developed in other countries could be directly transferred to Senegal. Experience has shown that the local dimension of fish farming is very important: farming techniques must be adapted to each environment.

The choice of the type of fish culture to be developed in the Senegal river basin is crucial for determining the success of future developments. Subsistence aquaculture is unlikely to succeed. The level of technicality required to develop fish farming makes subsistence aquaculture economically unattractive (Lazard et al. 1990). This explains why farmers rapidly lose interest in this kind of operation.

Concerning industrial fish culture, the experiment showed that most operations of this type have failed, the production costs remaining substantially higher than market prices (Lazard et al. 1990).

The type of fish farming that presents the best chances of success is certainly artisanal fish farming: small-scale commercial production, integrated with existing agricultural farming systems. This type of operation presents the advantage of providing farmers with additional income while not requiring large investments.

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Nile Tilapia (*Oreochromis niloticus*) Culture in Small Waterbodies under Different Feeding and Fertilization Regimes[a]

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Abstract

Oreochromis mossambicus and *O. niloticus* were introduced into Bangladesh in 1954 and 1974, respectively, but were not well established as cultured species due to poor management. The stocks have been mixed. A new founder population of *O. niloticus* was introduced from Thailand in 1986 and studies were undertaken for developing management practices for its culture in seasonal ponds and ditches. This paper describes the production economics of *O. niloticus* culture in these small waterbodies under different feeding and fertilization regimes. Production and benefits were high using oil cake and rice bran as supplementary feeds, but the shortage and high cost of oil cake probably preclude its use in tilapia culture in the near future. Problems encountered in the management of perennial ponds are discussed and the potential of *O. niloticus* culture for improving household incomes and nutrition in Bangladesh is assessed.

Introduction

Fish is the main animal protein source for the people of Bangladesh contributing 71% to their total animal protein intake. Bangladesh is a least developed country, one-third of which is inundated for about six months a year. In spite of its vast water resources, inland fish production in Bangladesh is very low. The low per caput fish consumption of 7.5 kg·year^{-1} (World Bank 1991) has led to protein malnutrition, especially in rural areas.

Indian and Chinese carps are the main species used for culture in freshwater ponds. In addition to over 1.3 million perennial ponds covering some 146,000 ha, there are many small seasonal ponds, ditches, borrow pits and roadside canals in rural Bangladesh. These retain water for periods ranging from four to seven months. Most households in rural areas possess backyard ponds or ditches, which are rarely utilized for aquaculture, as farmers think that these

[a]ICLARM Contribution No. 846.

are not suitable for traditionally cultured species, especially Indian and Chinese carps. To develop these small waterbodies for productive fish culture, research has been undertaken at the Fisheries Research Institute, Mymensingh to identify suitable species for short cycle aquaculture, and develop low-cost management systems for optimizing production. Nile tilapia (*Oreochromis niloticus*), a hardy fish that can survive in shallow and turbid water conditions, and a good converter of organic matter into high quality protein (Stickney et al. 1979; Pullin and Lowe-McConnell 1982) has been identified as one potential species.

O. mossambicus was introduced into Bangladesh in 1954 and *O. niloticus* in 1974. In spite of their long history in the country, neither species became established in aquaculture, as there were no effective management guidelines (Hussain et al. 1989). Stocks of the two species became mixed and, therefore, a new founder stock of *O. niloticus* was imported from Thailand in 1986: the Chitralada strain of Egyptian origin. Studies were undertaken on-station and on-farm, with the participation of farmers, to evaluate the production potential of *O. niloticus* in small seasonal waterbodies under different feeding and fertilization regimes (Hussain et al. 1989). The results of these studies are presented in this paper.

Materials and Methods

On-station Trials

Studies were carried out in 280-m^2 ponds, with an average water depth of 1 m, using fingerlings produced from the Chitralada strain imported from Thailand. Culture of *O. niloticus* was tried using two systems: (1) fertilization of ponds without supplementary feeding and (2) supplementary feeding without fertilization of ponds. Two supplementary feeds were tried, one consisting only of rice bran and the other consisting of rice bran and mustard (*Brassica campestris*) oil cake in the ratio of 3:2 by weight. All treatments had three replicates. Table 1 summarizes the treatments used.

Pond preparation for all treatments included prior draining of the ponds and application of lime to the pond bottom at the rate of 250 $kg \cdot ha^{-1}$. Three days after liming, the ponds were filled with groundwater and fertilized with cattle dung and inorganic fertilizers (urea and triple super phosphate [TSP] in a 1:2 ratio) at the rate of 750 and 25 $kg \cdot ha^{-1}$, respectively. Five days after fertilization, the ponds were stocked with 10 to 11-g *O. niloticus* fingerlings at 20,000 fingerlings$\cdot ha^{-1}$. For Treatments 1 and 2 that used supplementary feed, feeding was done six days a week at the rate of 5-6% of the estimated fish biomass. For estimating the feeding rate, the ponds were netted at monthly intervals and 10% of the fish was sampled. From this, the fish biomass in the pond was estimated and the feeding adjusted at monthly intervals. Fertilization of Treatment 3 was done at weekly intervals, with cattle dung and inorganic fertilizers (urea and TSP in a 1:2 ratio), at the rate of 750 and 25 $kg \cdot ha^{-1} \cdot week^{-1}$, respectively. Two months after stocking and every two weeks thereafter, fry produced in the ponds were removed using a fine mesh seine net to reduce competition for food. The ponds were harvested after six months rearing and the production estimated.

On-farm Trials

On-farm, farmer participatory trials were carried out in six formerly derelict seasonal farm ponds of 80-120 m^2 individual size. The water depth in these rainfed ponds varied from a maximum of 1 m during rainy season (June), the depth decreasing over time and finally drying by December-January. Before the onset of the rains, the ponds were cleared of weeds and lime was applied at the rate of 200 $kg \cdot ha^{-1}$. After rain

Table 1. Production (kg·ha^{-1}) of Nile tilapia (*Oreochromis niloticus*) stocked at 20,000 fingerlings·ha^{-1} in triplicate 280-m^2 ponds after six months farming with different feeding and fertilization treatments. For fuller details of treatments, see text. The data presented are the means of triplicate ponds. Ranges are between parentheses.

Treatments					Production		
Treatment no.	Average size at stocking (g)	Supplement feed	Fertilizers	Feed conversion ratio	Undersize fish (< 80 g)	Market-size fish (> 80 g)	Total
1	10.5	Rice bran	-	5.8	700 (582-864)	2,038 (1,904-2,068)	2,738 (2,486-2,932)
2	11.0	Rice bran 60% + mustard oil cake 40%	-	4.6	400 (296-529)	3,154 (3,036-3,250)	3,554 (3,332-3,707)
3	10.5	-	Cattle dung, urea and TSP	-	627 (586-689)	883 (775-1,068)	1,510 (1,382-1,757)

filled the ponds, 5-10 g fingerlings, procured by farmers who have perennial ponds and were raising the Chitralada strain, were stocked at the rate of 20,000·ha^{-1}. The ponds were divided into two groups: for the first group, pond owners were asked to fertilize the ponds with inorganic fertilizers, urea and TSP (1:2 ratio) at the rate of 25·kg·ha^{-1}·week^{-1}, whereas the other three pond owners were asked to fertilize the ponds with cattle dung at the rate of 750 kg·ha^{-1}·week^{-1}. This use of two fertilizer treatments was chosen because some farmers have easy access to cattle dung and its use can be considered a maximum use of on-farm inputs, whereas the other farmers do not have access to cattle dung and its transportation from distant places is difficult. In view of the small quantities needed per pond, these latter farmers preferred to use inorganic fertilizers—urea and TSP. Despite following these preferences, farmers could not strictly adhere to suggested rates of fertilization and feeding, hence the wide variation in use of inputs from pond to pond. All the pond owners were asked to feed the fish daily with rice bran at the rate of 5% of the estimated fish biomass. The culture periods ranged from four to six months, depending on the duration of water retention in the ponds. The ponds were harvested when the water depth went below 30 cm.

Results and Discussion

On-station Trials

Table 1 gives the production obtained for the three treatments. Fish of less than 80 g were treated as undersized and marketed at low prices.

In the financial analysis presented in Table 2, the cost of production (excluding pond rental and labor) was lowest (Tk 17,200·ha^{-1}) for Treatment 3 (fertilization, no feeding). However, net profits were higher with supplementary feeding. Getting an increased net profit of Tk 4,361·ha^{-1}, through inclusion of mustard oil cake in the feed, required an additional production cost of Tk 24,199·ha^{-1}, a high investment for a small increase in net profit. Thus, of the treatments tried, feeding with rice bran appeared to be a more economical operation with a benefit:cost ratio of 2.50, as against 1.41 with fertilization and 2.07 with rice bran and mustard oil cake as supplementary feed. Also, the use of oil cake as supplementary feed in *O. niloticus* culture would be beyond

Table 2. Financial analysis (in Taka) of Nile tilapia (*Oreochromis niloticus*) stocked at 20,000 fingerlings·ha^{-1} in 280-m^2 ponds and farmed for six months under different feeding and fertilization treatments. The data presented are averages of three ponds per hectare.

	Treatment 1 Supplementary feed: rice bran Fertilization: nil		Treatment 2 Supplementary feed: rice bran + mustard oil cake Fertilization: nil		Treatment 3 Supplementary feed: nil Fertilization: Organic + inorganic	
	Quantity (kg)	Cost (Tk*)	Quantity (kg)	Cost (Tk*)	Quantity (kg)	Cost (Tk*)
Operational Costs:						
1. Pond preparation						
- Lime	250	750	250	750	250	750
- Cattle dung	750	325	750	325	750	325
- Inorganic fertilizer	25	125	25	125	25	125
2. Fingerlings (nos.)	20,000	4,000	20,000	4,000	20,000	4,000
3. Feed and fertilizers						
- Rice bran	14,792	22,188	9,598	14,397	-	-
- Oil cake	-	-	6,398	31,990		
- Cattle dung	-	-	-	-	18,000	9,000
- Inorganic fertilizer	-	-	-	-	600	3,000
Total operational costs (Tk)		27,388		51,587		17,200
Gross production (kg·ha^{-1})		2,738		3,554		1,510
Gross sales (@ Tk 35 kg^{-1})		95,830		124,390		52,850
Net profit		68,442		72,803		35,650

* US$1=Tk 37.50.

the means of most poor rural farmers, due to the high price of oil cake.

On-farm Trials

Production after four to six months (Table 3) ranged from 1,500 to 2,343 kg·ha^{-1} with use of inorganic fertilizers, and from 1,441 to 1,925 kg·ha^{-1} with cattle dung. It is difficult to assess which fertilizer was more effective in increasing production, as the farmers did not adhere to suggested practices. Whereas the farmers applied inorganic fertilizers in excess (125-316%) of suggested rates, in the case of cattle dung, application was very low (6-21%) as compared to the suggested rate (Table 3). Use of supplementary feed (rice bran) was also very low and varied from pond to pond. In view of this, it is difficult to draw conclusions as to the efficiency of fertilizers used in increasing fish production.

In spite of these variable and generally low inputs, production of 1,441 to 2,343 kg·ha^{-1}, obtained in four to six months rearing, can be considered high as compared to the 100-200 kg·ha^{-1} of wild fish that the farmers were formerly harvesting from these ponds. The average cost of production worked out to Tk 11.73·kg^{-1} of fish produced, against a sale price of Tk 35·kg^{-1}, indicating high profit margins.

Table 3. On-farm culture trials of Nile tilapia (*Oreochromis niloticus*) stocked at 20,000 fingerlings·ha^{-1} in small, formerly derelict ponds, using different fertilization treatments and daily feeding with rice bran whenever possible. For further details, see text.

Pond size (m^2)	Fertilizers applied	Culture period (months)	Fertilizers suggested (kg/pond)	Fertilizers actually applied (kg/pond)	Average size at harvest (g)	Gross production (kg/pond)
80	Urea + TSP	6	4.8	12.0	82.2	16.00
120	Urea + TSP	6	7.2	11.5	89.0	28.12
80	Urea + TSP	4	3.2	4.0	95.7	12.00
120	Cattle dung	4	144	9.0	97.2	17.29
120	Cattle dung	6	216	45.0	98.1	23.10
120	Cattle dung	4.5	162	34	126.0	19.13

About 5,000 farmers have now adopted *O. niloticus* culture in their homestead ponds. A sample survey covering 113 farms revealed an average production of 1.4 t·ha^{-1} in six to eight months rearing, with a production cost of Tk 6.60·kg^{-1} (Gupta et al. 1991; Gupta 1992). The low cost of production was due to the very low levels of inputs used by farmers. Seventy per cent of such fish, produced on farms, are consumed by households, thus improving the nutrition and health of farm families. Average production of *O. niloticus* from a pond of only 170 m^2—the average size of ponds covered by the survey—was 23.5 kg, which is almost equivalent to the national annual consumption of fish by low-income rural households with six family members (World Bank 1991).

One of the problems encountered by the farmers, especially in ponds which are either perennial or hold water for longer periods of time (more than six months), is that of breeding and overpopulation, leading to the stunted growth of fish. This inhibits the culture of *O. niloticus* in perennial ponds. Studies are in progress to address this issue through introduction of carnivorous fish into the system.

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Rural Development of Tilapia Culture in Africa: from Myth to Reality

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Abstract

Aquaculture development initiatives have often emphasized the need for African farmers to integrate this activity in their farming system. Aquaculture (as can be observed in Southeast Asia) should have evolved alongside ongoing development processes, and even become the catalyst of a reorientation of agriculture (from shifting to fixed). The objective of this article is to contribute to the development of this dynamic in Africa.

This objective has practically never been achieved and some of the reasons for this failure are obvious: farmers were considered incapable to innovate and irrational in their choice of economic strategies. Moreover, the socioeconomic constraints of the farmers were totally unknown, and the internal economic systems of the rural development projects masked the need to propose a production unit really accessible to them. Aquaculture development remains a current concern.

Social sciences use many tools to understand farmers' strategies. Here, farmers are considered as economic agents whose rationality is explained essentially by their socioeconomic environment and the environment that they develop.

The relevance of this analysis is tested against a concrete case: the forested areas of Midwestern Côte d'Ivoire where this approach has been applied for the past five years and is giving interesting results. We begin with a rapid description of agriculture in terms of agrarian systems. This description emphasizes the agricultural dynamics referring to a typology of farming systems where fish culture is considered only on the basis of the constraints that it can help to eliminate. The work productivity in aquaculture is compared to that of other agricultural undertakings.

Introduction

Fish culture is considered by the rural populations of Côte d'Ivoire as a possible variant of the farmer's strategy to minimize expenses in animal proteins intended for consumption, to increase or maximize income and to diversify agriculture (Koffi 1989). From the socioeconomic perspective, fish culture development in Africa should therefore be considered in terms of economic efficiency and opportunity cost of the agricultural inputs, of accessibility and efficiency of the production systems, and of protection against risks and uncertainties.

Financial and Economic Efficiency of Fish Farming Systems

Financial and economic efficiency is appreciated both from the microeconomic (at the farmer's level) and the macroeconomic perspective (at the community or State level). Financial analyses assess the systems' financial efficiency for the farmer while economic analyses measure the contribution of fish culture to national revenues (gross domestic product or GDP). Economic analyses are absolutely necessary because grants and other economic policy measures adopted to protect the producers, by virtue of the thesis of a developing industry, are costs supported by the taxpayers at the national level. These grants have in general become chronic expenses without which the industry cannot survive. The progressive disengagement of the State demands that economic evaluations be made.

