The Second International Symposium on Tilapia in Aquaculture

Edited by

R.S.V. Pullin
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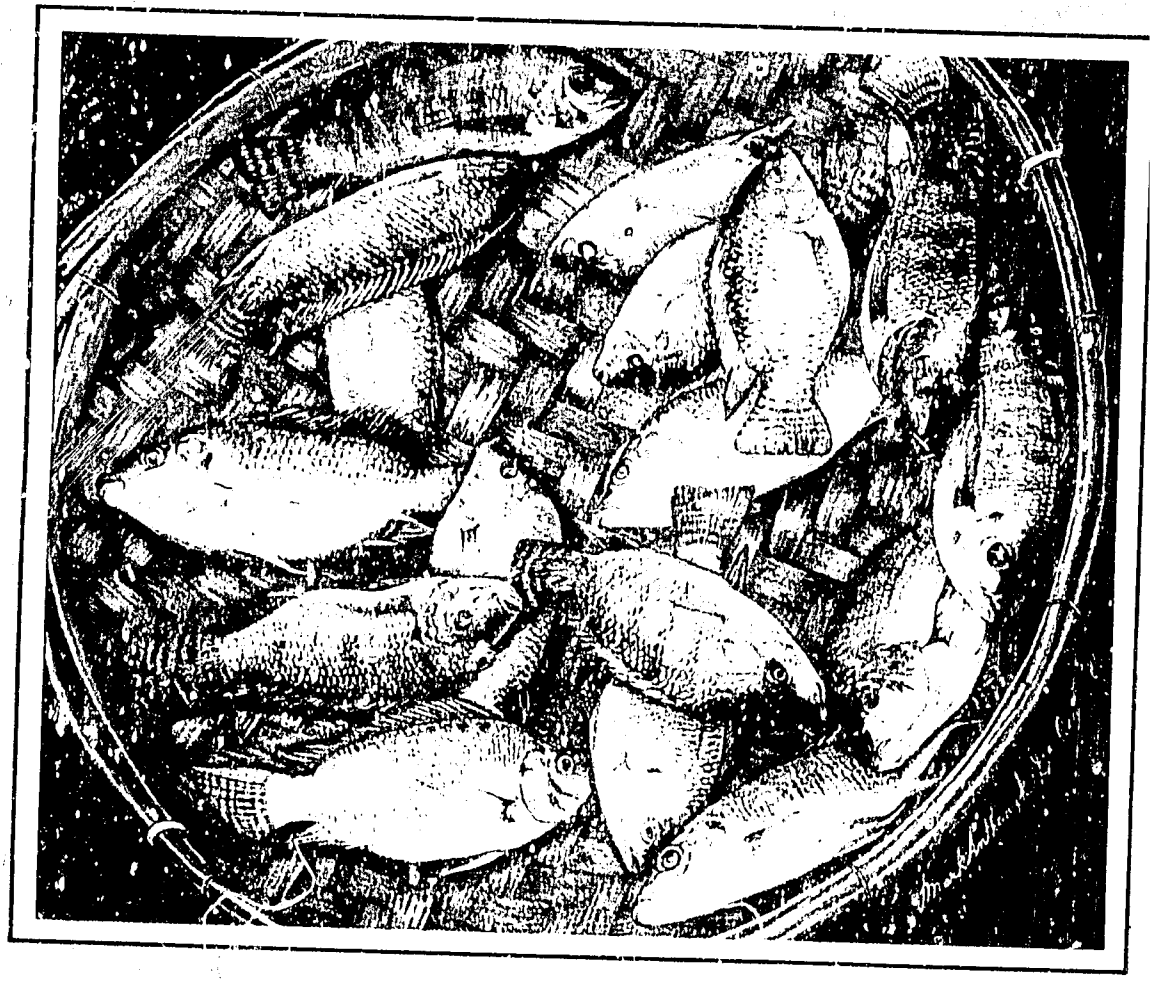
J.L. Maclean



DEPARTMENT OF FISHERIES, THAILAND

INTERNATIONAL CENTER FOR LIVING AQUATIC RESOURCES MANAGEMENT

The Second International Symposium on Tilapia in Aquaculture

Bangkok, Thailand
16-20 March 1987

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1988

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MANILA, PHILIPPINES

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Preface

The First International Symposium on Tilapia in Aquaculture (ISTA I) in Nazareth, Israel, 8-13 May 1983, drew over 150 participants from 47 countries. It was abundantly clear to the organizers, among whom Prof. Lev Fishelson played the leading role, that this should not be simply a 'one-off' conference, but rather the start of a series of ISTAs to provide for presentation of research results and discussion among the large and expanding international community of tilapia researchers. Somehow, ICLARM was later asked to take up the ISTA 'torch' and find an Asian venue.

Thailand was a natural choice for ISTA II, having an unrivalled diversity of tropical aquaculture systems, including an important tilapia culture sector. The Thai Department of Fisheries and ICLARM had long enjoyed a very close and fruitful working relationship and it was thus a foregone conclusion, given the strong support of Director General of Fisheries, Vanich Varikul, the hard work of an organizing committee chaired by Deputy Director General of Fisheries Mrs. Bung-Orn Saisithi, the warmth of Thai hospitality and the advantages of Bangkok as a conference venue, that ISTA II would be a resounding success. It was, bringing together over 250 participants. The opening ceremony, graced by her Royal Highness Princess Chulabhorn and the many events superbly organized by the Thai Department of Fisheries left indelible memories.

The main product of ISTA II's success is to be found in these proceedings. They demonstrate that tilapia culture and the research and development efforts that support it have come of age worldwide. No longer need the problems of stunting and overpopulation of ponds deter modern tilapia culturists. The accent now is on improvement of tilapia breeds and culture systems to suit a wide range of socioeconomic circumstances. Tilapia research now encompasses a wide range of disciplines from the biological to the social sciences.

As these proceedings go to press, it is already time to look ahead to ISTA III. ICLARM will again be involved in the organization. The consensus at ISTA II was that ISTA III should be in Africa, the 'home' of tilapias. In November 1988, the Ministry of Scientific Research of the Republic of Côte d'Ivoire and ICLARM signed a Memorandum of Agreement to hold ISTA III in Côte d'Ivoire in 1991. This forum will therefore shift to a continent that has great need of new development to improve human nutrition and livelihood. Where aquaculture can contribute to this, tilapia is likely to be the leading commodity. The organizers will strive to make ISTA III a lively forum for results and ideas that can help the expansion of tilapia culture in Africa and other regions.

The Editors

Royal Address

by

H.R.H. Princess Chulabhorn

at the Opening Ceremony of

The Second Symposium on Tilapia in Aquaculture (ISTA II)

at the Convention Hall, Ambassador Hotel

Bangkok, 16 March 1987

The Minister of Agriculture and Cooperatives
The Organizing Committee
Distinguished Participants to the Symposium
Ladies and Gentlemen

It gives me much pleasure to be invited to preside over the Opening Ceremony of the Second Symposium on Tilapia in Aquaculture this morning. I truly feel honored to be among so many distinguished scientists and culturists, gathered at another important meeting, with the view to finding ways and means to overcome problems, as well as to explore further fields of cooperation in a particular aspect of aquaculture.

I find it most interesting to hear that over a hundred papers will be presented for your consideration. In this respect, I wish to express my sincere appreciation for all the scholarship that will be contributed to the Symposium. As a scientist myself, I look forward with great expectation to receiving the Record of Proceedings of this meeting, which undoubtedly, would contain valuable information on this particular species of fish: the tilapia.

I should also like to take this opportunity to thank the Minister of Agriculture and Cooperatives for the reference to His Majesty the King's interest in fisheries and aquaculture, as well as to join him in extending a warm welcome to you all, particularly during the Visit Thailand Year.

From the Program, it seems to me that you will all have a busy schedule ahead. My request for everyone, especially the Thai participants is to participate actively in the deliberations, bearing in mind that the success of the Symposium will mean an increase in the food supply for the world's population, and most important of all, to the poorer sector of the people.

At this auspicious moment, I have the pleasure of declaring the Second Symposium on Tilapia in Aquaculture open. I wish you all every success in your noble cause and a happy stay in Thailand.

Welcoming Remarks

by

The Director General of Fisheries

Mr. Vanich Varikul

I am delighted to extend, on behalf of the Thai Department of Fisheries, a very warm welcome to all participants in the Second International Symposium on Tilapia in Aquaculture (ISTA II). The Department of Fisheries is deeply honored to be one of the organizers of ISTA II and to welcome such a distinguished gathering of experts.

Thailand is blessed with vast land and water resources suitable for aquaculture and boasts one of the largest and most varied aquaculture sectors in the world. Tilapias are very important in Thai aquaculture. Our National Inland Fisheries Institute and other institutions are pursuing a vigorous research program on the biology and culture of tilapias. It is my hope that the ISTA II participants will find opportunities to see something of the research and development efforts currently underway for the expansion of Thai aquaculture, as well as participating in the very important business of the conference itself.

The results of the ISTA II will be utilized by all who have interests in tilapia culture. I wish you all a successful meeting and a most enjoyable stay in Thailand.