Koffi (1989) assessed the economic efficiency of *Oreochromis niloticus* monosex farming as against mixed-sex farming in Côte d'Ivoire. In addition, the author reported that organic fertilization using wet pig manure contributes to a substantial increase in productivity without increasing production costs (under the current market conditions for this production input). This partly explains the economic efficiency observed in the monosex culture of *O. niloticus* using rice bran as feed and organic fertilizers on farms supported by a light, autonomous, extension structure (Tables 1 and 2).

In fact, the desired privatization of the production of nutritionally efficient feed (3A feed for example[a]) should contribute to a satisfactory increase in productivity to compensate for the State grant, and to obtain a cost price that would not be prohibitive for fish farmers (lower or equal to 50 F CFA[b] per kg for the 3A feed while it is currently over 80 F CFA [Lazard et al. 1991]).

Comparative Economic and Financial Ratios in the Use of Production Inputs between Aquaculture and Irrigated Agriculture

The results of the financial analyses tend to be optimistic regarding the possibility of developing monosex tilapia farming systems in inland environments. However, fish culture can be accepted in rural environments only if the financial and economic ratios compare favorably with those estimated for other irrigated agricultural undertakings.

Monetary Product per Unit of Production

In general, the undertakings that are competing with fish culture for land use

[a]The 3A feed is produced by the Rural Fish Culture Development Project. It is composed of 10% fish meal, 20% cottonseed cake and 70% rice white shorts with a protein content of approximately 25%.

[b]US$1=300 F CFA in 1991, currently 600 F CFA.

Table 1. Economic efficiency ratios for different fish culture models developed in Côte d'Ivoire (Koffi 1989).

Models	Ratios	Average farm (mean)	(ST)
	NP/T	14,189	5,117.4
M1	DWR	2,742	1,006.22
	RRC	47%	0.17
	RRA	47%	0.17
	NP/T	13,604	6,850.38
M2	DWR	2,501	1,410.6
	RRC	43%	0.26
	RRA	43%	0.26
	NP/T	22,009	6,369.24
M3	DWR	3,919	1,410.6
	RRC	522%	3.57
	RRA	126%	0.75

ST: Standard deviation.
NP/T: Net profit per 100 m^2 per year in F CFA.
DWR: Daily work remuneration and family management in F CFA.
RRC: Rate of return of capital.
RRA: Rate of return of assets.
Model M1: Heavy support structure, control fish farmers in Bouake, monosex male tilapia, predators: *Clarias*, 3A feed.
Model M2: Control fish farmers in Daloa, heavy support structure, monosex male tilapia, predators: *Clarias*, rice bran.
Model M3: Heavy support structure (PAPU CD), monosex male tilapia, predators + polyculture of *Heterotis* and *Heterobranchus isopterus* integrated with pig-rearing for organic fertilization.

Table 2. Economic profile of the different fish culture models developed in Côte d'Ivoire including support and extension costs.

Type of support structure	NEP/100 m^2 (1)	Balance/100 m^2 Support structure a	Balance/100 m^2 Feed b	Balance/100 m^2 Material c	Total extension e=a+b+c	Balance (1)-e
M3	20,138	-13,689	0	0	-13,689	+6,450
M1	-1,238	-23,855	-19	14	-23,860	-25,098
M2	8,878	-18,570	-19	14	-18,575	-9,697

NEP/100 m^2: Net economic profit·100 m^2·$year^{-1}$ in F CFA.

(lowlands) are essentially irrigated rice, maize and vegetable crops.

Comparison of the monetary products indicates that efficient fish culture models (Table 1) make a more efficient use of land than irrigated rice (904-2,204 F CFA·100 m^{-2}·$year^{-1}$ according to SATMACI and CIDT[c]) but not as efficiently as some vegetable crops (17,277 F CFA·100 m^{-2}·$year^{-1}$ for tomatoes [Kinimo 1988]).

It is worth noting that the studies on vegetable crops have an anticipatory character. The models that have been analyzed have the disadvantage of not integrating all the socioeconomic constraints that are specific to the target population. Ratio estimates are sometimes biased estimates compared to the real performances of the farmers.

Efficient Use of Labor

In terms of the efficient use of family labor, monosex tilapia culture models associated or not with predators and integrated with a variety of animal farming models compare favorably with food crops.

Ratios estimated by Ruf (1982) for rainfed rice (512-1,108 F CFA·day^{-1}) and yam (670-1,108 F CFA·day^{-1}) indicate a lower economic efficiency compared to efficient fish culture models.

Investment Return

The rate of projected average economic efficiency is 17% for rural development projects funded by the World Bank and particularly intended for small-scale farmers and the rural poor (Gittinger 1985). Economic efficiency reaches 20% for all other categories of agricultural projects. The minimum acceptable rate is 10% in most developing countries. The most efficient fish farming models give a higher economic efficiency rate for the farmer's own capital and assets; this rate is also higher than the traditional bank savings interest rate.

Note however that informal credit is practiced under conditions considerably more profitable than fish culture. This informal credit system or "margouillat" is widely practiced in urban areas and is accessible at a nominal interest rate of 30% per month.

Accessibility and Efficiency of the Different Systems

Aside from the models that consider financial and economic efficiency, there are other models that are effectively accessible to individuals from modest, even deprived populations.

The promotion of models that make use of production inputs accessible and available under conditions allowing production at a competitive cost price is highly advisable. For this, production inputs that are locally available for possible use in fish culture must be inventoried. The model must not be capital-intensive, i.e., it must encourage the use of labor as opposed to capital. In African rural environments, the most scarce production input is capital. Yet, the most efficient systems are those making intensive use of the most abounding production inputs, in this case, labor.

The chosen systems must therefore rely on productive combinations that take into account the limitations in production factors (financial, labor, land and marketing constraints). Regarding financial constraints, models requiring

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[c]SATMACI: Center of Technical Assistance for Agricultural Modernization in Côte d'Ivoire; CIDT: Ivorian Center for Textile Development.

an important working capital are not suited for rural development.

Between two possible choices, it is socially more interesting to choose models entailing no specific advantage for financially better-off groups. Failing this, fish culture would contribute, at best, to improved protein availability; but this would also intensify social inequalities in income distribution.

Protection against Risks and Uncertainties

The risks related to fish farming systems also constitute a fundamental criterion in the choice of technical models. Those involving lesser risks are preferred.

From the technical perspective, the choice of carnivorous fish associated with tilapia is justified. Their use eliminates the risk of suspending production of sexed tilapias due to the proliferation of fry (linked to unavoidable sexing errors). This occurs frequently as soon as control harvests are no longer or poorly performed.

In addition, in order to consider fish culture as an element of a strategy for diversifying sources of income, there must not be any positive correlation between its risks (risks with prices, yields and stocks) and the risks involved in the farmers' other traditional activities. For example, a fish farm depending on agricultural returns, on salaries or on State grants, will not survive the hazards associated with these activities.

The analyses referred to earlier cannot alone define the model to be adopted as major constraints are not taken in account (land tenure and dominant groups). It is necessary to do a comprehensive analysis of the agrarian system to understand the agricultural society at the regional level. On the other hand, using this approach contributes to the identification of target categories based on the farming system dynamics, and to the definition of the type of fish production units to be integrated in the development of these farming systems.

Conditions for the Integration of Fish Culture in Farmer Dynamics (Case of Midwestern Côte d'Ivoire)

Too often, the farming world is considered homogeneous, and only personal qualities (such as the ability to do physical work, intellectual aptitudes, level of education, etc.) are considered to explain the quality of the relationship with the support structure. Taken exclusively, these qualities do not explain the rationality of the farmers' choice.

The consideration of the socioeconomic environment automatically leads to the definition of target categories. If conducted properly, this analysis should partly explain why some groups succeed where others generally fail. It is also important to indicate that these criteria are only trend indicators. The concept of target categories, in order to become a relevant tool, should be developed in concert with socioeconomists and the support structure.

Any approach emphasizing the dynamics of rural development must rely on a socioeconomic analysis of the history of agriculture in order to characterize the region's specific agrarian system. Agricultural or even fish farming practices are not reduced to their sole productive dimension, but are also considered as social practices. One of the instruments of this approach is the definition of farming groups (typology) within which access to production inputs is comparable, the reproduction of their activity being limited by the same constraints. Farms of a similar type will

adopt similar behaviors towards certain constraints, the same issues determining the framework for their reproduction.

The challenge that farmers from the Midwestern region are faced with is analyzed hereafter.

Brief Historical Background

The agriculture of the Bété[d] was integrated fairly late in the economics of plantation, this being attributed to historical factors (linked particularly to colonization; Dozon 1985; Ruf 1988).

The determining factors of this evolution are basically twofold:

- independence, with its slogan "land belongs to those who till it"; and
- the excellent remuneration, from 1965 onwards, afforded by cocoa and also by coffee compared to other productions.

These conditions, coupled with low densities of indigenous populations and the often loose control of land tenure, contributed to mass immigration from the 1970s, the immigrant population today outnumbering the Bété's, 60% against 40%. This recent immigration has resulted in the constitution of three subgroups of populations whose economic behaviors are different:

- indigenous populations (Bété);
- immigrants from Central Côte d'Ivoire (Baoulé); and
- immigrants from Northern Côte d'Ivoire (Dioula, Burkinabé, etc.).

The third group has sometimes come as manual laborers and bought land or plantations (Forget 1982; Ruf 1984). Beyond this classification, farming systems (rotation cropping, production ratios and division of labor) have been organized around coffee and cocoa plantations, and family incomes depend to a major extent on the sale of these produce.

As a consequence of this evolution, two factors are at present determining the organization of farming systems:

- the labor invested or "tree capital" and the labor available on the farm, and therefore the type of plantation; and
- the availability of land (available fallows, lowlands, forests, plantations, etc.) (Ruf 1987).

Recently, the sharp fall of the coffee and cocoa markets, and the many problems affecting the marketing of these commodities have resulted in a loss of interest for this type of culture, and have plunged this agrarian society in an unprecedented crisis, questioning the relevance of the establishment of future plantations.

Moreover, as the "black" forest has almost totally disappeared, land becomes a limiting factor: certain regions are already experiencing relative land saturation.

Classification (Typology)

Many types of farming systems or typologies exist in Midwestern Côte d'Ivoire (Forget 1982; Forget and Chatellier 1984; Ruf 1985, 1988). These emphasize the dynamics of the production system in order to facilitate the possible integration of fish culture. Given this framework, five types of farming systems have been identified (Table 3):

Type 1. This type encompasses a number of large capital-intensive farms relying on an initial capital that is not generated by agriculture. These farms cover more than ten hectares in plantations composed of a combination of one or several other crops: cacao, cola, coffee, rubber tree, coconut, even mechanized food crops or extensive husbandry.

[d]Ethnic group from Midwestern Côte d'Ivoire.

Table 3. Typology of the agricultural undertakings in Midwestern Côte d'Ivoire.

Type	Size and crops	Characteristics
1	Several tens of hectares	Approach external to farmer's logic
2	Ten hectares of plantations Several hectares of food crops	Land control Efficient use of labor in plantations
3	A few hectares of plantations	Limited labor force
4-1	0.5 or 1 ha of plantations many annual food crops	Future availability of land (youth) Evolution towards Type 2
4-2	0.5 or 1 ha of plantations many annual food crops	Limited access to land No investment of labor possible in plantations
5	No land	Labor for sale

Type 2. These are large plantations (approximately 10 ha). They are composed of immigrants, who successfully mobilized an important work force when the land was available, and indigenous populations who have lived on these lands for a long time (former heads of family or village chiefs).

These plantations are all characterized by an easy access to an extensive labor force either through bonds that farmers have kept with their society of origin (immigrants) or through the sale of land by indigenous populations who accumulated enough capital to hire paid workers. These farms are characterized by coffee-cocoa plantations and the subsistence farming of food crops.

Type 3. These are small-scale farms that have limited access to labor and, because of this, have developed only a limited surface of plantation. The deficit can be of several origins: an old planter having long been the "little brother" of a large planter, or a laborer having had late access to land, for example.

This limited work force is more efficiently used in extensive plantation activities at the appropriate periods: gathering, cleaning of plantations during the low season for food crops, for example.

Type 4. These are indigenous small-scale farms with a structural surplus of labor that can only be used in the culture of food crops (access to plantations and to foreign labor is limited).

However, these farmers can have access to fallows or to lowlands for the culture of annual crops. As the work factor cannot be invested in plantations, it is invested in food crops and cotton. Following the decrease in cash crop revenues, this group of farmers is currently divided into two subgroups:

4-1. Those who, after receiving an inheritance (from the immediate family), possess or will possess an important piece of land from which they can earn a profit (they can, for example, rent it in exchange for services).

4-2. Those who only possess limited land and who work outside. They cultivate several annual crops on the same plot (example: rice-cotton; rice-maize-cassava). These limited land areas

do not allow them to "invest" their work in a permanent plantation.

The close relationship between marriage and access to land ownership must be emphasized. The land of a married man will not be questioned, which frequently occurs in the case of an unmarried young man (Dozon 1985; Ruf 1988). The head of a family is granted the right to land ownership by the village folks and his farm also benefits from additional labor.

Type 4 is found in areas dominated by Baoulé and Mossi (Burkinabé) populations and where there are no more forests to clear; however, this needs to be confirmed.

Furthermore, as long as land was not a limiting factor, Type 4 transformed itself into a Type 2 farm. The lower remuneration for work guaranteed by cash crops and the growing scarcity of available land have combined to keep this group away from the plantations.

Type 5. These are the last migrants who will no longer have access to land ownership and only have the usufruct from one plot cultivated with food crops for their own food requirements. This arrangement is part of the remuneration for their work.

Young Bétés, employed by Type 2 production units and waiting for their family situation to evolve, are temporarily integrated in Type 5 and evolve naturally towards Type 4.

It should be emphasized that this rapid presentation cannot explain the evolution of the Bété populations, particularly their transition from Type 2 to Type 3, Type 4-1 to Type 2 or Type 5 to Type 4. In fact, this evolution depends on the family organization (extended or immediate family) and on the function and role ascribed to each member of the family. The fact that Type 4 is distancing itself from Type 2, and seeks to constitute an independent production unit, can be partly explained by the relationship existing between young and older persons.

It is still unclear which among these systems is likely to integrate fish culture as a technical innovation supported by farmer dynamics, and therefore, the major characteristics of fish culture as production workshop.

It should also be reminded that in this region, the demand for fish is high. Fish being the primary source of protein for planters, local opportunities do exist.

Investing in this activity requires labor and vast amounts of money compared to farmers' income. The establishment of several farms by young people shows that such investment is accessible to all categories. This type of investment also requires access to land, and although the areas available are limited, land can be found in the lowlands which are not coveted by the plantations located in the uplands.

Farming operations require an important labor investment distributed throughout the year.

Efficient use of labor depends on the sources of production inputs: when conditions are suitable for rice bran, labor usually costs approximately 3,000 F CFA per day.

Consequences of this Classification

It is clear that all categories with limited access to labor force, although such is used as efficiently as possible by extensifying work on the plantations, cannot reduce it further.

The recent decline in purchasing power does not allow even Type 2 to farm fish using cash.

In a region where land tenure is dominated by Bété populations, the only categories that can undertake fish culture are Types 4-1, 4-2 and 5.

The instability of Type 5 and the availability of land in Type 4-1 contribute

to make Type 4-2 the most suitable group to establish a fish culture unit.

Comparison with the Actual Situation

The Daloa Periurban Fish Culture Project (PAPU-CD) has been supporting eight young Bété fish farmers established in the bush.

Group 4-2 is composed of four fish farmers. In general, these farmers have built most of their farms by themselves and are ready to invest more work. In Zalihouan, the fish farmer is expanding his farm; in Bolia, he is rearing rabbits to fertilize his pond and has accepted to experiment with acadjas; in Nioubuoua, the farmer has integrated rabbit culture with fish culture; and in Tahiraghue, he is interested in developing a new acadja.

Group 4-1 is represented by a fish farmer in Bla, who, following the recent death of his father, now belongs to Group 2. The ponds were entirely built by laborers and are now in a derelict state.

Group 3 is represented by a fish farmer in Zakoua who has entirely subcontracted his undertaking to contract workers.

Group 2 is represented by a fish farmer from Zakoua and one from Tchéblogué (the latter is a particular case as most of his plantations have burned down).

The motivation of Group 4-2 is explained partly by the fact that fish culture constitutes an alternative undertaking by making more efficient use of the available production inputs than in other undertakings. In addition, farmers are granted a social status that has become otherwise inaccessible following the recent developments already discussed. To renounce fish culture would be to renounce a social status equivalent to that of the planter. Here, the idea is to propose a technical model that is well-adapted to this type, becoming an instrument for social advancement (Oswald and Copin 1992). It should be emphasized that these young farmers who have limited access to land, but are good resource managers, have been chosen by the Project since they were qualified to work on a relatively sophisticated technical fish culture model.

Based on these hypotheses, a technical model is considered viable if it is characterized by the investment of labor in the fish culture undertaking (in Group 4-2), which should allow the farmers to increase their investment, i.e., expand the fish farm. A possible success scenario would be a technical model that gives access to outside labor force, contributing to faster investment. If these conditions are met, one will truly speak of rural fish culture development. As soon as the economic conditions for reproducing such undertakings are met, the social appropriation of these techniques by fish farmers will have to be examined as well as the spontaneous reproduction potential, in order to define the most appropriate type of support.

Conclusion

The absence of a real rural development in African fish culture must not lead to discouragement. It is clear that the farmer's situation is difficult to apprehend, as reflected by the failure of many agricultural projects. However, in many cases, the rapid evolution of agricultural practices show the capacity of the farmers to implement new systems of production. Actions seeking to mobilize this capacity of implementation must necessarily involve systematic research efforts on the efficient use of the production factors. These efforts are totally justified in a mid-term perspective where fish culture can perfectly and naturally find its place.

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Which Research for Which Development of Tilapia Aquaculture in SubSaharan Africa?

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Abstract

Over the past 30 years, no real national or regional research strategy seems to have been developed for the aquaculture of tilapia in subSaharan Africa. Research in aquaculture has always been very "opportunistic," mainly because of budget constraints. In some cases, research components were included in important development projects, but their biotechnical bases were weak and these companion research programs had to be discontinued.

Also, research in tilapia aquaculture has often been conducted independently of other research areas such as biology, agronomy, zoology and socioeconomics (farming systems) and mainly under the departments of forest and wildlife research, a legacy of the colonial era. This type of research has very rarely been part of a global aquaculture development plan which, on one hand, generally did not exist. As a result, many aquaculture scientists on the continent are compelled to work in isolation, and their results have often little impact on development. Furthermore, basic research has mainly been conducted with a view of developing intensive and/or industrial fish culture which, in most cases, does not conform with the technical and socioeconomic context prevailing on the African continent.

These different considerations are analyzed on the basis of a number of examples. The interface research-development is studied with reference to "pilot" projects and companion studies. To conclude, proposals are made for the future in terms of the adequateness between aquaculture development planning and fundamental and applied research strategies.

Aquaculture Research (Particularly on Tilapia) in SubSaharan Africa

Background and Current Situation

The African continent has a long history of aquaculture research which began in many countries before the days of independence. Research work then was mainly empirical and focused on applied research.

The major research stations on the continent were the following: Djoumouna in Congo; Landjia in the Central African Republic; Foumban in Cameroon; the Fish Culture Research Station in Bouake, Côte d'Ivoire; Sagana in Kenya; Anamalazaotra and Ampamaherana in Madagascar; Kipopo in Zaire; Kajansi in Uganda; Chilanga in Zambia; and the Henderson Research Center in Zimbabwe.

The Bouake Station (now the IDESSA Fish Culture Center) and that of Chi-langa are the only stations which were able to continue their research work without interruption since their creation (in 1957-1958 for Bouake). Others conducted research occasionally when development

projects included a research component (in Landjia, for example), usually funded by UNDP and implemented by FAO in cooperation with a European laboratory (Wageningen on *Clarias gariepinus* at the Landjia Station, for example).

Today, in francophone Africa, few research stations are really operational and able to contribute to development with real scientific and/or technical innovations. Among the stations that are still active (in varying degrees) are those implanted in Côte d'Ivoire (the Oceanological Research Center of Abidjan-Layo and the IDESSA Fish Culture Station in Bouake), the Djoumouna Station in Congo and the Foumban Station in Cameroon.

Besides these stations, some university laboratories are also conducting research in aquaculture or in applied hydrobiology.

Also, in addition to the research done in these large experimental stations funded by national or international agencies, research (or *researchers*, more specifically) is funded through a more flexible system developed 15 years ago: first through the International Foundation for Science (IFS), Sweden, then through the International Development Research Center (IDRC), Canada. Funding by these organizations is in the form of research grants for specific scientists and projects that are carefully identified and limited in time. This type of financial assistance allows many researchers to develop research programs but not to set up a station or a laboratory.

The major problem facing African research is its sustainability; it is expensive (as anywhere else) and researchers are few. Another problem is to decide whether research efforts should be focused on basic or applied research, or both. Basic research can be developed only in well-equipped laboratories and by highly trained scientists.

A temporary alternative could be to develop basic research on tropical species and basic issues in laboratories from the North while training African scientists, and gradually transferring these research activities to the South. The problem usually encountered is that upon return to their countries of origin, these researchers hardly or no longer have access to the literature or the analytical tools they were trained with. The profession "aquaculture scientist" must be created in Africa. Concerning applied research, it is usually closely associated with development and will be discussed later in the section on Research & Development. However, it should be emphasized that research and production are highly correlated: today, among the countries of black francophone Africa, Côte d'Ivoire is the country where aquaculture production is the highest and, at the same time, where research efforts have always been pursued and even intensified over the last years.

Aquaculture Programs and Major Results

The major programs in aquaculture research developed so far on the continent and their most significant results can be summarized as follows:

SPECIES

Tilapias. Tilapia, originating from Africa, is the species that is most commonly studied, constituting the major part of African aquaculture production. According to modern systematics (Trewavas 1983), tilapias fall under three genera: *Oreochromis*, *Sarotherodon* and *Tilapia*.

Paradoxically, after some limited, occasional tests conducted on *Oreochromis niloticus* in African stations, it is from Asia, the cradle of fish culture,

that research on these species really started, and where their economic and biological importance for aquaculture was demonstrated. Hickling (1960) developed crosses between various species of *Oreochromis* (*urolepis*, *hornorum* and *mossambicus*) giving all-male hybrids from species introduced from Africa at the Batu Berendam Station in Malaysia. These activities were continued in the 1960s at the Bouake Station (Lessent 1968), and it is from this moment that the *Oreochromis* genus was used in African aquaculture development (Lazard et al. 1990b).

More recent studies have also focused on indigenous species of lagoon tilapias, mainly *S. melanotheron* and *T. guineensis* (Legendre et al. 1990).

Other African Indigenous Species. At the same time, a number of research studies focused on indigenous species other than tilapia. So far, these are mainly three species of catfish (*Clarias gariepinus*, *Chrysichthys nigrodigitatus* and *Heterobranchus longifilis*) and *Heterotis niloticus*.

Fish Transfers within and to the African Continent. There are different problems according to whether the introductions concern genera or species that are not from the continent, or species (or strains) of an indigenous genus. The second type of transfer is very likely to induce interspecific hybridization or interfertilization between strains and original populations may not be maintained whether in the natural environment or on-station.

According to Welcomme (1988), introductions and transfers of fish within or to the African continent may have totaled 256 since 1850 (146 of which occurred between 1950 and 1980). Of these, tilapias account for 74 introductions, 62 of which occurred between 1950 and 1980. This inventory is probably incomplete as a great many transfers of fish, particularly within the continent, go unreported.

Discussions on the issue of introductions and transfers must take place as soon as possible, and be open and transparent. Each African country should make a comprehensive report on its specific situation regarding these matters so that a continentwide strategy for the conservation and the management of the genetic resources of African aquatic species be implemented. This strategy must be based on research programs on the population genetics of the potentially major fisheries and aquaculture species (Pullin 1988; Lazard 1990), particularly tilapias for which Africa today is considered the "genetic reservoir."

RESEARCH AREAS

Biology. Many studies have focused on the different aspects of the biology of tilapia, the greatest part of which is being done outside Africa. In some cases, tilapias were considered as models (particularly, *O. mossambicus*) and studies on these species were not primarily towards realizing their aquaculture potential.

Little basic research has been conducted so far on tilapias on the African continent focusing essentially on the reproductive physiology, particularly sex-determining mechanisms (Jalabert et al. 1974; Baroiller 1988) and on population genetics (Pouyaud and Agnese, this vol.; Rognon and Guyomard, this vol.) and hybridization, in close collaboration with laboratories from the North. However, most biological studies fell essentially under applied research.

Production Systems and Farming Technologies. It can be said that all the major farming structures (ponds, cages, pens and raceways) have been tested in Africa, not only with tilapia but also with other species.

Each structure may correspond to different farming systems developed on-station or under a Research & Development program.

In this area, however, much work remains to be done to improve the integration of fish farming with agricultural production systems.

The two avenues to explore in the future seem to be the economic and biotechnical optimization of the research already done, and the development of new, efficient farming systems adapted to the rural environment where an important vacuum can now be felt. Small-scale, commercial production systems in rural environments (the counterpart of peri-urban systems) are yet to be developed.

This model cannot be developed without the farmers. Therefore, system studies should preferably be done under the developer's authority for the global orientations of rural development and should not be disconnected from a regional reality. The term "system" contains a determining socioeconomic component: on-station research on systems is justified only if it represents, in the context of Research & Development, the shortest route between on-farm and basic research.

Socioeconomic Studies. In Africa, socioeconomic research in aquaculture is almost nonexistent. There are no real research programs in this field of investigation. This can be explained by the negligible impact of aquaculture on African economies, by a reluctance of the scientists concerned to be involved in programs that can only be multidisciplinary and by a slow awareness by the planners or aquaculture project officers of the necessity to integrate the economic or social dimension.

A few examples of such slow awareness (as manifested in the course of the projects) can be noted: the Rural Fish Culture Development Project and the Lagoon Aquaculture Development Project, both in Côte d'Ivoire. The first project studied the economic and financial efficiency of the different aquaculture farming systems. For this, 104 fish farmers were surveyed and the economic profile of each subsystem of production as well as several indices (cash and solvency) were analyzed to study certain scenarios considering natural and economic risks and uncertainties (Koffi 1992).

The second project focused on the pricing of cultured fish, the commercial channels and the commercial strategy implemented by the different actors operating at each level of the different circuits (Weigel 1989).

Concerning the social aspects of African aquaculture, three research avenues could be explored. These studies should increase the knowledge base of aquaculture which, in the past, was essentially based on quick and superficial research work.

- The objective of the first research avenue would be to analyze, with reference to specific local situations, the integration of aquaculture activities into the existing fisheries or agricultural production systems. In artisanal aquaculture, production inputs are allocated on the basis of the old system. Improved understanding in land, labor, capital and input use is the key to the successful "grafting" of aquaculture onto an already regulated social and economic organization. Once again, to avoid poor results because of inadequate research methodologies, this type of research requires a high level of expertise in social sciences which cannot be procured solely by biologists or technologists.
- The second research avenue could consist in a bioeconomic analysis of a number of existing or developing aquaculture undertakings. This type of

interdisciplinary analysis should, in essence, improve the understanding on the conditions necessary to increase aquaculture profitability. Such analysis requires the close monitoring of investment and, particularly, operating costs of the aquaculture units, and the relevance of these costs vis-à-vis the biotechnical parameters. In time, such research should provide enough elements on the optimum efficiency of the aquaculture ventures and their real unit costs.

- The third research avenue could be a macroeconomic analysis on a regional scale of aquaculture vis-à-vis the general fisheries industry. As such, this type of research goes beyond the aquaculture framework. Through this type of research, major quantitative elements could be identified, and the industry's various agents likely to be involved in aquaculture as well as their role and the organization of the producers and traders could be described. This type of study would also identify the financial institutions likely to be involved, and indicate the specific actions to be taken by governments and funding agencies.

Research & Development

The term "Research & Development" should only refer to the activities done at the interface between research and development. In fact, the analysis of aquaculture activities undertaken over the last 30 years on the African continent shows that except for some clearly defined research programs and clearly identified extension operations, such activities have been undertaken under both research and development programs.

Where does this confusion come from? The following explanations can be proposed:

- Weak scientific, biotechnical and socioeconomic knowledge base when launching a development project, describing it as "Research & Development," "Pilot," or "Pre-extension" Project. These terms often mean that the project has no real biological, technical, social or economic theme on which extension programs can be developed.
- The will of funding agencies to see projects lead rapidly to "significant" productions even if the technical basis of these projects is weak or nonexistent.
- Research stations and laboratories often work in isolation and their results are not always useful for development projects.

These different research conditions usually lead to the following results:

- Most pilot projects have adopted an approach going "from development to research" (Lazard et al. 1990a).
- The institutionalization of companion studies is the sign of a takeover of research work by development projects in order to solve urgent problems, usually of biotechnical character. Certainly, such takeover has positive aspects in that research is being stimulated both financially and thematically (as in the example of the reproduction in captivity of *C. nigrodigitatus* in Côte d'Ivoire). However, there can also be negative effects in that the often limited resources of a research station or laboratory can be all mobilized on a research theme apparently rich in development consequences, but that can prove to lead nowhere, at least temporarily, as in the case of the aquaculture of *Oreochromis* spp. in the Ivorian lagoons.

In addition to unfamiliar observers, this "takeover" often seems to be a perfect example of complementarity.

Future developments in fish culture Research & Development could be the following, based on the fact that while research and development are closely related, they should not be confused:

- research must be conducted in adequate laboratory facilities and in a stimulating scientific environment, hence the importance of creating regional poles linking critical masses of researchers, paired with research centers from the North;
- development must be carried out by professional developers;
- at present, there is no interface between research and development in Africa. It must be created, but there seems to be no general rule in this regard. It could take place within pilot stations acting as transmission belt between research and fish farmers. There are other alternatives through which pilot fish farmers could play the role of interface between research and development, which would contribute, on one hand, in solving the crucial problem of the future of pilot stations when projects end;
- confusion between research and development will be avoided if the respective professions of the different actors involved in fish culture development are clearly demarcated, as suggested earlier, yet allowing as much "permeability" between them as possible. Permeability between the different categories of involvement in aquaculture will be possible if the various institutions (in many countries, often numerous and antagonistic) taking part in this activity do not compete excessively. The real problem here is not so much having too many parties involved but lacking bridges between them: mobility will be necessary to achieve a harmonious development of aquaculture on the African continent;
- the use of human resources should be optimized: African scientists, trained at great costs abroad and also at the cost of personal sacrifice, should, upon return, be able to find a job in their fields of specialization. Failing this, scientific training through research is useless; and
- aquaculture research programs should logically be designed in cooperation with developers and should be given the necessary means of implementation without depending on existing projects where they would simply be considered as a research component.