Introductory Remarks

by

The Minister of Agriculture and Cooperatives

H.E. General Harn Leenanond

Greetings to the Second International Symposium on Tilapia in Aquaculture (ISTA II). The Ministry of Agriculture and Cooperatives of Thailand extends a warm welcome to all ISTA participants who have come from so many countries to confer together and to advance the science of aquaculture.

Aquaculture is a food producing system of great importance in Asia and has a very long history. Tilapias are important cultured fish in Thailand. The Nile tilapia (pla nil) was introduced through the vision of His Majesty the King of Thailand, who received a gift of pla nil from the Crown Prince of Japan in 1965.

I hope that all our visitors will find this timely conference and their stay in Thailand a rewarding and enjoyable experience. The meeting of so many distinguished experts in the rich climate of Thai hospitality, culture and traditions is sure to be a memorable success.

Welcoming Remarks

by

Dr. Ian R. Smith

Director General of ICLARM

May It Please Your Royal Highness

Please permit me, Ian Roger Smith, Director General of the International Center for Living Aquatic Resources Management (ICLARM), to say what a privilege it is for ICLARM to have the honor of co-convening with the Department of Fisheries under the Thailand Ministry of Agriculture and Cooperatives this Second International Symposium on Tilapia in Aquaculture.

Because we are convinced of the importance of tilapia culture, particularly as a major protein source in many developing countries, ICLARM has been looking forward for some time to this long awaited Symposium. We expect it will serve as an ideal venue for scientific exchange among colleagues interested in aquaculture and tilapias. We are delighted to see so many individuals representing various agencies and institutions throughout the world, sharing their excitement over the potential for tilapias. ICLARM is very much among those who firmly believe that this fish has tremendous potential, especially for small-scale farming systems, because capital requirements are less of a hindrance to widespread adoption. Of course, there are constraints to the expansion of this industry. However, solutions for some of these constraints are certain to be among the results of this gathering.

On behalf of ICLARM, therefore, I wish to stress to all participants the great importance of your ideas and recommendations for the future of the tilapia industry. As an international research center, ICLARM eagerly awaits the fruitful outcome of your discussions and the implementation of new technology for the benefit of producers and consumers in developing countries. May I thank all the distinguished participants for joining us at this Symposium.

A. PRESENTED PAPERS
SESSION I: CULTURE SYSTEMS, MANAGEMENT AND PRODUCTION

Relationships Between Primary Production and Yield of Tilapia in Ponds*

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DIANA, J.S., P.J. SCHNEEBERGER and C. KWEI LIN. 1988. Relationships between primary production and yield of tilapia in ponds, p. 1-6. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623 p. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Abstract

The purpose of this study was to determine relationships between physicochemical variables and primary production or fish yield using multiple regression analysis.

In Thailand, 8-12 ponds (250 m^2) were stocked with all-male *Oreochromis niloticus* (25-35 g) at a density of 1 fish/m^3. Experiments were run for approximately 5 months during the wet season of 1984 and the wet and dry seasons of 1985. Ponds received nutrient inputs according to three different management schemes: (1) low input inorganic fertilizer (8 kg/ha/month P_2O_5); (2) high input organic fertilizer (500 kg/ha/week chicken manure); and (3) high input inorganic fertilizer (100 kg/ha/week P_2O_5 and 30 kg/ha/week urea). Air and water temperature, solar radiation, rainfall, dissolved oxygen (DO), total phosphorus, nitrate-nitrite, ammonia, turbidity, chlorophyll *a*, primary production, fish weight, and fish survival were measured regularly through each experiment. Data were examined by regression analyses.

Ponds receiving high fertilizer inputs exhibited higher nutrient levels in water, higher primary production, and higher fish production than ponds treated with low inputs of fertilizer. Nitrogen and phosphorus appeared to be limiting factors for primary productivity, although multiple regression between these two factors and primary production indicated no significant relationship. The availability of nutrients may have been related more closely to regeneration rate than absolute concentration.

Fish yield was strongly correlated to rainfall, fish biomass, DO, water temperature and solar radiation. The correlation between net yield and fish biomass was a positive one, indicating that carrying capacity was not reached in the ponds.

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*Collaborative Research Support Program Aquaculture Project Contribution No. 87: 12.

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Introduction

The biological function of aquaculture ponds is extremely complex. While fish culturists generally know that increased nutrient supply in ponds will increase production of fish, the mechanisms for this increased production are poorly studied (Lannan et al. 1986). Thus, knowledge obtained at one site cannot necessarily be transferred to other regions. In response to this lack of predictability, a Collaborative Research Support Program in aquaculture was initiated with funding from the US Agency for International Development. The focus of this project was to do similar pond culture experiments in several countries, to evaluate the physical, chemical and biological dynamics of ponds, and to compare these dynamics among ponds of different regions. This paper presents data from the first sets of these experiments conducted in Thailand.

Nile tilapia *(Oreochromis niloticus)* are commonly grown in fishponds which are fertilized to increase primary production and fish growth. The food network is very complex in these ponds, as tilapia may consume algae, bacteria, zooplankton and the fertilizer itself under different conditions (Diana et al. 1985). The pathway of feeding will vary considerably with nutrient loading rate and with fertilizer type. Thus, increased primary production in a pond may not correlate well with increased fish growth if tilapia are mainly feeding on detritus.

The purpose of this study was to examine the relationships between pond function, primary production and fish yield under low and high nutrient inputs. Multiple regression methods were applied to pond dynamic data. The objective of the study was to determine if a regression model could adequately describe the relationship between pond dynamics and physicochemical parameters.

Materials and Methods

Data for this study were collected at the Ayutthaya Freshwater Fisheries Center located at Bang Sai, approximately 60 km northwest of Bangkok, Thailand. The site is located near the Chao Phraya River, has extensive diking and pumping facilities for flood control, and a pump reservoir system for water supply. Pond soil is slightly basic (pH 7.4) and impermeable. Ponds used in experiments were 400 m^2 in area when full and 250 m^2 in area when filled to a normal water depth of 0.9 m.

O. niloticus were spawned in brooding ponds and raised on site to appropriate sizes (25-35 g) for stocking at the beginning of experiments. During all experiments fish were initially stocked at 1 fish/m^3. Fish were manually sorted by sex and only male fish were stocked. For the low input wet season experiment, twelve replicate ponds were run from 4 August to 27 December 1984. The pond fertilization schedule called for triple superphosphate (TSP) to be applied to each pond at a rate of 8 kg/ha/month of P_2O_5. High nutrient input experiments were run in eight ponds under two different fertilization schedules. In each season, four ponds received chicken manure (NPK = 2.8-9.1-3.1) at 500 kg/ha/week. The other four ponds were treated weekly with TSP (0-4-0) at 100 kg/ha/week and urea (47-0-0) at 30 kg/ha/week. Application rates were determined such that loadings of nitrogen and phosphorus were identical between the two sets of ponds. This high input fertilization schedule was conducted in 1985 during the dry season (1 February to 30 June) and wet season (1 August to 25 December).

Physical, chemical and biological parameters were measured for each pond during all experiments. Weather data, including light, rainfall, wind speed and air temperature (max-min), were gathered daily. Pond parameters that were measured weekly included dissolved oxygen (DO) and pond temperature. Secchi disk depth and chlorophyll *a* were recorded weekly during the 1984 wet season and the 1985 dry season and twice-weekly during the 1985 wet season. Ammonia, nitrate-nitrite and total

phosphorus were all measured monthly during the 1984 wet season and weekly during both the wet and dry seasons of 1985. Primary production was determined once per month for 1984 wet season and 1985 dry season experiments and once per week during the 1985 wet season. Each month, DO and water temperature were measured at 4-hour intervals through a 24-hour period to determine diurnal fluctuations. Fish growth was evaluated monthly. Methods for these analyses were taken mainly from APHA (1980) and USEPA (1979), and are detailed in CRSP (1984).

Primary productivity was determined by light and dark bottle (Lind 1979) with bottles set at 25 cm below the water surface in each pond. The initial fish stock in each pond for each experiment was weighed as a group, counted and sexed individually. Fish growth was estimated each month by weighing fish from cast-net samples representing about 10% of the initial stock from each pond. At the termination of experiments, all fish were removed from ponds, weighed and counted. Monthly number of fish in each pond was determined by linear interpolation of the beginning and end fish numbers. Monthly total biomass of adult fish was calculated as the mean weight of the fish multiplied by the monthly number of fish for each pond. Net yield of adults each month (m) was calculated by subtracting biomass at month m - 1 from biomass at month m.

To compare parameters measured with different frequencies, daily, weekly and twice-weekly measurements were averaged to obtain one value per month. Monthly averages of each of the variables described above were entered into the computer and analyzed using step-wise regression with the level of significance for inclusion being 0.05. Dependent variables investigated included primary productivity and fish yield. Regression relationships were investigated for the overall data set, and for wet season *vs.* dry season data according to whether ponds received organic or inorganic fertilizer inputs. All statistics were run on the University of Michigan Terminal System using the program MIDAS (Fox and Guire 1976).