For an Aquaculture Research Strategy In SubSaharan Africa

Critical Analysis of Aquaculture Studies (Particularly on Tilapia) on the African Continent

THE ANALYSIS OF AQUACULTURE RESEARCH PROGRAMS CONDUCTED ON THE AFRICAN CONTINENT OVER THE LAST 30 YEARS DOES NOT REFLECT ANY REAL REGIONAL OR NATIONAL RESEARCH STRATEGY

Aquaculture research appears, *a posteriori*, to have been quite "opportunistic" in its approach and has resulted in the following:

- an independent, marginalized research effort. Aquaculture research has systematically been conducted outside the framework of other agricultural and zootechnical studies in the fields of biology, technology and socioeconomics;
- basic research (cognitive) is almost nonexistent on the continent for lack of an adequate scientific environment (intellectual and material), but also for lack of a real downstream demand sector;
- wrong assimilation of concepts from upstream research while research results are often approximate; and
- finally, aquaculture research has often proven to be opportunistic, particularly because of funding constraints. It has adapted to the major trends in the implementation of development projects (particularly by international

organizations) to make fish culture "everybody's business," generating adaptive resources lacking in creativity. On the other hand, "companion studies" have been done in an attempt to quickly solve major problems encountered in the implementation of projects developing more intensive, more sophisticated farming techniques that were initiated on weak or nonexistent biotechnical bases. In these conditions, studies were often discontinued, before achieving anything, together with the projects that initiated them.

THIS SITUATION IS PROBABLY BECAUSE, EXCEPT FOR A FEW CASES, AFRICAN FISH CULTURE HAS NOT YET ACQUIRED A REAL ECONOMIC DIMENSION

In other words, research and development started at the same time in the 1940-1950s and continued at the same pace. As over the last 40 years, both research and development counted on each other in order to progress, and both have lacked coherence. On the one hand, development, which was given important resources, had to yield rapid results in order to justify its continuation and amplification, without reliable biotechnical bases; on the other, research, which was given insufficient resources, was not able to produce a substantial basic research base (biological and technological), with its practitioners sensing that, in the absence of a sound development, their disciplinary approach would have limited impact. Development has remained experimental (or pilot) and research has remained essentially focused on farming systems, the replicability of which is limited for lack of sufficient knowledge regarding their mechanisms.

For the same reason, research has worked, from the beginning, on complex farming systems (polyculture, integrated farming and organic fertilization), most of them being the result of technological transfers from other continents (mainly Asia) where traditional empirical knowledge is essential.

AN ILLUSTRATION OF THIS ANALYSIS IS GIVEN BY THE EXAMINATION OF THE CONTENT OF THREE SYMPOSIA HELD OVER THE LAST 15 YEARS ON THE AFRICAN AQUACULTURE

- In 1975, the "Symposium on Aquaculture in Africa" (FAO 1976) was particularly concerned with the following:
  - regarding species, not only tilapias were given special emphasis but also new species (including *Clarias lazera*, mainly), in a clear attempt to diversify species for fish farmers and consumers. The symposium was also concerned with the introduction of exotic species on the African continent;
  - regarding technologies and farming systems, priority was given to the use of "traditional" inputs (mineral and organic fertilizers, integrated farming and rice-fish culture) based more on recipes than on a clear understanding of the mechanisms of these different treatments; and
  - the socioeconomic aspects of aquaculture were discussed only briefly and moreover by nonspecialists.
- In 1985, the "African Seminar on Aquaculture" (Huisman 1986) reflected a radical change in the research efforts on the continent over the previous decade. The proceedings on this seminar highlighted:
  - the results of intensive tilapia culture using artificial feed;
  - the studies on species other than tilapia: mainly, *Chrysichthys* and, to a lesser extent, *Clarias*; and
  - the near total absence of socioeconomic analysis in aquaculture.
- In 1988, the "Workshop on Applied Research in Aquaculture in Africa"

(Bernacsek and Powles 1992) reflected a development in the research efforts, particularly:

- a more scientific approach of organic fertilization and a concern to understand its mechanisms rather than to rely on simple recipes;
- a comprehensive biological approach of the culture of species such as tilapias in lagoon environments, improved knowledge of *Heterobranchus* and sex determinism in *Oreochromis*; and
- a comprehensive socioeconomic approach of the different technical, development models for extension.

Strategic Role of Research Efforts on the Development of Aquaculture In Africa

WHICH DEVELOPMENT?

There are many opportunities for aquaculture development in Africa, particularly in the farming of tilapias.

According to the Type of Environment. The type of environment largely determines the model of aquaculture and its intensification level:

- extensive fish culture in natural (flood plains) or artificial (hydro-agricultural and agro-pastoral reservoirs) waterbodies (fry stocking and fisheries management);
- semi-intensive fish culture in ponds; and
- intensive fish culture in earthen ponds with high water renewal, or in cages and in pens implemented in natural environments (lakes, rivers and lagoons).

According to the Type of Target Populations. The models of fish culture to develop will be different depending on the populations of fish farmers that are targeted for development: villagers, fishers, artisans, small-scale producers, medium-sized undertakings and commercial farmers, both in terms of investments and farming systems. In rural fish culture, models requiring labor as an investment and little operating funds will be preferred; for industrial/commercial farming, the approach will be radically different.

According to the Type of Market Targeted. The type of fish culture to develop will be different whether it is intended for urban markets where the purchasing power is relatively high, or for rural areas where it is low (or unequally distributed in time).

WHICH RESEARCH?

In the absence of clearly defined development schemes or even a general research framework, research and development must improvise. These conditions are favorable for creative scientists or those with wide-ranging research competence, as they are free to study the species and farming systems of their choice and move freely from upstream to downstream research. However, in view of the limited development of African aquaculture today and the lack of clear direction for research programs, this can be a source of great frustrations for those researchers wishing to investigate a specific aspect of a given species. In this context, research is compelled to work in multiple directions.

WHICH STRATEGY?

Here are a few examples of the strategic role research can play on the development of aquaculture in a given country or region.

Population Genetics. Studies on population genetics are fairly recent, focusing mainly on tilapias. This type of research was essentially motivated for its use in aquaculture on other continents (Asia).

A real African policy for the use of indigenous species for aquaculture should promote research on population genetics and on-station testing (comparison of strains, populations and species) using parameters such as growth rate in captivity, artificial feed conversion and tolerance to environmental factors such as temperature, salinity, etc., which could later lead to the creation of genetic improvement programs.

Such programs could have the following results:

- increased knowledge on the preservation of genetic resources of African species of importance for aquaculture and the fisheries;
- optimum use of these resources in aquaculture; and
- decreased rate of introduction of exotic species.

All reflecting a real strategic impact.

Choosing species of aquaculture potential. The choice of species in view of their use in aquaculture is not a neutral choice. Except for tilapias that show potential for low-cost aquaculture, the other species on which research has been conducted so far do not generally present the same characteristics, particularly in their mode of reproduction and feeding habits.

In this field of research, it seems that a continental program should focus on acquiring knowledge on the major biological parameters for the farming of species with a potential for aquaculture. In these conditions, a real strategy for the development of the aquaculture of these species could be developed taking into account the major farming constraints, the potential market and target populations.

At this junction, it is necessary to determine whether research should be focused on many species to be screened for aquaculture or on the biological and technological aspects of the culture of a few species.

Farming system. No in-depth study has been conducted so far on the role that fish culture can play in African farming systems and on its spaciotemporal integration into these systems. Such studies are very important and should not be limited to the transfer of technologies such as integrated farming systems, for example, which tend not to last longer than the projects that initiated them. The integration of aquaculture in a given region is possible only if the different farming systems and their respective dynamics are sufficiently understood.

To conclude, fisheries production in African countries should be planned before a strategic research planning is developed to achieve development objectives. In reality, however, it is often the opposite situation that prevails: research is left on its own to rescue hazardous development operations. No research strategy is possible in this context; research is condemned to remain opportunistic and supportive of a development that has not yet itself proven successful.

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Peri-urban Aquaculture in Midwestern Côte d'Ivoire

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Abstract

Repeated failures to develop aquaculture in tropical Africa in the past few years have discouraged many of those working in this field. Today, although aquaculture is not by itself the sole alternative to financially-stricken agricultural operations, it represents an interesting diversification of agriculture at the periphery of large cities. Some fish farmers have been devoting most of their time to aquaculture for the past six years using a more farm-oriented than enterprise-oriented approach.

Ponds are built along lowland ricefields. The coexistence of the two operations has shown some positive interactions (better water management, a rising level of the water table and water fertilization).

Oreochromis niloticus is used as dominant species in a monosex polyculture developed in three stages: broodfish production, fingerling production and production of marketable fish. Fish are fed rice bran and slaughterhouse residues or wet manure are used as fertilizers. The results of this polyculture are presented along with the economic profile of various farms. These results demonstrate the relevance of this activity for the fish farmers. A comparison of fish farms and rice farms is also made.

The authors emphasize the importance of peri-urban aquaculture because the response of the aquaculture market and the advent of new mechanisms of assistance between fish farmers may lead to many economic developments.

Introduction

In 1990, out of 230,000 tons of fish consumed in Côte d'Ivoire, 150,000 tons were imported. This deficit has increased consistently in the last few years and, despite all the efforts to develop aquaculture, few producers continue to practice it in the rural areas.

The conditions of aquaculture (Lazard et al. 1991), particularly the need for agricultural inputs and access to city markets (Koffi 1990), have led operators to move their activities to peri-urban

zones, for example the AFVP/SATMACI[a] Project at Daloa and at Gagnoa in Midwestern Côte d'Ivoire.

Due to demographic pressures (Daloa's population rose from 125,000 to 175,000 between 1984 and 1988), the local authorities have dispossessed traditional landusers, and land unsuitable for construction that still officially belongs to the municipalities has been transformed into ricefields and vegetable gardens. These peri-urban lowlands are worked by a small, mainly foreign peasantry that is originally from northern Côte d'Ivoire and the Sahelian countries (Mali and Burkina Faso). The water system of Midwestern Côte d'Ivoire allows the construction of many ponds on the slopes of valleys, contiguous to ricefields.

In December 1986, for the first time, a farm supported by the Daloa Peri-urban Fish Project began to yield a livelihood for a fish farmer. Following this, others decided to begin semi-intensive aquaculture based on a rigorous technical model (broodfish ponds, fingerlings ponds and marketable fish production ponds) that required a certain level of technical work (manual sexing, organic fertilization, use of carnivores and polyculture) and an efficient production tool (well-constructed ponds).

For many of the new fish farmers, aquaculture was to become their major—or even exclusive—activity.

Although this analysis is based on a still small number of fish farmers, it aims to describe the beginnings of a peri-urban fish farming dynamic, and to analyze the strategies developed by fish farmers in terms of construction (investment) and technical models.

[a]AFVP: Association française des volontaires du progrès (French Association of Volunteers for Progress); SATMACI: Société d'assistance technique à la modernisation agricole de la Côte d'Ivoire (Technical Assistance Association for the Agricultural Modernization of Côte d'Ivoire).

Ponds and Lowlands

Construction

ISSUES

In humid West African tropical zones, farmers have not generally had to be confronted with water problems for agricultural production. Knowledge about water and water management practices is therefore recent, particularly in the forested south of Côte d'Ivoire. The earliest establishment of ricefields in lowlands dates from the 1970s, for which irrigation channels were dug and maintained by SODERIZ[b]. Later, these channels were abandoned in favor of earth- and tree-branch dams placed on the main drain, directly feeding the ricefields. Depending on the particular situations, several factors can explain this deviation from the proposed model: lack of understanding between rice farmers, inadequate water supply and additional work. For example, efficient water supply in a ricefield located far from the drain requires much more work: maintenance of the irrigation channel, construction and surveillance of the dams, etc. Under these conditions, the creation of aquaculture ponds for which water management is essential (installation of a weir, pond maintenance, feeding and drainage channels, etc.) may seem bound to fail.

Yet today, several core groups of fish farmers have formed in some peri-urban rice lowlands, and over the years they have improved and extended their operations (for example, the "Gako" rice

[b]SODERIZ: Société pour le développement de la riziculture (Ricefarming Development Company). A state-owned company responsible for ricefarming development and the conversion of lowlands to irrigated ricefarming. Its activities in Midwestern Côte d'Ivoire were taken over by SATMACI.

lowlands at Daloa and those of Barouhio at Gagnoa) despite the absence of water management traditions and despite the size of the necessary investment. This development indicates a real interest in aquaculture.

INTERACTIONS BETWEEN RICE AND FISH CULTURE

Water is supplied either by a diversion from the main drain of the rice lowland or by the tapping of a secondary water table feeding the ricefield, for ponds located upstream from ricefield.

There are many positive interactions between rice culture and fish culture: increased water supply for the rice, improved water management in the lowland, increased areas for irrigated rice, relatively reduced crop losses caused by floods and fertilization of the rice by the pond's water.

Surveys of rice farmers located near fish farms, taken in late 1990, showed that:

- 6.6% think fish culture competes with rice for space and water;
- 13.3% think fish culture could affect rice production if aquaculture management does not allow for the requirements of rice; and
- 80% are satisfied to have ponds close to their ricefield (Barmoy 1990)[c].

In time, the development of fish ponds along the rice lowlands will raise all the water tables that feed them. This is why support service agencies must favor an integrated approach to lowland management that will conserve water and reduce work as fishponds and ricefields will be progressively extended.

[c]In an extreme case, a fish farmer has even had rice farmers help pay for repairing his dam after a flood washed it away.

APPROPRIATION OF POND CONSTRUCTION METHODS

A description of fishpond construction methods was presented in the "Manuel de construction des étangs" (Pond Construction Manual) (Oswald 1989b).

The extent of appropriation of pond construction methods by farmers will depend on attempts aimed at improving their farm's technical quality and at minimizing the time needed to manage and maintain it. The end result is sometimes disappointing; however, experience is gradually building up, allowing fish farmers to design improved ponds and to minimize the effect of climatic hazards. For example, improved grading of the weir, a new canal layout to limit water losses by strengthening the downstream dike and reducing the impact of dry season water shortages are among the possible improvements.

Frequent mistakes by beginners often contribute to their understanding of pond construction logic: how many times have heavy rains shown fish farmers the usefulness of weirs by washing away dikes and fish?

Contrary to popular belief, the fish farmers rapidly understand the benefits of high quality construction, as reflected by their receptivity to the proposed standards. They quickly accept quality criteria even though they require additional work (strengthening of the downstream dikes, 2% bottom slope, dike slopes, 0.60 m minimum pond depth and more than a meter at the monk drain).

A well-built pond facilitates water control, including frequent drainage, and allows rational fertilization.

In this context, the monk drain is an essential tool: it serves as overflow and as a drainage tool and allows the regulation of the water level, thus facilitating

the capture of the last fish before complete drainage.

For example, the map of the Gako lowland (Fig. 1) includes the construction dates of the different farms, which is a good illustration of the expansion of this peri-urban artisanal aquaculture.

Construction Costs

ACCESS TO INVESTMENT

The construction of ponds requires a cash investment to buy materials and tools. It also requires an investment of work by the fish farmer or subcontractors.

- Minimum expenses by heading and by pond are as follows:
  - drainage work: on average 20,000 F CFA[d] or approximately US$70 (125 mm PVC pipes, bags of cement and rental of mold for creating the monk drain); and
  - digging material: the price of new material is 30,000 F CFA (wheelbarrow, daba[e], machete and file); the fish farmer often already has these tools.

The chronic lack of cash in rural areas makes it difficult to raise the funds needed during construction of a five-pond farm. It is often the inability to produce this money on time that hinders construction.

- The amount of work needed to build a five-pond farm varies according to the nature of the terrain, the shape of the pond and the quantity of earth to be excavated. A single full-time worker takes between two and three months to build a 400 to 500-m^2 pond (where amounts of cuts and fills are equal). The slow periods of the agricultural calendar make such construction by farmers possible.

[d]In 1991, US$1≅300 F CFA; in 1996, US$1=600 F CFA.

[e]This African tool is a short-handled hoe.

Based on the two extreme situations possible, the costs can be estimated as follows (Table 1):

- Situation 1: between 45,000 and 60,000 F CFA for a 400 to 500-m^2 pond built by a subcontractor, paid by the fish farmer (following the crisis, labor costs have tended to decrease).
- Situation 2: between 21,000 and 36,000 F CFA for a pond of the same size built with unpaid labor by a worker who is starting his own fish farm; the opportunity cost of one day of work has been valued at 350 F CFA (Koffi 1989).

Furthermore, it is interesting that the rare sales of ponds have involved significantly higher prices (more than 200,000 F CFA per pond).

Technical Aspects

Summary of the Proposed Technical System

The farming of *Oreochromis niloticus* follows a three-step process in three different ponds for the production of broodfish, fingerlings and marketable fish (ponds of male tilapias, controlled by some carnivorous fish, with yields of fish of at least 200 g mean body weight). This technical model is based on Lazard (1984) and has already been described several times (Copin and Oswald 1988; Oswald 1989a; Oswald and Copin 1992).

This technical model is based on control of the factors of production by the fish farmer (fry, inputs and marketing). The inputs are rice bran as "feed" and farm or slaughterhouse organic residues as "nutrient."

The model is made for a simple and rational management procedure. The cycles follow a logical order: three production ponds are successively stocked with males from the fingerlings pond,

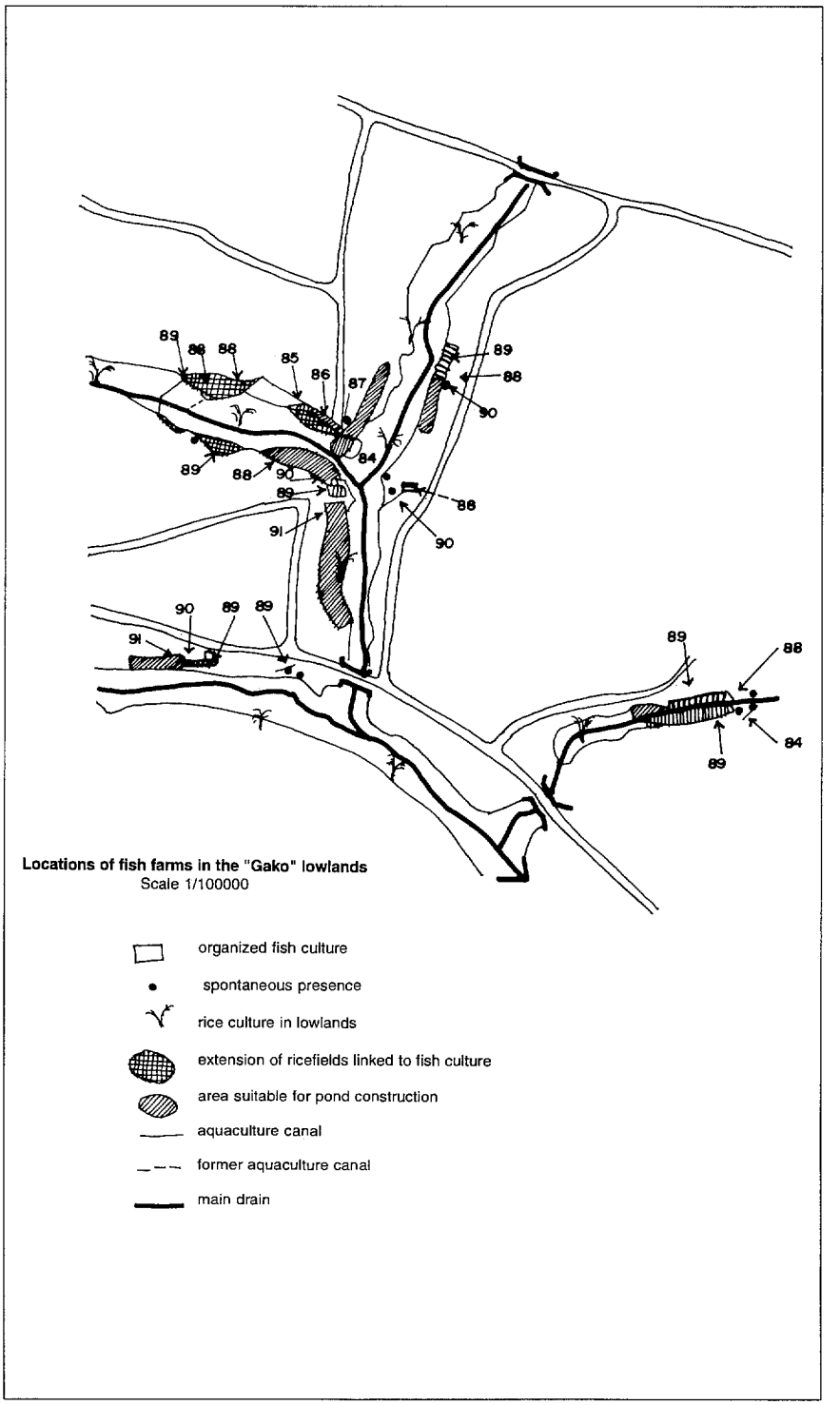

Fig. 1. Aquaculture setup in rice culture lowlands (Gako). Numbers indicate the year of construction.

Table 1. Construction costs of a 400-500 m^2 pond using different methods.

Cost of one pond	Situation 1 (subcontractor)	Situation 2 (fish farmer)
Maximum	80,000 F CFA	56,000 F CFA
Minimum	65,000 F CFA	41,000 F CFA

which is restocked each time from the broodfish pond. The ponds are drained for each harvest of marketable fish and for each sexing procedure. All ponds are compatible with all stages of the cycle, which facilitates the reorganization of the operation.

Aside from being rational, the approach to operations management is flexible insofar as the cycle lengths are adapted to technical performances and market demands. In practice, a three-month delay does not significantly disturb production. The same technical model applies to a fish farmer doing three or six harvests of marketable fish a year.

Requirements

Mastery of this technique by the farmer requires a certain "know-how." Fertilization is only possible if the water supply is sufficiently controlled and if the pond depth is adequate (0.60 m). It depends on considerations like water color, fish behavior of trying to breathe air at the water surface when DO concentration is too low, trial harvests, etc.

The use of strict carnivores (*Hemichromis fasciatus* or *Parachanna obscura*) contributes to the improved control of the sexed tilapia population (uncontrolled reproductions being linked to sexing errors), but it is difficult to maintain adequate stocks of these carnivores.

Heterotis niloticus and *Heterobranchus isopterus* are generally used in polyculture. *Heterotis* has its own three-stage production cycle. The broodfishes are placed in a tilapia production pond, the fingerlings pond is also used for tilapia fingerlings production and the other ponds are used to produce both *Oreochromis* and *Heterotis*. *Heterobranchus isopterus* taken from the wild (flood plains) are introduced into the tilapia production ponds at the maximum density of $0.2 \cdot m^{-2}$.

Extent of Appropriation of the Proposed Technique

EVOLUTION OF SOME PRACTICES

Observing the evolution of fish farmers' practices allows us to understand the extent of appropriation of the techniques described above.

- Protection against wild fish:

The search for discarded material to use in feeding and drainage grids is constant; air filters are used as well as refrigerator shelves.

- Fertilization practices:

The need to use fertilizers becomes clear only after a few months during the production of the first batch of sexed tilapias. Later, ponds are fertilized immediately upon stocking and cases of overfertilization are also reported. Also, some fish farmers go to the extent of renewing the water excessively, canceling out the effects of fertilization.

In draining their ponds, some fish farmers have regretted not being able to reuse their already well-fertilized water for another cycle.

- Use of carnivores:

Novice fish farmers see the usefulness of carnivores only after three or four months of culture, when they notice stunted growth in the ponds, coupled

with the presence of undesired fry. Only then do the fish farmers begin to look for an adequate supply of carnivores from the flood plains.

- Management of fry production:

The number of fry produced in a broodfish pond increases if fish farmers shift from one harvest every two months to two harvests each month. The trade-off is that the mean body weight of the fry will be lower (Lazard 1984).

In contrast, in a five-pond farm, it is in the fish farmer's interest to harvest every two months to obtain enough fry of a higher mean body weight.

Choosing fry size over number of fry becomes a constraint when the farm expands because the fish farmers tend to wait long enough between two harvests to maintain the same fry size. They are therefore unable to satisfy their own demand for new fry. One fish farmer was faced with this problem and initially solved it by resorting to the fry surpluses of his neighbors. The neighbors, having tired of this unbalanced relationship, forced him to reconsider the management of his broodfish pond. Obliged to harvest his small fry, he is again in a situation where fry production is not a limiting factor. Table 2 illustrates this change.

These observations show the extent to which techniques are only learned and used when they help to remove an obstructive constraint experienced by the fish farmer.

CHOICES MADE BY FISH FARMERS ABOUT THE TECHNICAL MODEL AND JUSTIFICATIONS

For the counting of the fry transferred to the fingerlings pond, support service agencies recommended an evaluation based on biomass and mean body weight. Fish farmers opted from the outset for a more reliable exhaustive counting that was ultimately easier since the number of mortalities observed remained unchanged.

Improved control by the most efficient fish farmers of sexing starting at a mean body weight of 18 g contributes to reduce the fingerling cycles in favor of production cycles.

Following various climatic events (e.g. a dry spell) or social events (e.g. village funerals), some farms have seen their breeding cycle totally disrupted. The affected fish farmers, without the aid of support services, chose to wait and rapidly re-established their cycle from available fish stock.

Fish farmers are very interested in mastering species other than tilapia (especially *Heterotis niloticus*, *Heterobranchus longifilis* and *H. isopterus*, *Labeo coubie*, etc.). In fact, they keep broodfishes of these species even if this particular choice contributes to reduced yields or to unproductive occupation of pond space. This increased interest in other species has contributed to the development of a reproduction and production technique for *Heterotis niloticus* associated with tilapias.

It seems that fish farmers want to improve the model to provide for the production of bigger commercial tilapia, the optimization of polyculture by reproducing other species and some specialized ponds to produce fish of high commercial value (*Heterobranchus longifilis*).

Economic Overview of Fish Farming Operations

Fish Farm Economic Profile

Two economic profiles are presented here (Tables 3 and 4):

- a ten-pond fish farm in operation for several years, of which the 1987 results have already been published (Copin and Oswald 1988); and

Table 2. Technical evolution of broodfish pond management (mean values over several cycles). Situation 1: the fish farmer gives priority to the mean body weight of the fry; situation 2: the fish farmer gives priority to the number of fry. (Treatment: rice bran at 4 kg·day^{-1}; 0.5 broodfishes·m^{-2}; and mean body weight of broodfishes: 30 g.)

	Situation 1	Situation 2
Harvest frequency (·month^{-1})	0.61	2
Cycle average duration (month)	5.5	2.75
No. of fry·month^{-1}·100 m^{-2}	446	2,265
Production of fry (kg·month^{-1}·100 m^{-2})	1.9	3.2
Mean weight of harvested fry (g)	4.2	1.4

- a fish farm that began production in 1991.

In the first farm, a total of 229 working days were devoted to farming and these were marketing activities on 260 days (movements between the house and farm excluded).

By 1990, the productivity of the working day (F CFA per day) was 1,913 F CFA and the productivity per 100 m^2 was 20,790 F CFA per annum.

This fish farmer repaid 50,000 F CFA of his debt during the year.

In the case of the five-pond farm, 183 working days were devoted to farming and 37 to marketing (movements between the house and farm excluded).

By 1990, the productivity of the working day (F CFA per day) was 1,710 F CFA and the productivity per 100 m^2 (F CFA·100 m^{-2}) was 15,040 F CFA per annum.

Note that this farm was not yet fully operational and that the fish farmer was devoting a good part of his earnings to the repayment of his debt (124,100 F CFA).

Comparison of Aquaculture Revenues with Rice Culture Revenues

The comparison of aquaculture earnings with rice culture earnings shows that aquaculture has a higher work productivity as well as a higher revenue per unit of surface area.

The working capital requirement of aquaculture is lower than that of rice culture (Table 5).

It is worth noting that some rice farmers are gradually becoming involved in aquaculture.

Social Dynamics and Aquaculture

Role of the Project

The role played by the "project" structure has an impact on several levels and should not be underestimated.

It guarantees a certain technical reliability in exchange for the commitment asked of fish farmers. Attempts to reproduce this model without outside support have all failed for lack of an efficient production tool. One of the main roles of the Project is the study and monitoring of construction methods; today, the fish farmer alone is incapable of building good quality ponds.

Moreover, the project financially sustains the investment in aquaculture by making credit[f] available to the fish farmer and it also sells seine nets, among other things, to the farmers.

[f]Credit is not discussed in detail. In contrast with the first fish farmers (Copin and Oswald 1988), some fish farmers today build their farms without credit. Credit therefore no longer has the deciding role that it did initially.

Table 3. Economic profile of a ten-pond, 450-m² farm (rice bran for feed and fertilization with slaughterhouse residues).

Expenses F CFA		Returns F CFA	
Feed:		Sale of *O n* (Mw 150-220 g)	
Rice bran 23,850 kg x 2 F CFA	47,700	1,321 kg x 500 F CFA	649,000
Marketing:		Sale of *Het* (Mw 2,000-3,000 g)	
100 F CFA·day^{-1} x 260 day·year^{-1}	26,000	380 kg x 500 F CFA	160,000
Shop rental		Sale of *H is* (Mw 400-600 g)	
3,000 F CFA·month^{-1} x 12 months	36,000	150 kg x 530 F CFA	80,000
Purchase of fry *H is*		Sale of *H lg* (Mw 2,000-5,000 g)	
400 fry x 25	10,000	80 kg x 800 F CFA	64,000
Amortization of light equipment[a]		Sale of *O n* (Mw 30 g)	
	21,250	300 kg x 150 F CFA	45,000
Pond amortization[a]	40,000	Sale of fry *O n*	
		5,000 fry	5,000
		Sale of fry *Het*	
		50 fry	
	7,500		
		Self-consumption	36,000
Total	220,950		1,046,500
Net profit	935,550		

(*O n*: *Oreochromis niloticus*; *Het*: *Heterotis niloticus*; *H is*: *Heterobranchus isopterus*; *H lg*: *Heterobranchus longifilis*; and Mw: mean weight.)
[a]The amortization of the light equipment and the pond is computed for 5 and 20 years, respectively.

Table 4. Economic profile of a five-pond, 500-m² farm (rice bran for feed and slaughterhouse residue for fertilizer). The case of a young man working for himself.

Expenses		Returns	
Feed:		Sale of *O n* (Mw 150-220 g)	
Rice bran 13,250 kg x 2	26,500	620 kg x 453	281,000
Purchase of fry *H n*		Sale of *Het* (Mw 2,000-3,000 g)	
40 fry x 150	6,000	103 kg x 450	45,000
Depreciation of light equipment[a]		Sale of *H is* and *H lg*	
	21,250	99 kg x 610	60,300
Pond depreciation[a]	14,000	Sale of *O n* (Mw 30 g)	
		240 kg x 190	45,600
		Self-consumption	12,000
Total	67,750		443,900
Net profit	376,150		

(*On*: *Oreochromis niloticus*; *Het*: *Heterotis niloticus*; *H is*: *Heterobranchus isopterus*; *H lg*: *Heterobranchus longifilis*; and Mw: Mean weight.)
[a]The depreciation of the light equipment and the pond is computed for 5 and 20 years, respectively.