Results

Most nutrient factors in the water directly reflected the nutrient input rate. Total inorganic nitrogen and total phosphorus differed considerably between low input ponds (0.05 and 0.06 mg/l, respectively) and high input ponds (0.165 and 0.48 mg/l). These nutrients also differed between organically and inorganically fertilized ponds at high input (0.13 *vs.* 0.20 mg/l for nitrogen, 0.13 *vs.* 0.84 mg/l for phosphorus). These nutrient factors resulted in higher primary production and fish yield in high input ponds compared to low input ones, but no large difference between organically and inorganically fertilized ponds. The ponds stratified lightly in temperature and largely in oxygen during the high input experiments.

Multiple regressions were run between the dependent variables of primary productivity and net fish yield and all of the other physical and biological variables already described, to evaluate factors affecting yield. These regressions were also run on two scales: a global scale (all treatments and ponds combined), and a treatment scale (all ponds for one treatment and season).

Primary production was significantly correlated with chlorophyll *a* concentration and rainfall on a global level ($r^2 = 0.67$) (Table 1). If the same regression was restricted to only physicochemical variables, the regression included rainfall, Secchi disk depth and wind speed ($r^2 = 0.57$). If only biological variables were analyzed, then chlorophyll *a* and fish yield were included ($r^2 = 0.60$).

When analyzed on the differences within a treatment and season, primary production was usually correlated to physical variables (Table 1). Variance was explained equally well within a treatment (mean $r^2 = 0.70$) and for global data ($r^2 = 0.68$). Fertilizer type had little consistent

effect on the regressions, although the coefficient of determination and factors included in regressions varied considerably for organically and inorganically fertilized ponds in a season.

Monthly net fish yield was strongly correlated to both physical and biological factors on a global basis ($r^2 = 0.74$, Table 2). These factors included rainfall, adult fish biomass, dissolved oxygen, water temperature and solar radiation. When restricted only to physicochemical variables, rainfall, Secchi disk depth, total phosphorus and water temperature were included in the regression ($r^2 = 0.60$). When restricted to only biological variables, primary production and adult fish biomass were included ($r^2 = 0.44$). The regressions restricted within a given treatment and season for fish yield were generally less predictive than the global regression (mean $r^2 = 0.64$), and mainly included rainfall and adult fish biomass.

Discussion

The data from the three experimental runs clearly indicate that increased fertilization rates resulted in larger yields and higher primary production in the ponds. These levels of input in our experiments could probably be considered as low and medium loadings, and higher fertilization rates might have even larger effects. Hepher (1962) indicated that pond waters never exceed 0.5 mg/l orthophosphate or 2.0 mg/l nitrogen. As our nutrient levels were well below these, fertilizer input could have been raised considerably, but the impact of higher levels of fertilization on oxygen levels in the ponds may well be detrimental to fish production. Tilapia growth rate averaged 1.2 g/day for our high input ponds, which was below the maximum for a variety of cage culture experiments (approximately 2.4 g/day, Coche 1982) and about equal to the average for those experiments (1.36 g/day). Thus, growth observed in our experiments could possibly be further enhanced by higher fertilization rates.

Multiple regression between primary production and physicochemical and biological factors indicated that differences in rainfall and chlorophyll *a* concentration accounted for 67% of the variance in primary production. Nutrient factors did not enter into any of the multiple regressions except for low nutrient, wet season runs. Possibly the rapid use of available phosphorus and nitrogen made those nutrients always at low abundance, thus obscuring any correlation with primary production. The obvious correlation of chlorophyll *a* (algae biomass) with primary productivity was noted consistently in our analyses.

Rainfall regularly appeared as a factor influencing primary production and net fish yield in the regressions, and did so in a positive way so a dilution effect was not the reason for this correlation. Little runoff could occur in this area, as the only watershed for each pond is the dyke itself. Thus, enrichment from runoff is unlikely. Possibly rainfall caused greater mixing and nutrient resuspension as temperature stratification was negatively correlated with rainfall. Rainfall is generally low in most nutrients, although dissolved nitrogen levels may be high (Haines 1981).

Monthly net fish yield was strongly correlated to rainfall, fish biomass, DO, water temperature and solar radiation. Most of these variables are physical ones which affect fish growth rate or food availability (Webb 1978). However, differences in primary production, fish biomass, and chlorophyll *a* alone explained 44% of the variance in fish yield. This probably indicates that fish growth was mainly due to ingestion of autotrophic material rather than predominantly heterotrophic food sources. Correlations of net yield with adult biomass were positive, indicating that carrying capacity was not reached in the ponds, but rather that fish yield was still increasing with increased biomass.

Multiple regressions do not prove cause and effect relationships, but only show correlations (Carpenter et al. 1985). For example, a constant plankton production, cropped differentially by

Table 1. Results of multiple regressions between primary production and other physical and biological variables. Treatments examined include: global (all data from both years); physicochemical (global data with biological variables excluded); biological (global data with physicochemical variables excluded); and the separate fertilization and season combinations.

Treatment	Included variables	r2
Global	Chlorophyll *a* Rainfall	0.67
Physicochemical	Rainfall Secchi disk depth Windspeed	0.57
Biological	Chlorophyll *a* Net fish yield	0.60
Low input, Wet season	Chlorophyll *a* Fish biomass Ammonia Solar radiation	0.88
High input, Inorganic Wet season	Rainfall Secchi disk depth	0.79
High input, Organic Wet season	Net fish yield Secchi disk depth Rainfall	0.86
High input, Inorganic Dry season	Wind speed	0.52
High input, Organic Dry season	Chlorophyll *a*	0.47

Table 2. Results of multiple regressions between monthly net fish yield and other physical and biological variables. Each scale analyzed is included. Treatments as in Table 1.

Treatment	Included variables	r2
Global	Rainfall Adult fish biomass Dissolved oxygen Water temperature Solar radiation	0.74
Physicochemical	Rainfall Secchi disk depth Total phosphorus Water temperature	0.60
Biological	Primary production Adult fish biomass Chlorophyll *a*	0.44
Low input, Wet season	Adult fish biomass Rainfall Wind speed	0.59
High input, Inorganic Wet season	Rainfall Adult fish biomass	0.91
High input, Organic Wet season	Rainfall	0.74
High input, Inorganic Dry season	Rainfall Adult fish biomass	0.65
High input, Organic Dry season	Primary production	0.33

increased fish biomass, could show a significant negative correlation between those two factors. This might be interpreted as fish not being dependent on plankton biomass, when actually fish dependence caused low plankton biomass. Conversely, nutrients which may always be in limited supply may show no correlation to plant growth, as the nutrient level would be kept low by plant use. Thus, nutrient availability may be related more closely to regeneration rate than to absolute concentration.

Acknowledgements

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Tilapia Raised on Septage as High Protein Animal Feed

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Abstract

Net extrapolated yields of 5 to 6 t/ha/year of Nile tilapia *(Oreochromis niloticus)* were obtained at the end of 5- to 7-month experiments in sixteen 200-m^2 earth ponds stocked at an initial density of 1 fish/m^2 and fertilized with septage at an organic loading rate of 150 kg chemical oxygen demand/ha/day. Studies were subsequently conducted in three, more commercially realistic, 1,740-m^2 earth ponds with the same fish stocking density and septage loading rate but with the fish seined at 2 to 4 week intervals over a period of 15 to 18 months, starting 3 months after tilapia were stocked when they were breeding prolifically. Sustainable extrapolated net yields of 6.2 to 7.3 t/ha/year were obtained by intermediate harvesting. Higher yields may be feasible with better fish stock management although the maximum standing stock of tilapia in ponds fertilized with septage was only 2 to 4 t/ha. Two additional constraints were limits to recruitment and the difficulty in seining larger, slow growing fish.

Introduction

Human excreta have been used as an organic fertilizer for fishponds for centuries, particularly in certain parts of Asia (Prowse 1967; Allen 1972; Edwards 1980a) although there are constraints to more widespread excreta reuse. Excreta reuse and resource recovery systems need to be developed that are socially acceptable and that do not pose unacceptable risks to public health (Edwards 1985).

A research program at the Asian Institute of Technology has been concerned for the past 10 years with the development of bioengineering design criteria for tilapia production on human waste as feed for high market value carnivorous fish such as snakehead *(Channa striata)* and walking catfish (*Clarias* spp.). Septage (septage tank and cesspool slurry) is added to a single stage stabilization - fishpond for simultaneous waste treatment and resource recovery. The system incorporates the concept of lengthening the food chain because the tilapia from the excreta-fed pond are used to feed fish of a higher market value raised in a separate pond system rather than directly as human food. This concept also has socio-

logical relevance because it may permit excreta reuse in societies in which it is traditionally unacceptable.

A major problem in the culture of tilapias for table fish is the control of prolific breeding to obtain fish of a minimum market size because of the inverse relationship between stocking density and size of harvested fish (Hickling 1963; Guerrero 1982; Hepher and Pruginin 1982). However, precocious spawning of tilapias is used to advantage in the proposed system. Harvest size is unimportant in tilapias used for animal feed and yields should be high in a freely breeding population: a large number of small fish increases at a faster rate than a smaller number of larger fish of the same total weight because the specific growth rate of small fish is greater than that of large fish (Weatherly 1972; Hepher 1978). Precocious spawning also reduces the need to purchase fry which are continually generated within the system (Edwards et al. 1981).

It was suggested that it may be possible to produce high yields of small Nile tilapia suitable for animal feed by stocking an excreta-loaded fishpond with high initial fish densities (Edwards 1980a, 1980b). The hypothesis was based on the cultivation of tilapia in small concrete tanks through which phytoplankton was pumped from a sewage-fed high rate stabilization pond. The mean weight of individual harvested fish decreased with an increase in initial fish stocking density but the final yield or harvest of the fish population increased; the reduction in mean individual fish weight with an increase in initial stocking density was more than compensated for by the much higher numbers of fish stocked at higher initial densities. At a high initial stocking density of 10 fish/m^2, the extrapolated fish yield approached 20 t/ha/year (Edwards et al. 1981). The feasibility of attaining such a high fish yield in commercial size ponds is supported by the literature. Tapiador (1973) considered it conceivable to produce 50 t/ha/year of small fish of 20-40 g using sewage, if several crops of fish were raised each year. According to Bardach et al. (1972), maximum yields of tilapia may be as high as 18 t/ha/year although comprised of small fish.

Five experiments were run over a period of 5 years in sixteen small-scale 200-m^2 experimental ponds to investigate the effects of various organic loadings of septage and fish stocking densities on growth and yield of Nile tilapia *(Oreochromis niloticus)*. These were followed by an experiment in three 1,740-m^2 earth ponds in which the effect of intermediate harvesting on fish yield was assessed. Data concerning the first four experiments are reported in detail elsewhere (Polprasert et al. 1982; Edwards et al. 1984).

Materials and Methods

Small-scale experimental ponds

Five experiments were run in the sixteen 200-m^2 earth ponds stocked with small fingerlings of maximum weight 3.9 g (Table 1). Ponds were fertilized with septage twice a week at three times the daily organic loading rate to give an equivalent septage loading of six days/week. Five to ten per cent of the initial stocked fish were weighed at monthly intervals and all fish (stocked fish and progeny) were sorted into five length classes (<10 cm, 10-<15 cm, 15-<20 cm, 20-<25 cm, 25-<30 cm), and counted and weighed on draining the ponds at the end of each experiment. The pond mud was allowed to dry and was removed at the end of each experiment to avoid residual fertilizer effects between experiments. The ponds could not be dried at the end of Experiment 3 because of the southwest monsoon but residual fertilizer effects were probably minor in Experiment 4 because all ponds received the same relatively high septage loading rate.

The optimal organic loading of septage for fish production should have been established before experiments were run with a range of stocking densities at the optimal organic loading rate. Unfortunate-

Table 1. Mean fish production data for experiments in 200 m^2 experimental ponds. *Triplicate;. + Duplicate.

Experiment	Dates	Duration (months)	Treatment: Organic loading (kg COD/ ha/day)	Initial fish stocking density (number/m^2)	Final stocking density (number/m^2)	Standing stock (t/ha)	Extrapolated net yield (t/ha/year)
1*	7/80-4/81	9.4-9.8	Varied	3	9	3.8	4.8
				6	12	3.5	4.4
				9	12	3.2	4.0
				12	11	2.7	3.3
				15	14	3.7	4.6
2*	4/82-10/82	5.1	0	1	5	0.4	0.8
			50		11	1.0	2.3
			100		15	1.7	3.8
			150		14	1.5	3.4
			200		24	2.5	5.8
3+	1/83-5/83	4.2	0	1	9	0.3	0.9
			50		29	1.3	3.6
			100		14	1.5	4.1
			150		10	1.7	4.6
			200		11	1.4	3.8
			250		25	2.0	5.7
			300		26	2.3	6.5
4*	7/83-2/84	7.0	150	1	8	2.9	4.9
				3	8	1.5	2.4
				5	7	2.1	3.4
				10	6	1.7	2.5
				20	9	2.2	2.9
5*	9/84-2/85	5.0	150	1	6	2.7	6.5

ly, there was sufficient time initially to run only one experiment with the funding then available. The amount of septage added to the ponds was gradually increased during Experiment 1 to determine the maximum loading that allowed the water to remain aerobic; the ponds were underloaded during the first half of the experiment but during the latter half the ponds were usually loaded at a rate of 100 kg COD/ha/day, a better but still inadequate rate of loading. However, experiments to determine the relationship between organic loading and fish production (Experiments 2 and 3) preceded a study of the effect of variation in stocking density at an optimal organic loading (Experiment 4) when additional research funds were secured. A constant fish stocking density of 1 fish/m^2 with a range of organic loadings of septage from 0 to 300 kg COD/ha/day was used in Experiments 2 and 3. A constant organic loading rate of septage of 150 kg COD/ha/day with a range of fish stocking densities from 1 to 20 fish/m^2 was used in Experiments 1 and 4. Experiment 5 was a study of the relative contribution of stocked fish and recruits to total biomass. All ponds were stocked at 1 fish/m^2 and fertilized with septage at 150 kg COD/ha/day. Fish were harvested by draining ponds at successive monthly intervals.

Commercial-scale experimental ponds

The three 1,740-m^2 earth ponds were stocked initially with 3.5 g fingerlings at 1

fish/m^2 and were loaded with septage at a rate of 150 kg COD/ha/day, twice a week at three times the daily organic loading rate. The ponds were only about 20 m wide to facilitate seining, using a net with mesh size of 2 cm (2.5 cm stretched), large enough to permit most small recruits to pass through the net. The experiment was not preceded by detailed experiments on the optimum frequency and size of intermediate harvests. Four harvesting strategies were employed during approximately 15 to 18 months to determine a sustainable level of intermediate harvesting. One to three seines were used for each pond during harvesting. It was considered unwise to use more than three seines because this harvesting effort was adequate to attain a realistic harvesting target, and to avoid unduly stressing the fish.

Results

Small-scale experimental ponds

The rate of increase of mean individual fish weight and the final mean individual fish weight both increased with an increase in rate of organic loading with a constant fish stocking density of 1/m^2 in Experiments 2 and 3. However, the growth rate of stocked fish slowed down considerably after 3 to 4 months, even at the higher organic loading rates, with the mean weight of stocked fish at harvest usually ranging from 120 to 180 g. Extrapolated net fish yield also increased linearly with an increase in organic loading rate (Table 1). An organic loading rate of 150 kg COD/ha/day was selected as the optimal loading rate based on the results of Experiments 2 and 3. Higher fish yields were obtained at higher organic loading rates but it was considered prudent to select a lower loading rate to minimize the risk of overloading with a possible reduction in fish growth rate and fish mortality. Equivalent total Kjeldahl nitrogen and dry matter loading rates at an organic loading rate of 150 kg COD/ha/day ranged from approximately 5 to 8 and from 100 to 125 kg/ha/day, respectively.

There was a pronounced inverse relationship between mean size of stocked fish and initial stocking density in Experiments 1 and 4 in which fish stocking density was varied with a constant organic loading rate (Table 1). The organic loading rate was increased with time during Experiment 1 but the rates were similar at different treatments.

The extrapolated net fish yield did not increase with an increase in fish stocking density in Experiments 1 and 4 as expected. Net yields did not vary much with stocking density and the highest yields occurred at the lowest stocking density (Table 1). There was a marked effect of initial stocking density on the mean individual weights of sampled fish but the final stocking densities of fish harvested from each pond on draining were similar, 9-14 and 3-9/m^2 in Experiments 1 and 3, respectively. The size range distribution of fish on draining the ponds suggested that breeding took place. There may have been a relatively high mortality at the higher initial stocking densities because the final stocking densities at the higher initial stocking densities were often lower than the initial densities.

In Experiment 5, to assess the relative contributions of stocked fish and recruits to total pond biomass, breeding was first observed at the end of the second month in two of the three ponds drained. Recruits could only be separated from stocked fish up to month 3 when there were no fish in the 10-<15 cm size class, but not in months 4 and 5 when the size classes overlapped. A survival rate of 77% of stocked fish from month 3 was used to differentiate between the biomass of stocked fish and recruits. The total biomass in the pond continued to increase in months 4 and 5 but this was mainly due to growth of recruits because the rate of increase of stocked fish had slowed down considerably as observed in previous experiments. However, the rate of increase of the biomass of the recruits had not slowed down after 5 months and the recruits comprised 33% of the total fish biomass at the end of the experiment.

The maximum extrapolated net fish yields in the five experiments ranged from 4.8 to 6.5 with a mean of 5.7 t/ha/year. The extrapolated maximum standing stock, a more realistic indicator of fish production because the experiments were less than one year duration, ranged from 2.3 to 3.8 with a mean of 2.8 t/ha/year, in the 4.3- to 9.5-month experiments. The maximum final stocking density, irrespective of size class, was 29/m^2 with a mean of 13/m^2 for the treatment means in the five experiments (Table 1).