Table 5. Economic data for several rice farms in the Gagnoa region of Côte d'Ivoire (François 1991).

Situations	1	2	3	4
Surface (ha)	1.5	1.5	0.5	0.25
Ownership (land tenure)	yes	yes	yes	no
Production ($t \cdot year^{-1}$)	7	7.2	4	0.6
Capital investment	medium	medium	high	low
Net profit ($F\ CFA \cdot year^{-1}$)	420,000	520,000	280,000	40,000
Time worked ($days \cdot year^{-1}$)	390	540	140	90
Work productivity ($F\ CFA \cdot day^{-1}$)	1,070	960	2,030	460
Land productivity ($F\ CFA \cdot 100\ m^{-2} \cdot year^{-1}$)	2,800	3,470	5,600	1,600

Although self-development therefore seems impossible today, significant changes are nevertheless taking place, especially the improved control of many production factors by fish farmers (production of fry, supply of inputs and production techniques) and exchanges of services (assistance in sexing and assistance with marketing).

The model has also been transferred without outside support to temporary ponds stocked with some sexed tilapia and some predators.

Organization Dynamics

- Supply of rice bran:

Some fish farmers have resorted to the services of an accredited husker with whom a relationship is established that leads to credit and deferred payments. A five-pond fish farm consumes between seven and 20 tons of rice bran per year, depending on the associated fertilizer.

- Marketing:

Informal marketing arrangements are developing among fish farmers. For example, the produce of three farms in the Gako lowland is currently marketed by the wife of only one of these fish farmers.

- Fish supply:

Fish farmers sometimes experience disruptions in their cycles for a lack of fry or a lack of males. Initially, they seek to solve the problem by requesting extra stock from neighboring fish farmers as a favor to be repaid.

The quantities of fish exchanged are surprisingly large: data for 1990 from the notebooks of the Gako lowland fish farmers indicate:

- 10,565 fry of 4 g (total weight: 45.86 kg); and
- 2,304 male fingerlings of 27 g (62.92 kg).

The actual figures are probably higher (exchanges are not always recorded) and the figures for the first half of 1991 are still higher.

These arrangements (totally independent of the support services) illustrate the dynamism of the trade which contributes to improve the results of the various farms. By 1990, a quarter of the fish produced on this lowland originated in other fish farms.

The fish are transported on foot in basins. One fish farmer, who had stocked up on fry at a fish farm 4 km away from the lowland, even resorted to a taxi to transport his fish (2,000 fry in three trips at 100 F CFA per trip!).

Farm-oriented Logic or Enterprise-oriented Logic?

Everyone sees aquaculture as a definitive way of appropriating a small plot of land. Support services are seen as

guarantors of the investment made. In extreme cases, traditional landowners have even claimed an interest in aquaculture solely as a pretext to recover their traditional lands.

Aquaculture also seems to represent transferable capital. Fish farmers often point out that they work for their families and can therefore exploit family members.

The management of fish farm labor, which is essentially constituted by the family, depends on the type of social organization. Great disparities exist between fish farmers of different ethnic origins. In contrast with the Bété[g], who have had trouble mobilizing family labor, the Dioula[h] find it relatively easy.

During the difficult initial stage of production, some fish farmers favor self-sufficiency and outside work at the expense of a rapid increase in their own enterprise's production (their ponds are used for the storage of fingerlings or wild fish).

Although this type of aquaculture has generally been labeled "artisanal" or "entrepreneurial," some of these observations suggest that these producers are using a "farm-oriented" logic.

Conclusion

Although aquacultural development is still limited in size and fragile, some significant elements of an endogenous dynamic can be identified.

The investment of labor, which is considerable for a five-pond farm, seems compatible with the agricultural calendar. Even if it takes several years to build the farm, the presence of a core of efficient fish farmers means that the farm will be operational from the very beginning. The majority of fish farmers choose to expand their farm once cruising speed has been achieved.

The technique is easily reproduced as soon as fish farmers attain a certain level of experience and technical expertise. Once established, fish farmers do not depend on any structure for the operation of their farm.

This type of aquaculture is financially and economically profitable. Moreover, it does not suffer from a lack of cash, which is a particularly chronic phenomenon in the current socioeconomic context. While these strengths pave the way for a real aquacultural expansion, fish culture is already justified by the development of agricultural inputs utilized by this enterprise and because it represents a socioeconomic improvement for the social groups concerned.

The existing dynamic core groups of fish farmers are an excellent base for a renewed development of farming models adapted to the rural context, provided that research agencies can establish a fruitful dialogue with interested parties. Given the position that we predict aquaculture will occupy in the farming systems of humid tropical zones, the existence and development of such a technical, social and economic yardstick appears to be a challenge of the greatest importance for agricultural development.

Acknowledgements

The authors wish to thank Damien Colin, head of the SATMACI-PAPU CD aquaculture project, for his kindness in locating a great deal of useful information for this paper.

[g]Bété: indigenous ethnic group of central western Côte d'Ivoire.

[h]Dioula: the northern ethnicities of Malinké origin; they control most of the trade.

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C. POSTERS

Growth and Food Conversion of Five Strains of Nile Tilapia (*Oreochromis niloticus*) Fry

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Abstract

The growth and food conversion of five strains (Egypt, Senegal, Thailand, Israel and Singapore) of Nile tilapia (*Oreochromis niloticus*) fry were determined under laboratory conditions. The fry (0.0256 g mean body weight and 1.15 cm mean total length) were stocked in fifteen 20-l aquaria and fed everyday on powdered artificial food containing 30% crude protein. The feeding rate was 20% of total fish biomass daily. Fish samples were taken every seven days for five weeks for length and weight measurements.

Specific growth rates and food conversion ratios did not differ significantly among strains ($P>0.05$).

Historical and Technical Aspects of the Introduction of Tilapia Culture in Colombia

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Abstract

The first tilapia introduced to Colombia in 1953 was *Oreochromis mossambicus* from Jamaica. Tilapia farming at that time was conceived as an activity for rural populations. Its dissemination, culture or distribution were later forbidden, due to its high capacity to invade natural waters below 1,200 m. In 1962, *Tilapia rendalli* was introduced from Panama, in the hope of taking advantage of its feeding behavior to control weeds in ponds, reservoirs and channels. However, its establishment in natural waters below 1,600 m had serious effects on native flora and fauna and its culture was restricted. In 1979, *Oreochromis niloticus* (Ghana strain) was introduced from Brazil. It gained acceptance due to its good taste, fast growth, large size, and suitability for mono- and polyculture in lakes, reservoirs and fishponds below 2,400 m. The problem for private tilapia farmers was that they had to compete with the tilapia capture fisheries in natural waters. The solution was to aim for high production levels year-round. This evolution from essentially family subsistence activities in 1980 to profitable businesses was achieved by 1987. In 1985, *Oreochromis urolepis hornorum* was introduced from Brazil for the production of an all-male progeny by crossing with *O. niloticus*. This was not very successful. In spite of their fast growth and large size, the fish had an unattractive black coloration. In 1982, a red tilapia from Florida (*O. mossambicus* albino x *O. urolepis hornorum*) was introduced from Alabama (USA) for more intensive culture. Demand for red tilapia increased slowly at first, but by 1987 the first more-intensive fish farm was established using its own red tilapia strain called red tilapia yumbo (red Florida x *O. niloticus*) in the Valle del Cauca. This facility has aeration and relies on exchange of underground water and feeding with high quality pelleted feeds. The farm produces two crops, totalling 40-50 $t \cdot year^{-1}$, from domestic and export markets. Nationally, farmed tilapia production grew from 100 t in 1985 to 700 t in 1988. In 1989, several Israeli tilapia farmers formed a joint venture with Colombian investors for two large intensive tilapia farms: one in Nariño (45 ha) and another in the Valle del Cauca (101 ha). Both have been in operation since 1990. They have "1985 red Florida tilapia," red *O. aureus* from Jamaica and Israel, *O. niloticus* from Egypt (Lake Manzalah) and *O. aureus* from Israel. These farms have modern facilities for highly intensive tilapia culture. In 1991, more red tilapia farms were constructed and about 65 farms were in operation by 1992, covering 635 ha. This made Colombia, in 1992, the third largest producer of tilapia in Latin America (11,050 t), after Mexico (75,000 t) and Cuba (16,500 t).

Studies on the Growth Performance and Gonadal Development of Triploid Tilapia *Oreochromis aureus*

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Abstract

Heat shock treatment at 41°C induced 100% triploidy in *Oreochromis aureus*, using the method of Chang and Liao (this vol.). Diploid (control) and triploid fry were reared from first feeding in triplicate hapas (2x1.2x0.9 m net cages) in the same pond with a water depth of 60 cm. Twenty fish were kept in each hapa and fed twice a day with commercial eel feed for the first 12 weeks and with commercial tilapia floating pellets for the next 12 weeks. The crude protein content of the feeds were 44 and 23%, respectively. Twenty diploid and 20 triploid fish were then measured and examined for gonad development. The gonadosomatic index (GSI) was calculated as: GSI = (gonad weight/body weight) x 100.

Diploid and triploid fish did not differ significantly ($P>0.05$) in size (body weights were 119.79±40.00 and 109.50±21.70 g, respectively), but the triploids were of more uniform size than the diploids. The genital papillae of triploid fish showed no development compared with diploids at 18 weeks old. Mean gonad weights of females and males were similar for diploids (1.02±0.93 and 1.01±0.34 g, respectively), but different for triploids (0.08±0.10 and 0.34±0.31 g, respectively). Some testes of triploids developed well with many sperm of variable appearance accumulating in the lobular lumina. Yolk accumulation was pronounced in the oocytes of diploids, but the threadlike triploid ovaries contained mainly oogonia with only a few yolky oocytes.

The Malaŵi Central and Northern Regions Fish Farming Project: Research, Progress and Prospects

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Abstract

The Central and Northern Regions Fish Farming Project started in 1989 for five years with funding from the European Development Fund of the European Community (US$3.6 million) and the Malaŵi Government (US$1.2 million). The main aim was to increase the supply of fresh fish through fish farming in those areas of Northern and Central Malaŵi where it is scarce. The primary objective was "to establish the technical and economic parameters for developing fish farming in Northern and Central Malaŵi." This is being carried out through research on fish farming methods, and extension of these methods to farmers. The project headquarters is in Mzuzu, Northern Region.

The main areas of research have been:

- finding alternative indigenous species to culture. The main species used in Malaŵi has been *Oreochromis shiranus*, which shows very bad growth characteristics. *Oreochromis karongae* grows to a larger size before maturity and has faster overall growth rates. *Clarias gariepinus* and *Bathyclarias* spp. also look like promising species for use by farmers in the future; and
- investigating the potential of small waterbodies and dams for fish production.

The extension program is carried out through a team of field-based extension workers. Simple methods are advocated: single species culture, maize bran and animal manures, and harvesting by pond draining. Farmer numbers have increased to 300 around Mzuzu, operating 458 ponds. The main problem has been lack of fingerlings.

The main activities planned for the future are:

- continue research on appropriate fish farming methods;
- expand coverage of the extension service; and
- develop models for exploration of small waterbodies.

Interactions between Nile Tilapia (*Oreochromis niloticus*) and the Pond Community at Various Fish Densities

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Abstract

Nile tilapia is primarily an algal feeder. In a rearing pond, it may compete or, in other ways, interact with herbivores among the invertebrates, and change the structure and the processes in the system. The interaction between tilapia and the rest of the biological community is, however, poorly understood. In this study, the succession in the biological community was followed in nine experimental fertilized ponds stocked with high density, low density and without fish. The experiment lasted for 74 days.

The ponds were 10x4 m^{-2} with a water level of about 1 m. Superphosphate was added daily to all. Temperature, oxygen, transparency, water level, pH, conductivity, phosphate, nitrate, phytoplankton and zooplankton were sampled each day. Fish was sampled six times during the study. Diet, growth rate and production in the tilapia were measured and estimated.

The fish diet was mainly desmids, green algae (some filamentous) and some cyanobacteria. Zooplankton occurred scarcely with *Bosmina longirostris* being dominant. The communities reacted differently to the treatments. The transparency was higher in ponds without fish. Differences in community structure in the various ponds appeared. These differences seem not to have a significant effect on the growth rate of tilapia.

Studies on the Control of Tilapia Recruitment Using a Tilapia-Predator Polyculture System in Southwest Nigeria

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Abstract

Many methods have been used to control undesirable tilapia population, but the combined stocking of tilapias with predators is the most desirable method in Africa. In this study, *Parachanna obscura*, *Clarias gariepinus*, *C. isheriensis* and *Heterobranchus bidorsalis* were evaluated in concrete ponds (160 m^2, water depth = 1.4 m) using different tilapia-predator stocking combinations in four successive production cycles, each lasting 180 days, to control recruitment and to produce market-size *Tilapia guineensis*. Tilapia fingerlings were stocked first followed by the predator fingerlings 60 days later, to allow the tilapias enough time to grow and breed. Ponds were fertilized with dry poultry manure (60 $kg \cdot ha^{-1} \cdot day^{-1}$) and fish were fed 1:1 mixture of macadamia presscake and blood meal (56.8% protein) at 5% body weight per day. At harvest (180 days after stocking predators), ponds were drained; all fishes were removed and sorted according to species, counted and weighed. The market weight of tilapia was set at 200 g and effective recruitment control at an A_1 value (% weight of market-size tilapia in the population) of >90%. Treatment means were subjected to ANOVA and Duncan's multiple range tests (P=0.05).

Tilapia mortality was <10% in any pond and was not related to the treatments, rather it was due to handling stress during periodic weighing. All predators stocked were recovered at harvest. Tilapia fry were observed as from 90 days after stocking. The stocking of predators had a direct effect on tilapia production. Generally, the fewer the predator stocked, the lower the adult tilapia yield. The combinations that gave effective control of tilapia recruitment were 10:1, 6:1 and 20:1 with *P. obscura*, *C. gariepinus*, *C. isheriensis* and *H. bidorsalis*, respectively, and produced tilapia A_1 values >90. Differences in A_1 value, mean final weight and tilapia yield between treatments were significant (P<0.05) for each predator tested and were due to the consumption of pond-spawned tilapia fry by the predators. This reduced the competition between adult tilapias and pond-spawned juvenile tilapias for food, thus enabling growth of adult tilapia. This demonstrates effective reduction of juvenile tilapia by all the predators tested. This technique improved tilapia yields, an economic benefit to the farmer.

Observations on the Possible Effects of Salinity, Pond Regime Practices and Behavior on the Culture of *Tilapia guineensis* and *Sarotherodon melanotheron*

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Abstract

Attempts at the polyculture of *Tilapia guineensis* and *Sarotherodon melanotheron* in the Buguma fish farm of the African Regional Aquaculture Centre, Nigeria, showed that no matter in what ratio the two species were initially stocked, *S. melanotheron* was always preponderant in the harvest, accounting for 70-95% both numerically and by weight. Investigation of the abundance ratio of the two species in the farm's semi-enclosed main channel gave a ratio of 1:1.8 in favor of *S. melanotheron*. However, in the creeks around the farm, the ratio was reduced to 1:1.1, suggesting that the abundance of *S. melanotheron* in the ponds was probably a product of the pond culture system. Further investigation of the natural abundance pattern of the species in the polyhaline zone (average annual salinity, 20 ppt) gave a ratio of 2.1:1 in favor of *T. guineensis*. Also in the oligohaline zone (average annual salinity, 5 ppt), *T. guineensis* was almost exclusively present.