Commercial-scale experimental ponds

An attempt was made to remove the stocked fish in three successive harvests at monthly intervals after they had ceased to increase in weight, starting from 4 months after the fish were stocked in the first of the four harvesting strategies employed. The 3-month period over which the harvesting took place also gave recruits time to grow and reproduce. Fixed low, medium and high weight harvests of 37.5, 75.0 and 150.0 kg/pond/2 weeks were allocated to Ponds 1, 2 and 3, respectively, in the second harvesting strategy. These were determined assuming sustainable extrapolated yields of approximately 5, 10 and 20 t/ha/year with intermediate harvesting at 2-week intervals. The high level harvest was sustainable for only four harvests, and decreased by about 50% in the fifth fortnightly harvest. The low and medium level harvests appeared to be sustainable over the period of harvesting. The low and high level harvests were reversed and the medium level harvest maintained in the third harvesting strategy. The high level harvest in Pond 1 exceeded 100 kg for only three harvests and then fell to about 50 kg, indicating again that the high level of harvest could not be sustained. The third harvesting strategy was maintained for only four fortnightly harvests. The fourth and final harvesting strategy involved a 75-kg limit from each pond and each fortnightly harvest using a maximum of two seines because data from Pond 2, which had been continually harvested at this level, indicated that it was sustainable with time. The fourth harvesting strategy was maintained for six to nine consecutive fortnightly harvests.

The extrapolated net fish yield from the ponds ranged from 6.2 to 7.3 with a mean of 6.8 t/ha/year. The standing stock ranged from 2.1 to 2.8 with a mean of 2.4 t/ha (Table 2). Most of the fish biomass harvested at regular 2-week intervals were in the second (10-<15 cm) and third (15-<20 cm) size classes although considerable biomass of smaller and larger fish was occasionally harvested.

Discussion

When tilapia were cultured in septage-fed 200-m^2 earth fishponds, there was little variation in fish yield with initial stocking density although there was a strong inverse relationship between mean fish weight and initial stocking density. The maximum extrapolated net fish yields

Table 2. Fish production data for the three 1,740 m^2 commercial-scale ponds with an initial stocking density of 1 fish/m^2 and an organic loading of 150 kg COD/ha/day.

Pond number	Dates	Duration (months)	Final standing stock (t/ha)	Extrapolated net yield (t/ha/year)
1	9/84 - 2/86	17.5	2.8	7.3
2	9/84 - 1/86	16.3	2.3	6.2
3	9/84 - 12/85	15.2	2.1	7.0

from the series of five experiments ranged from 4.8 to 6.5 with a mean maximum yield of only 5.7 t/ha/year, less than one-third of the suggested attainable yield.

Insufficient natural food may have been produced in septage-loaded ponds to support high fish yields. Maximum mean phytoplankton concentrations were usually only about 25 to 30 mg/l, less than half the 70 mg/l obtained in tanks fed with the effluent of the high rate stabilization pond (Edwards et al. 1981). Natural food in septage-fed ponds may also have limited the maximum size of harvested fish to usually less than 200 g. According to Hepher (1978) and Hepher and Pruginin (1981), production of natural food may be insufficient to sustain both body maintenance and maximum potential growth rate of fish.

Intermediate harvesting of the breeding, mixed aged tilapia population in the three 1,740-m^2 ponds was a successful strategy because the mean extrapolated net yield of 6.8 t/ha/year was 290% higher than the mean extrapolated standing stock of 2.3 t/ha when the ponds were drained at the end of the 15- to 18-month experiment. The standing stock of the three large ponds was not much less than the mean maximum extrapolated standing stock of 2.8 t/ha obtained in the five experiments in the small ponds. The yields obtained were similar to the maximum yields of 6.5 to 7.8 t/ha/year reported from sewage-fed ponds in Tainan, Taiwan, with 8 to 9 intermediate harvests of tilapia (probably *Oreochromis mossambicus*) ranging from 20 to 80 g during the March to November growing season (Huang 1968).

A further constraint to high yields was the relatively low level of recruitment in the freely breeding population. The mean stocking density on harvesting the 200-m^2 ponds in the five experiments was only 13/m^2, including recruits. The smallest size class (<10 cm) comprised a mean of only 4% of the total standing stock on draining the three 1,740-m^2 ponds. Tilapia reproduction has been reported to be reduced at higher fish stocking densities (Van der Lingen 1959; Allison et al. 1979; Fishelson 1983). The fortnightly seining might have disturbed breeding behavior to a sufficient extent to limit fry production. The predation by tilapia on fry of their own species (Iles 1973) might be expected to increase in intensity at high density. The difficulty of harvesting the larger, slower growing tilapia with the conventional seine net may also have reduced the potential yield. The two largest size classes (20-<25 cm and 25-<30 cm) comprised a mean of 33% of the standing stock on draining the three 1,740-m^2 ponds. Further increases in yield in septage-fed fishponds may be expected by stock manipulation to maximize recruitment and by more efficient seining to remove larger fish, perhaps, using electricity.

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Polyculture of the Tiger Shrimp *(Penaeus monodon)* with Nile Tilapia (*Oreochromis niloticus)* in Brackishwater Fishponds

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Abstract

A study was conducted in fifteen 500-m^2 ponds to determine the growth, survival and production of *Penaeus monodon* (Fabricius) in polyculture with *Oreochromis niloticus* (Linnaeus) and the extent of competition between shrimp and tilapia in brackishwater ponds. The treatments consisted of: (I) *P. monodon* at 6,000/ha; (II) *O. niloticus* at 6,000/ha; (III) *O. niloticus* at 4,000/ha; (IV) *P. monodon* at 6,000/ha plus *O. niloticus* at 6,000/ha; and (V) *P. monodon* at 6,000/ha plus *O. niloticus* at 4,000/ha. A completely randomized design with three replicates was used.

Treatment V gave the highest total production (283.32 kg/ha) followed by Treatment IV (221.24 kg/ha). Treatment I had the lowest total production. Analysis of variance on total production showed significant differences ($p < 0.05$) among treatments. Polyculture treatments (Treatments V and IV) were not different in terms of production but significant differences were observed between polyculture (Treatment V) and monoculture treatments (Treatments I, II and III). Mean net production of shrimp alone was highest in Treatment V followed by Treatment I and Treatment IV but were not significantly different between treatments. A similar trend was observed on the mean weight gain and percentage survival of *P. monodon*. Mean net production of *O. niloticus* was relatively low in all treatments. The low production of *O. niloticus* in all treatments was due to low survival (33% to 52%) and slow growth.

Competition was evident between *P. monodon* and *O. niloticus* at a stocking combination of 6,000 *P. monodon*/ha plus 6,000 *O. niloticus*/ha. Total yield from polyculture was better than monoculture. Polyculture of *P. monodon* at 6,000/ha and *O. niloticus* at 4,000/ha appeared feasible.

Introduction

The culture of shrimp *(Penaeus monodon)* in combination with other finfishes such as milkfish and mullet has been tried in brackishwater ponds with encouraging results (Eldani 1979; Manzano 1981; Pudadera 1980). Besides increased fish production, the presence of secondary species generates additional income for shrimp farmers, and at the same time, provides a cheaper source of protein for consumers.

Another promising species for culture in combination with *P. monodon* is Nile tilapia *(Oreochromis niloticus).* The culture of this fish has gained wide acceptance among fishpond operators in the Philippines (Guerrero and Abello 1976). The fish are appreciated by consumers in many countries and can provide acceptable yields on relatively low inputs (Hepher and Pruginin 1982). Recent findings indicate a rapid growth rate for this species in brackishwater ponds, with the fish attaining market size within a 90-day culture. An added benefit is that reproduction is inhibited at high salinities (> 15 ppt) (Corre 1981). Aside from being a plankton feeder, *O. niloticus* cleans the pond bottom of detritus and decaying algae, and thus contributes in a way to the improvement of the pond (Reich 1975).

Considering the growth potential and feeding behavior of *O. niloticus*, the addition of this species to ponds containing *P. monodon* would likely increase production per unit area. One important problem, however, in polyculture is to determine the optimum stocking combination that would be most efficient in utilizing the pond's natural productivity. Hence, this study was conducted with the following objectives:

1. To evaluate the growth and survival of *Penaeus monodon* and *Oreochromis niloticus* stocked at varying combinations in brackishwater ponds.
2. To determine the production of *P. monodon* and *O. niloticus* in monoculture and in polyculture systems.
3. To determine the extent of competition between *P. monodon* and *O. niloticus* at certain stocking combinations.

Materials and Methods

Experimental set-up

The experiment was conducted in fifteen 500-m^2 rhomboid-shaped ponds at the Southeast Asian Fisheries Development Center (SEAFDEC) Leganes Brackishwater Station, Leganes, Iloilo, Philippines, from 30 October 1981 to 1 March 1982.

Five treatments (I-V) with three replicates each were tested in a completely randomized design (CRD) as follows:

I. Monoculture of *P. monodon* (stocked at a rate of 6,000/ha)
II. Monoculture of *O. niloticus* (stocked at a rate of 6,000/ha)
III. Monoculture of *O. niloticus* (stocked at a rate of 4,000/ha)
IV. Polyculture of *P. monodon* (stocked at a rate of 6,000/ha) and *O. niloticus* (stocked at a rate of 6,000/ha)
V. Polyculture of *P. monodon* (stocked at a rate of 6,000/ha) and *O. niloticus* (stocked at a rate of 4,000/ha)

Pond management

The ponds were prepared by draining and drying the pond bottom for 2 days. Nylon screen enclosures were installed on the PVC water inlet and outlet of each pond to prevent escape of cultured species and entry of unwanted fishes. Tobacco dust was applied prior to stocking at a rate of 250 kg/ha to further eradicate pests and predators. Lime and chicken manure were simultaneously applied by broadcast method on the pond bottom at the rate of 2 t/ha each. Inorganic fertilizer (mono-ammonium phosphate) was also applied at 50 kg/ha at every 2-week interval to initiate and maintain the growth of natural food organisms.

The water supply depended mainly on tidal fluctuation. At least 60% of the pond water was replaced at every 2-week interval. Pond water depth was maintained at a minimum of 60 cm.

Stocking

Hatchery-produced *Penaeus monodon* juveniles (PL 45) were stocked 35 days ahead of tilapia fingerlings. During

stocking, the juveniles were released gradually near the coconut palm leaves previously installed in the ponds to allow them to cling and take refuge.

O. niloticus fingerlings (with average body weight of 3.01 g) were taken from a freshwater fishpond off the station. The fingerlings were transported to the station in oxygenated plastic bags inside a "pandan" bag. Upon arrival at the SEAFDEC Leganes Brackishwater Station, the fish were conditioned in an aerated fiberglass tank. Acclimation was done over a 12-day period by increasing the salinity by 5 ppt every 2 days until 28 ppt was attained.

The *P. monodon* juveniles and *O. niloticus* fingerlings were stocked at random but in accordance with the experimental design of each pond on 30 October 1981 and on 5 December 1981, respectively.

Harvesting

The experimental animals were harvested from all ponds on 1-2 March 1982, after a culture period of 120 days for *P. monodon* and 85 days for *O. niloticus*. Harvest of *P. monodon* and *O. niloticus* was done by completely draining the ponds and collecting the animals in a bagnet installed at the outlet pipe of each pond. *P. monodon* and *O. niloticus* found confined at the harvest/drain box were scooped while those remaining in the pond bottom were handpicked, after which they were counted individually and mass weighed.

Results and Discussion

Growth and survival of P. monodon and O. niloticus

The mean weight gain, daily weight increment and percentage survival of *P. monodon* and *O. niloticus* in the monoculture and polyculture systems are presented in Table 1. Polyculture of *P. monodon* at 6,000/ha with *O. niloticus* at 4,000/ha (Treatment V) achieved better growth rate of *P. monodon* when compared with the monoculture experiments. The percentage survival of *P. monodon* was highest in all ponds of Treatment V, indicating that *O. niloticus* can be cultured at these densities with *P. monodon* without any harmful effect.

Better growth and survival of *P. monodon* obtained in the presence of 4,000 *O. niloticus* could be due to the addition of undigested food particles excreted by *O. niloticus* that served directly as food for *P. monodon* and simultaneously as fertilizer of the pond bottom. Reich (1975) pointed

Table 1. Mean weight gain, daily weight increment and percentage survival of shrimp *(Penaeus monodon)* and tilapia *(Oreochromis niloticus)* cultured for 120 days a. l 85 days, respectively, in five monoculture and polyculture treatments.

Treatments	Shrimp *(P. monodon)*				Tilapia *(O. niloticus)*			
	Initial body wt. (g)	Mean wt. gain (g)	Daily wt. increment (g/day)	Survival (%)	Initial body wt. (g)	Mean wt. gain (g)	Daily wt. increment (g/day)	Survival (%)
I	0.047	27.91	0.23	75	—	—	—	—
II	—	—	—	—	1.71	86.34	1.02	33
III	—	—	—	—	2.42	64.27	0.76	52
IV	0.043	25.77	0.21	52	1.93	69.99	0.83	36
V	0.042	29.79	0.25	76	1.98	73.21	0.86	50

out that only 50% of the food taken by *O. niloticus* are digested, while the remainder goes to the bottom where it undergoes an additional decomposition and serves again as fish food. Another favorable effect of *O. niloticus* is its grazing activity on detritus and decaying algae which improves the dissolved oxygen regime for the benefit of the *P. monodon*. On the other hand, lower growth and survival of *P. monodon* with *O. niloticus* at 6,000/ha was probably due to competition for food and space considering an increase of 50% from the stocking density of 4,000 to 6,000/ha. It was observed that although *O. niloticus* is primarily a hervibore, under crowded conditions it would avidly consume microfauna, crustaceans, worms and small fishes (Rabanal and Hosillos 1957). These food items are likewise important in the diet of the *P. monodon*.

Both the monoculture and polyculture treatments produced market size tilapia (p > 50 g) within 85 days culture period without supplementary feeding. The rate of growth, however, was slower than expected probably due to physiological stress that affected *O. niloticus* during the process of acclimation from freshwater to seawater (28 ppt). Likewise, survival rates were generally low for all treatments. During the 2nd and 3rd months of culture (January to February), at least two to three dead *O. niloticus* were observed in the morning in each of the ponds. It was suspected that the low water temperature coupled with high salinity (Table 2) experienced during the culture period adversely affected *O. niloticus* production.

Table 2. Monthly averages and ranges of selected physio-chemical parameters during the culture period.

Parameters	Time (hour)	Treatments	Monthly averages during 1981 and 1982 Nov	Dec	Jan	Feb	Range
Water temperature (°C)	0530-0600	I	25.7	23.0	22.7	22.0	20.0 – 26.0
		II	25.8	23.1	22.8	21.9	20.0 – 26.1
		III	25.7	23.3	22.8	21.9	19.5 – 26.1
		IV	25.9	23.0	22.8	21.9	19.5 – 26.0
		V	25.9	22.2	22.9	22.0	20.0 – 26.0
	1430-1500	I	29.5	28.2	27.1	25.8	23.5 – 31.5
		II	29.3	28.0	27.0	25.5	23.3 – 31.3
		III	29.5	27.9	27.0	25.4	23.1 – 31.4
		IV	29.6	28.1	26.9	25.5	23.5 – 31.4
		V	29.6	27.9	26.9	25.5	23.5 – 31.5
Dissolved oxygen (mg/l)	0530-0600	I	3.5	4.0	2.7	3.6	1.4 – 5.8
		II	3.0	3.1	2.5	3.3	1.3 – 4.6
		III	4.5	3.2	3.3	3.5	2.2 – 5.7
		IV	4.0	4.6	2.9	3.4	2.1 – 4.1
		V	3.8	3.6	3.0	3.4	1.7 – 4.6
	1430-1500	I	8.7	10.6	9.6	9.1	6.8 – 14.0
		II	9.4	10.8	11.2	9.0	6.3 – 13.2
		III	10.6	9.5	11.2	8.8	6.9 – 14.5
		IV	8.9	10.0	9.2	8.6	7.0 – 13.9
		V	9.7	9.8	8.3	8.7	6.5 – 13.0
Salinity (ppt)	0800-0830	I	24.2	27.4	36.3	38.8	20.3 – 42.0
		II	24.0	26.4	35.6	38.2	21.0 – 41.0
		III	24.6	25.3	34.1	37.8	20.3 – 40.3
		IV	24.5	27.9	35.7	38.4	21.3 – 41.3
		V	24.1	26.9	34.8	37.9	22.0 – 40.3
pH	0800-0830	I	7.9	8.0	8.1	8.2	6.8 – 8.6
		II	8.1	8.3	8.3	7.7	7.2 – 8.8
		III	7.8	8.1	8.0	7.9	7.1 – 9.2
		IV	7.9	7.9	7.8	7.9	6.6 – 9.1
		V	8.0	8.1	8.1	7.8	6.9 – 8.5

Production

Table 3 shows the mean net production (kg/ha) of the *P. monodon* and *O. niloticus* in monoculture and polyculture systems. The best yield of *P. monodon* was obtained in Treatment V where it was combined with *O. niloticus* at 4,000/ha. A further increase in stocking density of *O. niloticus* (Treatment IV) apparently decreased final body weight, survival rate and consequently, net production of *P. monodon*. On the other hand, *O. niloticus* mean net production which ranged from 128.20 kg/ha (Treatment III) to 167.60 kg/ha (Treatment II) was relatively low as a result of slow growth and low survival of the fish.

In general, total production in polyculture was higher than in monoculture. Polyculture of 6,000/ha *P. monodon* and 4,000/ha *O. niloticus* proved beneficial in that a total production of 283.32 kg/ha *P. monodon* plus *O. niloticus* was achieved representing an increase of 130% in total yield over *P. monodon* alone. Since the polyculture yield exceeded the monoculture yield, it appeared that the criteria of successful polyculture, i.e., the secondary species *(O. niloticus)* did not adversely affect the primary species *(P. monodon)* while increasing the total production (Yashouv 1969 cited by Hepher and Pruginin 1982), were realized.