It is thought that *S. melanotheron* benefits more from the practice of fertilizing the ponds since it is, among other things, a plankton feeder. Secondly, its mouthbrooding practice enables it to increase its numbers in the pond while it preys on the fry of *T. guineensis*. It is suggested that polyculture of the two species should be avoided and that monoculture of *T. guineensis* is not advisable in mesohaline situations, since the abundance of *S. melanotheron* favors its infiltration and the normal pond culture practices give it a competitive advantage. It is also suggested that monoculture of *T. guineensis* is best attempted in the oligohaline and polyhaline zones where *S. melanotheron* is less abundant.

Periphyton Composition and Physicochemistry in an Artificial Habitat (Acadja-enclos) for *Sarotherodon melanotheron*—Adiapote Area, Ebrié Lagoon, Côte d'Ivoire

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Abstract

Sarotherodon melanotheron is a grazing fish which colonizes artificial habitats like the acadja-enclos (parks where branches are placed in shallow fresh- or brackishwaters). This lagoon tilapia feeds essentially on the periphyton that develops on the poles of bamboo acadjas.

Monthly sampling of the periphyton and dosage of the physicochemistry (temperature, oxygen, turbidity, pH and nutritive salts: PO_4, NO_2, NO_3 and NH_4) was conducted in open waters and in acadja-enclos from November 1988 to December 1989 in Adiapote, a brackishwater site with fluctuating salinity in the Ebrié Lagoon, Côte d'Ivoire.

The dominant periphyton species were: *Mougeotia floridana* (green algae), *Lithococcus schizodichotomum* and *Lyngbya rivulariarum* (cyanobacteria) and diatoms such as *Nitzschia*, *Melosira*, *Fragilaria* and *Achnanthes*. Generally, green algae were dominant throughout the year. However, although they were abundant during the dry season, cyanobacteria (February) and diatoms (March, April) were dominant. Diatoms were abundant during eight months of the year and were replaced during the dry season (February-July-November and December) by cyanobacteria. The periphyton biovolume varied from 0.06 to 0.72 $ml \cdot cm^{-2}$. The highest levels were found from March to August (including the rainy season) with a biovolume of 0.53 $ml \cdot cm^{-2}$. Temperature (25-31°C), oxygen (3.8-7.2 $mg \cdot l^{-1}$), salinity (1-25 ppt) and pH (6.7-8.6) had the same profile in open waters and in the acadja system. However, turbidity (0-55), PO_4 (0.5-12 $mole \cdot l^{-1}$), NO_2 (1.2-8.5 $mole \cdot l^{-1}$), NO_3 (0-21 $mole \cdot l^{-1}$) and NH_4 (2-70 $mole \cdot l^{-1}$) presented different profiles in lagoon waters and in the acadja system.

In July, nitrates and ammonia showed very high levels both in the acadja system and open waters. The highest nitrite levels were found in December and only in open waters. Phosphates showed very high levels in August with higher bottom values (February-July). Bottom turbidity is higher from November to May while bottom and surface values are identical from June to November.

Production of Pseudo-females of *Oreochromis aureus* Using Ethynyloestradiol

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Abstract

The objective of this study was to develop a technique for the mass production of "pseudo-females" (female phenotype, male genotype) of *Oreochromis aureus* to produce populations of fry with a high percentage of males (90-100%) and to avoid using classical sex reversal methods (using methyltestosterone). Batches of fry of *O. aureus* were treated with 17 α-ethynyloestradiol. Two doses were tested: 100 and 200 $mg \cdot kg^{-1}$ of feed, yielding 94 and 98% females, respectively, after 40 days of treatment, compared with 51% females obtained with the control batches. A few hermaphrodite individuals were found in the batches (2%) which received treatment.

Several females (n=53) from the fry batches were crossed with normal males (genotype ZZ) to select the "pseudo-females" (genotype ZZ). Out of 53 fish tested, 25 gave progenies with a high percentage of males (72.7-100%). These fish are considered "pseudo-females." Hybridizations were repeated (n=2-4) to verify the sex-ratio stability in several successive progenies (variation from 0-9.1% of the male ratio).

Fry (genotype ZZ) from "pseudo-females" hybridizations producing systematically a high percentage of males (98-100%) and normal males were sex reversed using ethynyloestradiol (200 $mg \cdot kg^{-1}$) to increase the number of "pseudo-females." These fish will be used as female spawners for the production of broodstock with a high percentage of males in ponds.

Consumption of Phytoplankton by *Oreochromis niloticus* in Lake Muhazi (Rwanda)

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MICHA, J.C., J.P. DESCY and F. LAVIOLETTE. 1996. Consumption of phytoplankton by *Oreochromis niloticus* in Lake Muhazi (Rwanda), p. 546. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

The 34.1-km^2 Lake Muhazi has eutrophic waters with a high phytoplankton biomass dominated by blue-green algae. However, the lake ichthyofauna has little diversity and low productivity. Samples of plankton and stomach contents of *Oreochromis niloticus* were collected in the same spots and at the same time to verify the ingestion of phytoplankton from the lake by this species. Principal component analysis, comparison for the frequency histograms and of coefficient of electivity indicate that the lake water and the stomach contents are dominated by cyanobacteria and green algae. There is little variation in the relative composition of the lower taxa (genus and species) with season and at different locations in the lake. However, it was observed that *O. niloticus* feeds more on certain categories of algae such as filamentous cyanobacteria and rarely on diatoms and green algae. To conclude, it seems that phytoplankton in Muhazi Lake is used selectively by *O. niloticus* and that most of the algal production is not consumed by the fish.

Documentation and Evaluation of *Oreochromis niloticus* Populations in Ghana for Aquaculture

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Abstract

Two wild populations of Ghana strain *Oreochromis niloticus* from the Volta Lake designated KU and KG 1, and a commercial stock, FS, were evaluated for growth and survival in a two-phase test trial. Mixed-sex fingerlings derived from the different tilapia stocks were tagged and tested communally in 0.2 ha-ponds for 77 days (Phase 1). Hand-sexed, all-male juveniles sorted at the end of Phase 1 were used in a similar experiment (Phase 2) for 105 days. All ponds were routinely manured and fertilized using dry chicken or cow manure, NPK (15:15:15) and urea. Supplemental feeding with a powdered mixture of fishmeal, copra cake and wheat bran (1:2:7 by weight) was also applied to ponds.

The results indicated differences in the growth and survival performance within the stocks tested. Survival decreased in Phase 1 in the following order: KU>KG>FS, and for Phase 2: KG=FS>KU. Ranking of growth in Phase 1 was KG=KU>FS, and for Phase 2: KU>KG>FS. However, performance differences among the three populations were not statistically significant (P>0.05).

Induced Spawning in *Oreochromis niloticus* L.

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RODERICK, E.E., L.P. SANTIAGO, M.-A. GARCIA and G.C. MAIR. 1996. Induced spawning in *Oreochromis niloticus* L., p. 548. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Chemical induction of spawning in tilapia could enable synchronization of spawning resulting in increased fecundity and controlled fry production. This would be useful for small-scale commercial hatcheries and for research purposes. This study evaluated the efficacy of single dose treatments of four chemical inducers—human chorionic gonadotropin (HCG), luteinizing hormone releasing hormone analogue (LHRHa), buserelin acetate (Receptal™) and fertirelin acetate (Ovalyse™)—specifically to induce timed spawning to enable reliable egg collection. HCG and buserelin acetate were ineffective inducers, although the doses applied for buserelin acetate were much lower than those recommended for other species. LHRHa (30 mg·100 g^{-1} body weight) and fertirelin acetate (1-22.5 mg·100 g^{-1} body weight) injections were effective in significantly increasing the proportion of females spawning over an eight-day period. However, the single doses applied were not sufficient to induce closely synchronized spawnings.

Acute Toxicity of Potassium Permanganate, Petroleum Product and Textile Effluent to *Oreochromis niloticus*

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Abstract

The acute toxicity of potassium permanganate ($KMnO_4$), petroleum product (engine oil and petrol in a 3:1 ratio) and textile mill effluent was determined for *Oreochromis niloticus*. The test substances were added as single doses in still water aquaria. The 96-hour LC_{50} of the test substances was determined for the fishes using spring water (alkalinity: 70-80 ppm) and pond water (alkalinity: 160-180 ppm). The LC_{50} for the potassium permanganate test for *O. niloticus* was 5.0 $mg \cdot l^{-1}$ and 3.0 $mg \cdot l^{-1}$ for both pond and spring waters, respectively. The results of the test using petrol and engine oil (3:1) gave a value of 13.0 $ml \cdot l^{-1}$ (pond water) and 12.5 $ml \cdot l^{-1}$ (spring water). The 96-hour LC_{50} value of 31.2% (pond water) and 24% of the textile mill effluent was determined for *O. niloticus*. The LC_{50} values for the fishes in both the spring and pond waters were statistically different ($P=0.05$). The fishes showed increased hyperactivities, body feature deformation, lesions and necrosis during the period of exposure to test substances. The effects of the test substances on the gill epithelia, liver and kidney were examined.

Analysis of the Morphometrics of Three Tilapias (*Tilapia zillii, Sarotherodon galilaeus* and *Oreochromis niloticus*) and Their Intergeneric Hybrids

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SCHWANCK, E. and K. RANA. 1996. Analysis of the morphometrics of three tilapias (*Tilapia zillii, Sarotherodon galilaeus* and *Oreochromis niloticus*) and their intergeneric hybrids, p. 550. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Understanding the modes of inheritance of morphological characters in tilapia is of prime importance when suspected hybridization in farmed or wild stock has to be confirmed, as well as when hybrids are intentionally bred for better characters. Twelve morphometric and meristic characters were measured in laboratory populations of *Tilapia zillii, Sarotherodon galilaeus* and *Oreochromis niloticus* and their intergeneric hybrids (mixed cross-sectional data).

Hybrids showed frequent deviations from intermediarity (especially those between *S. galilaeus* and *O. niloticus*) and differential inheritance between the sexes. The allometric ("growth") coefficients of different characters were counted separately for "small" (SL, 9-13 cm) and "large" fish (13-20 cm) to identify patterns of rapid growth in correlated characters. The analysis indicates a major dichotomy between the growth of weight and depth, on the one hand, and that of certain length-related characters (eye, jaw and fins), on the other. The second type of growth seems to be related with sexual activity and occurs in *T. zillii* in small fish, but in *O. niloticus* not before SL=13 cm. In *S. galilaeus*, there appears to be two rapid bursts of this growth pattern, one at a very small size (SL<9 cm) and again at SL=20 cm. An attempt is made to interpret the deviations from intermediarity and sex-specific differences in hybrids through desynchronization of the growth patterns of the parental species.

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Development of an Autonomous Pilot System in Recycled Water for the Integrated Production of Tilapia and Garden Crops Behaving as Environment Purifier

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SOULEYMANE, K., M. DIDIER and I. QUILLERET. 1996. Development of an autonomous pilot system in recycled water for the integrated production of tilapia and garden crops behaving as environment purifier, p. 551-552. *In* R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (eds.) The Third International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 41, 575 p.

Abstract

Since 1982, the authors have been studying the possible solutions to the problems of animal production related to the climatic conditions prevailing in Sahel and to the lack of water resources. A pilot system was developed at the University of Niamey, Niger, in 1986 and at the National Institute of Agricultural Research (INRA) in Versailles, France, in 1988 to collect data which form the basis of this study.

The association of fish, bacteria and plants constitutes a farming system within which the integrated production of animal (fish) and plant (tomato) proteins can optimize the use of nitrogen introduced through fish feed. This farming system is also based on the use of recycled water as a base for fish culture and as vector of nutrient through a physical cleansing process and biological purification by bacteria whose role is to transform a highly toxic product for fish (NH_3) into a nontoxic product (NO_3).

The Niamey pilot system, established on the grounds of the Department of Science, was composed of cemented circular tanks, a decanter, tanks for the treatment of water (bacterial filter) and a pumping pit, also cemented, containing an immersed pump for the circulation of water. Water was brought tangentially into the tanks, then evacuated towards the center by a system of overflow. Water was recycled approximately every three hours in each tank with an inflow of approximately 0.30 $m^3 \cdot h^{-1}$, i.e., eight times per day. The decanter was a cylindroconical aluminum tank at the bottom of which the coarsest particles in suspension were deposited. These were essentially composed of feces and feed which had not been consumed. The bacterial hydroponic filter was formed by two aluminum tanks containing 2.8 m^3 of gravel graded between 15 and 20 mm enabling the trapping of the finest particles, the nitrification of excreta and the fixing of plants. Culture tanks were fed by pumping, the other tanks were fed by gravity.

The project, with a major objective to demonstrate the applicability of the Hydroponic Recycling System in the Sub-Sahelian region, gave encouraging qualitative results. Water quality was excellent and conversion rates were close to 1.2 using feed produced on-station (15% fishmeal).

Tilapia (*Oreochromis niloticus*) and *Clarias* sp. were tested in this system. For plants, several tests on gumbo, maize (*Zea mays.* L.) and tomato (*Solanum lycopersicum*) gave very good results.

The Versailles pilot system consisted of culture tanks, a purification level composed of a decanter and a fixed biofilter, and a level of cultured plants. The dimensions of the structure were 1.90x1.10x1.10 m with a volume of 1,800 l. A rotary flowmeter gave a flow rate of 50 l per minute, i.e., 3 $m^3 \cdot h^{-1}$ which passed

through an aeration column before entering the culture tank. Tanks were thermoregulated during the winter using a 1 kW resistance piloted by a proportional regulator.

For added safety, oxygen was provided using two low-powered air pumps. Water was removed using a system of overflow. One side of the tank was made of glass to facilitate the observation of fish.

In the Versailles system, fish grew from 12 to 250 g (commercial size) in 150 days for an average temperature of 29°C. Conversion rates varied from 1.36 to 1.65 depending on the initial fish size in our culture conditions. Growth between 250 and 600 g gave conversion rates of 1.65 (some of our animals reached 1.1 kg). In these experiments, water quality was suitable for the tilapia incubation, nursing and grow-out phases.

Maximum total biomass was 60 kg, i.e., 31 $kg \cdot m^{-3}$. This depended on the water system of the culture tanks which could be easily modified and give an increase of approximately 30%. Plant production reached 201 kg over a six-month period, i.e., approximately 5 kg of tomato per plant.

Over a period of 359 days, the Versailles system recycled 25,843 m^3 of water and used 75 m^3 of new water, including water for cleaning and for plants. This volume of water represents the recycling of 8.6% of the total volume of the system per day and 0.29% of the recycled water. Total power consumption for the 359 days was 6,500 kW, i.e., 0.25 $kW \cdot m^{-3}$ of recycled water. This rate can be significantly reduced with the use of a more efficient pump.

We showed the impact, on juvenile stages, of a mixture of toxic products such as ammonium nitrogen and nitrites. Such impact had been overlooked in earlier studies despite the negative effect of these products on the growth, survival and potential weakening of the fish in the presence of pathogens. We observed through our different culture experiments the influence of stocking conditions in recycled systems on the reproduction of tilapia. Predictable obstacles in the production of fish with plants are a consequence of a fragmented interpretation of the traditional criteria of the fish environment (pH, O^2, T°, ions and dissolved substances). Each level should be adapted to optimize production. This static view does not reflect the complete process of a system in production. Our observations showed cycles or periods which govern the different flows of the system. Therefore, the fine-tuning of these techniques requires the synchronization of the variations of the flows in order to optimize the chain of production.

The three major levels (fish, bacteria and tomato) each have different cycles and variations. The main aim of our experiments was to establish the main synchronization clock that would take into account their frequencies.

The results of Versailles and of Niamey led to the formulation of analyses that must be confirmed. In comparing different sites, thus eliminating climate influences, we should be able to provide important keys for the design and management of systems in varied conditions without being bound by conclusions drawn from the results of a single site.

With regard to the animal component, we should study the upper limit of the system by increasing the stocking rates of fish and test other species. This study should be done with our reference feed and with feed adapted to local resources. For the plant component, a diversification of species and of varieties is possible.

The Development of a Tilapia Strain Registry as Part of FishBase

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Abstract

The Zoologisches Institut und Zoologisches Museum Hamburg (ZIM) and the International Center for Living Aquatic Resources Management (ICLARM) collaborate in developing and maintaining a Tilapia Strain Registry. ICLARM compiles a large biological database on fish (named FishBase) which gathers all available information on tilapiine species (e.g., habitats, genetic data, electrophoretic data, origin of strains, aquaculture performance, etc.). ZIM is setting up a collection of tilapiine species and assembles information on the availability of tilapiine types in other museums. ZIM also verifies and supplements the information on tilapiine species as contained in FishBase. As of August 1996, 117 tilapiine species plus 47 strains are documented on the FishBase CD-ROM, available from ICLARM.

Meanwhile, the Food and Agriculture Organization of the United Nations (FAO) and ICLARM have signed a Letter of Agreement to cooperate on the further development of the strain registry.

Effects of Dietary Levels of Carbohydrate, Lipid, Phosphorus and Zinc on the Growth and Feed Conversion of Nile Tilapia (*Oreochromis niloticus*)

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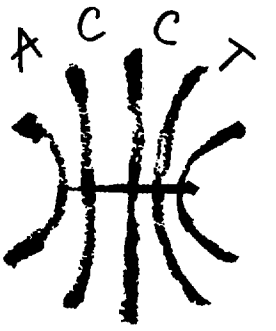
Abstract

A 54-day feeding experiment was conducted on juvenile Nile tilapia (*Oreochromis niloticus*) using purified diets containing equal contents of digestible energy (3,200 kcal·kg^{-1}) and 20% protein. The carbohydrate (dextrin) content in the test diets was 9, 32 or 50%; the corresponding lipid (soybean oil) content was 22.2, 12 or 4%. The diets were supplemented with 0.85 or 1.5% phosphorus and 40 or 100 mg·kg^{-1} zinc. The experiment was carried out in flowthrough aquaria using dechlorinated tap water at 24°C. Increasing the carbohydrate content in the diet resulted in 43 to 249% increases in weight gain and 27 to 59% increases in feed conversion ratio. The dietary phosphorus was important for the growth of the fish fed high carbohydrate diets. In fish fed diets of 36-50% carbohydrate, the higher supplementary phosphorus level (1.5%) greatly increased the weight gain. On the contrary, higher supplementary zinc (100 mg·kg^{-1}) inhibited growth. Higher supplementary zinc had a positive effect on feed conversion only in the group fed 50% carbohydrate diets.

D. SPONSORS AND COLLABORATORS

ACCT
Agence de coopération culturelle et technique



The Agence de coopération culturelle et technique (ACCT) was created in Niamey in 1970. It is the only intergovernmental organization for Francophone affairs and the main operator of the biannual conferences of heads of States and of governments of countries having French as a common language, also known as Francophone Summits.

The ACCT serves as the Secretariat for all Francophone representations. Its multilateral activities are concerned with education and training, culture and communication, technical cooperation and economic development, legal and judiciary cooperation and various specific operations under its Special Development Program.

In addition to its headquarters in Paris, the ACCT maintains an international school in Bordeaux, France, where its general direction for education and training is located; an Institute for Energy for countries having French as a common language (IEPF) in Quebec, Canada; liaison offices with international organizations in Geneva, Switzerland; with the European Union in Brussels, Belgium; with the United Nations in New-York, USA; and regional offices for West Africa in Lome, Togo; for Central Africa in Libreville, Gabon; and for Asia-Pacific in Hanoi, Vietnam.

ACCT brings together 44 countries or governments: Benin, Bulgaria, Burkina Faso, Burundi, Cambodia, Cameroon, Canada, Canada-New-Brunswick, Canada-Quebec, Central African Republic, Chad, Comoros, Congo, Côte d'Ivoire, Djibouti, Dominica, Egypt, Equatorial Guinea, France, the French community in Belgium, Gabon, Guinea, Guinea-Bissau, Haiti, Laos, Lebanon, Luxembourg, Madagascar, Mali, Mauritania, Mauritius, Monaco, Morocco, Niger, Romania, Rwanda, Saint Lucia, Senegal, Seychelles, Togo, Tunisia, Vanuatu, Vietnam and Zaire (the Kingdom of Belgium, Cape Verde and Switzerland bring to 47 the number of countries and governments participating in the Francophone Summits).

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CIRAD
Centre de coopération internationale en recherche agronomique pour le développement

The Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) is a French research organization specialized in the agricultural development of the tropical and subtropical regions. In 1984, CIRAD became a governmental institution with the consolidation of French agricultural, veterinary, forestry and food technology research organizations for the tropics and subtropics.

CIRAD's mission is to contribute to the economic development of these regions through research, experimentations, training, and dissemination of scientific and technical information.

The Centre employs 1,800 persons, including 900 senior staff, who work in about 50 countries. Its budget amounts to approximately 1 billion French francs, more than half of which is derived from public funds.

CIRAD is made up of seven departments: CIRAD-CA (annual crops); CIRAD-CP (permanent crops); CIRAD-FHLOR (fruit and garden crops); CIRAD-EMVT (livestock production and veterinary medicine); CIRAD-Forêt (forestry); CIRAD-SAR (food technology and rural systems); and CIRAD-GERDAT (management, common services and laboratories, and documentation). CIRAD operates through its own research facilities, and in cooperation with national agricultural research systems and development projects.

The mission of CIRAD-EMVT is to contribute to the development and upgrading of livestock production and industry in the warm regions of Africa, Asia, South America and the Pacific.

In the field of living aquatic resources, CIRAD-EMVT is conducting through its Aquaculture Research Unit (ABEL: aquaculture, biology and culture) research and development programs on three continents: Africa, South America and Asia. Research activities are focused on the biology of fish species for aquaculture and on the ecology of fishponds. At the same time, several aquaculture techniques and production systems are studied in the framework of pilot projects. CIRAD-EMVT assists many tropical countries in the design and implementation of their development strategy. In addition, experimental facilities are maintained in Montpellier by CIRAD-EMVT to further studies on tropical species; to train its partners from tropical countries; and to disseminate scientific and technical information.

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CRO
Centre de recherches océanologiques

Created in 1958 as a Government Service under the administrative umbrella of ORSTOM until November 1991, the Center was reorganized by Decree no. 91-646 of 9 October 1991 as a National Public Institution whose basic mission is to conduct research in the fields of oceanology, limnology and aquaculture.

With the aim of providing policy- and decisionmakers with elements of reflection based on a wider understanding of these ecosystems, the Centre de recherches océanologiques of Abidjan has, since 1958, focused its research on the interactions between the individual and the environment. Three major research directions were developed around a unifying theme, fish:

- to increase the knowledge base on ecosystems, focusing on the effects of climate change, the trophic relationships between fish communities and plankton and benthic resources, and the degradation of the environment near urban areas;
- to continue evaluation of pelagic and demersal stocks to serve as a basis for proposals to rationalize resource management and respect the renewal of resources; and
- to study the potential for aquaculture of local species based on biology and physiology of these species and to present the research results to developers.

These major directions all aim to improve the management of fragile aquatic environments and should, in time, lead to a wiser use of the natural resources. Extension efforts should contribute to populations becoming progressively responsible for the management of their environment, with the aim of sustaining their activities in a protected environment.

CTA
Technical Center for Agricultural and Rural Cooperation

The ACP-EU Technical Center for Agricultural and Rural Cooperation (CTA) was created in 1983 and operates under the Lome Convention between Member States of the European Union and the African, Caribbean and Pacific States (ACP).

CTA collects, disseminates and facilitates the exchange of information on research, training and innovations in the spheres of agricultural and rural development and extension for the benefit of the ACP States.

To achieve this, CTA commissions and publishes studies; organizes and supports conferences, workshops and seminars; publishes and co-publishes a wide range of books, proceedings, bibliographies and directories; strengthens documentation services in ACP countries; and offers an extensive information service.

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ICLARM
International Center for Living Aquatic Resources Management

ICLARM is an autonomous, nongovernmental, nonprofit, international scientific and technical center which has been organized to conduct, stimulate and accelerate research on all aspects of fisheries and other living aquatic resources. It was incorporated in Manila in 1977. It became a member of the Consultative Group on International Agricultural Research (CGIAR) in 1992.

ICLARM is an operational organization, not a granting entity. Its program of work is aimed to resolve critical, technical and socioeconomic constraints to increased production, improved resource management and equitable distribution of benefits in economically developing countries. The center's work focuses on tropical developing countries in both marine and freshwater areas. Research is carried out on the population dynamics, on alternative management schemes and on improving the productivity of key species. The work includes cooperative research with institutions in developing countries and

supporting activities in information and training. The programs of ICLARM are supported by a number of private foundations and governments.

Policies are set by a Board of Trustees with members drawn from the international community. Direction of ICLARM, under the policies set by the Board, is the responsibility of the Director General.

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INRA
Institut national de la recherche agronomique

At the service of agriculture, food industry and environment, INRA is one of the major world agricultural research organizations.

Legal Identity and Missions

Created in 1946, INRA became, in 1984, a public institute with a scientific and technological mandate under both the French Departments of Research and Agriculture.

Its main areas of research are:

- resource identification and management in the physical environment (soil, micro-climate and water resources);
- improvement of vegetal and animal production relevant to agricultural economy, including forest and aquatic species;
- conservation, transformation of agricultural products into food products and adaptation to consumer needs.
- utilization of non-food agricultural products through development of specific cultures or the use of by-products;
- protection and rational management of natural resources and rural environment; and
- increased awareness of the agricultural and rural world and its transformation through the development of social sciences.

Structures and Operation

A permanent staff of 8,700 including 1,800 scientists and 2,100 engineers is working in 300 laboratories and 170 experimental facilities located in most regions of France, including French Guyana and Guadeloupe.

Its total budget of over 3 billion francs is covered essentially by public funds (87%) and by INRA's own resources (13%), including revenues from germplasm, licenses, contracts, etc.

Scientific Cooperation

To achieve its objectives, the Institute develops important partnerships with:

- institutes of higher education, particularly schools of agriculture and veterinary studies (35 associated laboratories), but also with universities (10 associated laboratories) and other major schools (ENS, INSA, etc.);
- other French scientific organizations: CNRS, INSERM (particularly on human nutrition), CEMAGREF (forestry and water resources), CEA (environment) and CIRAD (tropical agriculture); and
- foreign research organizations, particularly European (BBSRC and DLO) and American (AAC and USDA) institutes as well as international organizations (FAO and CIRA).

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The French Ministry of Cooperation

Coopération française

With 0.57% of its gross national product dedicated in 1995 to official development assistance in 1994, France ranks, in absolute value, second among donor countries. An important part of France's state aid is aimed at Africa, particularly Francophone Africa.

The Ministry of Cooperation is active in 71 countries of subSaharan Africa, the Caribbean and the Pacific regions. Because these countries are signatories to the Lome Convention, greater synergy and efficiency are achievable with projects of the European Union.

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These proceedings constitute the first publication prepared at ICLARM using a translation software and a fisheries terminology database developed by the Translation Services Unit of the Center. Jean-François Baroiller, Eva Claudio, Carl Dawson, Beth Eleccion, Daniel Pauly and Xavier Rognon are thanked for their assistance in the various stages of the translation work.

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Agustin, L. 553
Ahmed, M. 471, 476
Akhteruzzaman, Md. 500
Albaret, J.J. 488
Ambali, A.J.D. 223
Auperin, B. 449
Avella, M. 461
Avit, J.L.B. 46
Baijot, E. 28
Baroiller, J.F. 229, 238, 246
Barry, I. 28
Beardmore, J.A. 314, 349
Behrends, L.L. 257
Bimbao, M.A.P. 471, 476
Bolivar, H.L. 290
Bouda, S. 28
Bourgois, M. 354
Bulls, M.J. 257
Capili, J.B. 266, 290
Castillo Campo, L.F. 538
Cauty, C. 246
Chan, J.R. 168
Chang, C.F. 539
Chang, S.L. 273, 539
Chikafumbwa, F.J.R. 175
Chimatiro, S.K. 183
Cissé, A. 193
Copin, Y. 525
Costa-Pierce, B.A. 129, 183, 280
Cuvelier, R. 354
Danting, J.C. 290
Deekae, S.N. 543
Dembele, I. 361
Descy, J.P. 546
Dickson, M.W. 108, 540
Didier, M. 551
Dionisio, E.E. 290, 537
Diouf, P.S. 488
Doudet, T. 461
Durand, J.R. 33
Ecoutin, J.M. 326
Edwards, P. 92
Eknath, A.E. 290, 415, 537
Elhigzi, F.A.R. 541
Fagbenro, O.A. 542
Falter, U. 299, 354
Foraste, M. 308
Fostier, A. 246
Froese, R. 553
Garcia, M. -A. 548
Gautier, J.Y. 308
Gayanilo, F. Jr. 433
Gilling, C.J. 314
Guerrero III, R.D. 42
Gupta, M.V. 471, 500
Guyomard, R. 398
Head, W.D. 160
Hem, S. 33, 46, 118
Hopkins, K.D. 152
Hörstgen-Schwark, G. 377
Hussain, M.G. 320
Igonifagha, G.D. 543
Jalabert, B. 246
Jamu, D.M. 54
Janagap, C.C. 415
Kabore, K.H. 28
Kaunda, E.K.W.H. 62
Kingsly, J.B. 257
Knud-Hansen, C.F. 70
Koffi, C. 505
Kohinoor, A.H.M. 500
Konan, A.A. 544
Kwei Lin, C. 70
Langston, S.A. 135
Larrson, P. 541
Laviolette, F. 546
Lazard, J. 17, 77, 505, 515
Le Faucheux, B. 308
Legendre, M. 77, 326, 339
Liao, I.C. 273, 539
Little, D.C. 92, 223
Macaranas, J.M. 415
Macgowan, I. 391
Macintosh, D.J. 92
Mair, G.C. 100, 548
Maluwa, A.O. 108
Marioghae, I.E. 442, 543
McAndrew, B.J. 320, 391

McConnell, S.K.J. 349
Meyer, D.E. 112
Micha, J.-C. 354, 546
Middendorp, A.J. 198
Mélard, CH. 545
Montferrer, D. 525
Moreau, J. 433
Moreau, Y. 204
Morissens, P. 118, 361
Msiska, O.V. 54, 129
Mueller, K.W. 160
Muraille, B. 354
Mutambo, J.T.B. 135
Noble, R.P. 141
Ofori, J.K. 547
Oguyeni, O.O 549
Olla, B.L. 168
Oswald, M. 118, 505, 525
Padi, J.N. 547
Palada-De Vera, M.S. 290
Palomares, M.L. 211
Pante, M.J.R. 415
Pauly, D. 211, 433
Penman, D.J. 320
Pouyaud, L. 368
Prunet, P. 449
Puckhaber, B. 377
Pullin, R.S.V. 1
Quilleret, I. 551
Rana, K.J. 383, 391, 550
Reyes, R.A. 290
Richard-Yris, M.A. 308
Roderick, E.E. 548
Rognon, Y. 246, 361, 398
Rouger, Y. 407
Salami, A.A. 542, 549
Sanchez, F. 118
Santiago, L.P. 548
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Skibinski, D.O.F. 266, 314, 349
Smith, S.J. 160, 168
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Tilquin, Ch. 354
Toguyeni, A. 238
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