Competition index

Table 4 presents the degree of influence of *O. niloticus* over *P. monodon* and vice versa calculated using the competition index formula. The competitive relationship between the *P. monodon* and *O. niloticus* at equal stocking density of 6,000/ha was positive (0.340) indicating that *O. niloticus* at 6,000/ha exerted significant competition for the *P. monodon*. On the contrary, a negative value (-0.12) obtained from the stocking combination of 6,000/ha *P. monodon* and 4,000/ha *O. niloticus* indicates absence of competition and, at the same time, a beneficial relationship between the primary and secondary species.

Competition for food was more likely at a stocking combination of 6,000 *P. monodon*/ha plus 6,000 *O. niloticus*/ha. The stocking density of *O. niloticus* at 6,000/ha was probably beyond the carrying capacity of the *P. monodon* pond, hence the food suppy became a limiting factor. It was observed that in polyculture there is always a certain degree of competition for food even between species which feed in nature from completely different niches, especially if the ponds are stocked at a high density (Reich 1975).

Table 3. Mean net production of *Penaeus monodon* and *Oreochromis niloticus* in mono- (treatments I-III) polyculture (treatments IV and V) syatems.

Treatments	Net production (kg/ha) *P. monodon*	*O. niloticus*	Total production (kg/ha)
I	123.21	—	123.21[a]
II	—	167.60	167.60[a]
III	—	128.52	128.52[a]
IV	80.91	140.32	221.24[ab]
V	137.69	145.88	283.32[b]

Means with the same superscript are not significantly different at 5% level.

Table 4. Competition indices between *Penaeus monodon* and *Oreochromis niloticus* cultured at varying densities in brackishwater fishponds.

Specifications	Mean net production (kg/ha) Monoculture	Polyculture	Competition index
Competition of *O. niloticus* with *P. monodon*			
1. 6,000 *P. monodon*/ha + 6,000 *O. niloticus*/ha	123.21	80.91	0.34
2. 6,000 *P. monodon*/ha + 4,000 *O. niloticus*/ha	123.21	137.44	−0.12
Competition of *P. monodon* with *O. niloticus*			
1. 6,000 *O. niloticus*/ha + 6,000 *P. monodon*/ha	167.60	140.32	0.16
2. 4,000 *O. niloticus*/ha + 6,000 *P. monodon*/ha	128.52	145.88	−0.14

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Role of Tilapia *(Oreochromis andersonii)* in Integrated Farming Systems in Zambia

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Abstract

The potential for development of aquaculture as an industry in Zambia has been found to be appreciable because of the favorable climate, soil, water supply, availability of land and the spontaneous interest generated in the private sector. The old system of cultivating several species of tilapia together in the same pond has been changed to monospecies culture and *Oreochromis andersonii* has been shown to yield good growth and production rates. A broodstock (Kafue strain) of the species was introduced into the Chilanga Fish Farm and separate ponds for the production of fry, fingerlings and market-size fish have been maintained.

Among the various systems of aquaculture tried during the past three years in three farms established by the project in different regions of the country, integrated fish farming using fish-cum-pig and fish-cum-duck combinations has given very good production results. Modest averages worked out for the fish-cum-pig system are between 4 and 6 t/ha/year and for the fish-cum-Peking duck system between 3.5 and 4.5 t/ha/year. The paper describes details of the integrated farming technology with special reference to the role of *O. andersonii*, pond management practices and related economic considerations.

Introduction

The importance of aquaculture development as a component of integrated rural development in the Republic of Zambia has only been fully recognized in recent years (Gopalakrishnan 1986a). Based on the successful results obtained by the FAO/UNDP Pilot Fish Culture Development Project in Zambia, it has been established that when fish farming is integrated with other types of agricultural production, including animal husbandry, the benefits accruing to the rural community as a result of full utilization of land and water resources, by-products and residues could be significant and attractive (Gopalakrishnan 1986b). The major gains anticipated for the community from development of integrated fish farming are food self-sufficiency for the family, increased income from the fish produced,

diversification of income sources, availability of comparatively less expensive stock feed and manure in the rural sector itself and increased agricultural production by using pond humus as fertilizer. Integrated fish farming can be practised in different parts of the country as a primary occupation supplemented by other crops, as a secondary undertaking or as a side-line activity, depending on the resources available. The soil, water and other environmental conditions prevalent in Zambia have been found to be good for the development of aquaculture as an industry.

For rational and scientific development of integrated fish farming in Zambia, the right species of fish had to be chosen. All evidence available indicated that members of the tilapia group should be the first priority, taking into consideration their high culture potential in southern Africa and consumer preference. Field trials conducted during 1980-1982 clearly indicated that *Oreochromis andersonii* was the best candidate. Compared with other local tilapia, the growth potential of this species under semi-intensive culture conditions and its resistance to handling and cold temperatures had been shown to be high. A monoculture production system based on *O. andersonii* was demonstrated to be appropriate for economically viable fish farming.

Tilapia is well represented in Zambia by *Tilapia sparrmanii* and *T. rendalli*. The more common species in the *Oreochromis* group cultivated in Zambia are *Oreochromis andersonii, O. macrochir* and *O. mossambicus*.

Of these, *O. andersonii* (three-spotted bream; local name 'njinji' - Fig. 1) occurs naturally in the lagoons of the Upper Zambezi and Kafue river systems of Zambia. The male of the species is bigger than the female and develops specific maroon red coloration on the top of the head during the breeding season. The breeding habits of the species observed in different areas of the country are similar to typical characteristics of the group. From the experimental trials conducted in Chilanga it has been observed that spawning of the species starts in October and ends by April/May (Cayron-Thomas 1985).

Although earlier reports describe *O. andersonii* as a bottom feeder, observations made in Chilanga indicate that the species feeds at all levels. In trials using supplementary feeding and integrated farming systems, this species showed much better growth and survival rates than other tilapias. The average number of fry/fingerlings obtained per female

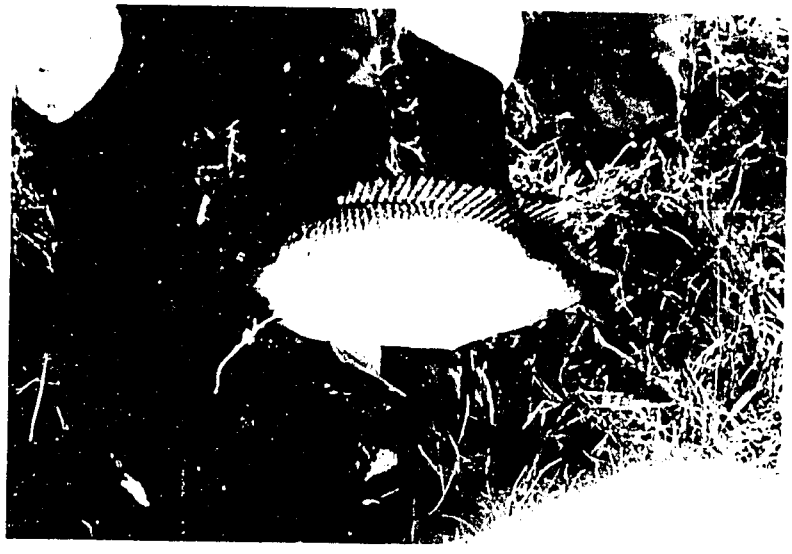

Fig. 1. *Oreochromis andersonii*.

under pond culture conditions has so far reached a maximum of 634. Under these circumstances the old system of cultivating several species of tilapia together in the same pond was changed to monoculture of *O. andersonii*.

O. andersonii in Integrated Farming Systems

Breeding of O. andersonii in integrated farming systems

The *O. andersonii* fingerlings used for integrated farming were obtained from a natural stock introduced into the Chilanga Fish Farm from the Charvanya lagoon in the Kafue River system. The broodfish were originally stocked at a density of 3,000/ha in two ratios; either 4 or 5 females to 1 male. The ratio has now been standardized to 5 females to 1 male (Cayron-Thomas 1986). During the first set of trials, 20% of the broodstock were lost. This loss was reduced to less than 10% during subsequent years by rigorous management measures, especially prevention of predation by otters. It has been shown also that the loss of broodstock can be minimized by draining in pond in mid-season followed by selection and restocking. A summary of the fry/fingerling production results obtained is given in Table 1.

Simultaneously with the breeding trials, a system of maintaining nursery, rearing and production ponds was developed. The breeding system in earthen ponds has been developed as an integrated farming exercise with 'Peking' ducks. In the duck breeding activities, which were undertaken simultaneously in the same pond, 190 birds (150 females and 40 males) were generally used. The females were changed every two years and the males every year. They were fed with layers' mash and maize (7:3) at 200 g/duck/day.

Integrated farming - O. andersonii *and pigs*

Pig weaners of the crossbreed between Landrace and Large White were purchased from commercial farms. The weight of the weaners ranged between 22 and 28 kg. Pigsties built on stilts over water and on the dikes were used for the trials (Fig. 2) with manure brushed or washed into the pond. At the end of each growing cycle, the pigs were sold as baconers or heavy hogs. Commercial pig feed available locally was used and was generally given dry in feeding troughs fixed to the floor of the pigsty, at the following rates:

Ave. wt. of pig (kg)	25	30	40	50	60	70-100
Feed/pig/day (kg)	1.2	1.4	1.9	2.3	2.6	2.8

The average feed : pig conversion ratio obtained was 4.4:1. A summary of *O. andersonii* production rates obtained from the different trials made during 1982-1986 is presented in Table 2. These observations indicate that under normal conditions and with adoption of proper management practices, *O. andersonii* produc-

Table 1. Production of *O. andersonii* seed in integrated farming systems of the Chilanga Experimental Fish Farm.

Season	No./female broodstock	No. harvested	No./ha
1983-84	188	77,800	389,000
1984-85	380	129,320	646,000
1985-86	684	286,671	1,433,400

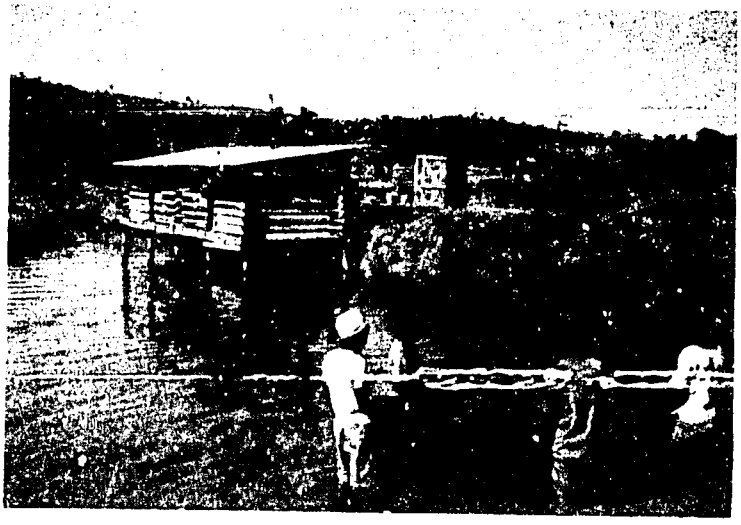

Fig. 2. Integrated fish farming system with pigsty built on stilts. *O. andersonii* production rate reached 9.8 t/ha/year in this pond.

Table 2. *O. andersonii* production trials (1982-1986) in integrated systems with pigs.

Pond area (ha)	No. pigs (total)	Stocking rate (no./ha)	Growth period (d ys)	Net prod. rate (kg/ha/ year)[1]	Harvest rate (kg/ha/ year)[2]
17	20	27,900	173	7,220	8,640
17	20	36,900	196	6,380	7,340
17	20	43,500	134	9,840	11,970
17	16	21,500	152	5,070*	6,290*
17	16	25,400	53	9,880	19,320
17	12	35,600	252	3,380*	4,300*
17	15	32,200	284	4,540	4,730*
17	15	23,100	185	4,420	6,200*
16	25	25,500	87	2,390	10,830
16	12	25,600	225	3,340*	3,390*
16	14	28,700	212	2,680*	3,630*
16	14	38,000	133	4,910	8,420
32	20	43,000	204	3,490	5,950

*Results vitiated by poaching/drought

[1]Based on final wt. less stocked wt.

[2]Based on gross wt. of fish harvested. Harvest rate is taken into consideration by farmers for determining economic indicators.

tion rates can average between 4 and 6 t/ha/year in the integrated farming system with pigs. It was determined that the ideal number of pigs to be grown is between 80 and 100/ha, except during the cold season when it would be advisable to reduce the manuring rate, and hence the pigs, to the minimum level.

Integrated farming - O. andersonii *and ducks*

Preliminary trials of integrated fish farming with ducks were conducted using *T. rendalli* and the locally available 'Muscovy' ducks. The production results

were not satisfactory, caused by a combination of factors. These trials indicated that 'Muscovy' ducks are not suitable for semi-intensive integrated fish farming as they do not stay in the water for long enough periods, thus producing insufficient manuring. Therefore, 225-day-old 'Peking' ducklings were procured from Hungary and the progeny derived from them fully acclimatized to local conditions. The duck breeding program has yielded successful results and approximately 20,000 ducklings have been produced so far.

The ducklings are transferred to fishponds when 3 weeks old and stocked at 300-500/ha, according to availability, the higher density having been found to produce the maximum tilapia production.

All further trials were conducted using 'Peking' ducks and *O. andersonii*. Two types of duck houses were tried out successfully. In the first system, the houses were built on stilts above the water surface with the floor made of wire mesh (Fig. 3). In the second system, the house was built either on dikes or nearby land area. The manure accumulated in the shed during the night was either washed down into the nearest ponds or collected and transported according to requirements in other ponds.

When 3-5 weeks old, the ducks were generally fed with a 1:1 mixture of broiler finisher and whole maize at 0.20 kg/duck/day. When the ducks were 6-9 weeks old, this was increased to 0.25 kg/duck/day. The results obtained so far indicate that in integrated systems with *O. andersonii* and 'Peking' ducks, fish production rates between 3.5 and 4.5 t/ha/year can be obtained with proper management practices (Table 3).

Economics of Integrated Farming Using *O. andersonii*

A study of economics of tilapia farming in Zambia indicates that integrated farming with *O. andersonii* and pigs or 'Peking' ducks could be an attractive financial proposal (L'Heureux 1985; Gopalakrishnan 1986a). The potential rates of return for a standard 5-ha fish farm are presented in Table 4. These estimates were based on rather high construction costs quoted by an engineering company, mainly because other reasonable estimates were not available at that time. Further observations, however, have shown that pond construction costs will be much lower than those used in the

Fig. 3. Duck house built over water in integrated duck-fish culture.

computations. Profit and loss accounts based on current costs have been estimated as shown in Table 5.

Conclusions

The scope and potential for development of integrated fish farming in different regions of the world have been demonstrated through several studies (Pullin and Shehadeh 1980; Hopkins and Cruz 1982). It is also known that even empirical methods of cultivating fish along with other animals have been successfully practised for many years (FAO 1983). However, it is only in recent years that such systems have been considered for adaptation in the African region. Members of the tilapia group are certainly

Table 3. *O. andersonii* production trials in integrated systems with ducks.

Pond area (ha)	No./ha	Growth period (days)	Net Prod. rate (kg/ha/year)[1]	Harvest rate (kg/ha/year)[2]
0.16	30,000	185	3,730	4,610
0.16	43,000	139	7,040	9,097
0.16	19,400	240	6,160	7,740
0.49	38,000	170	2,130*	2,260*
0.27	17,000	341	3,350	3,600
0.25	25,000	176	3,150	4,940
0.25	25,000	188	1,690*	4,090*

*Results vitiated due to poaching/drought.
[1]Based on final wt. less stocked wt.
[2]Based on gross wt. of fish harvested.

Table 4. Investment costs and rates of return of integrated fish farming in 5-ha farm (costs in Kwacha; 1 US$ = 8.00 Kwacha).

Size of ponds		5,000 m^2	2,500 m^2	1,500 m^2
Base investment costs				
Ponds		280,650	378,845	473,398
Buildings		50,989	50,906	50,590
Broodstock and contingencies		18,000	18,000	18,000
Fish-cum-pig				
Additional investment costs		36,450	36,450	36,450
Working capital		117,400	117,400	117,400
Rate of return:	4 t/ha	23.0%	19.0%	16.3%
	6 t/ha	30.6%	25.4%	21.4%
	8 t/ha	38.1%	31.8%	27.4%
Fish-cum-duck				
Additional investment costs		60,000	60,000	60,000
Working capital		20,000	20,000	20,000
Rate of return:	4 t/ha	24.1%	19.2%	16.0%
	6 t/ha	33.2%	26.8%	22.5%
	8 t/ha	42.3%	34.2%	26.8%

Table 5. Profit and loss statement of integrated fish farming in Zambia (based on a currently operating 5-ha farm): US$1.00 = 8.00 Kwacha.

Fish-cum-pig

1. *Annual production and revenue (in Kwacha)*

	Quantity (kg)	Unit price*	Revenue
Production sold:			
Fish (at minimum 4 t/ha)	20,000	7.5	150,000
Pigs	1,080 x 75	8.5	688,500
Total			838,500

2. *Annual operating costs (in Kwacha)*

Variable costs:		
Labor		30,000
Weaners (1,080 x 25 kg x K 6.50)		175,500
Feed (1,080 x 50 kg x FCR 4.4 x K 1.41/kg)	335,000	335,000
Transportation		8,000
Supplies		20,000
Miscellaneous		20,000
Fixed costs:		
Management		10,000
Depreciation		31,200
Interest (10%)		72,920
Total cost		706,620

3. *Investment costs* (in Kwacha)

Fixed capital costs	418,000
Additional capital costs	72,000
Working capital	239,200
Total	729,200

4. *Key economic indicators*

Profit = K 838,500 - 702,620 = K 135,880
Rate of return on investment = 18.63%

Fish-cum-duck

1. *Annual production and revenue (in Kwacha)*

	Quantity (kg)	Unit price*	Revenue
Production sold:			
Fish (at minimum 4 t/ha)	20,000	7.5	150,000
Ducks	9,600 x 2.5	10.0	240,000
Total			390,000

2. *Annual operating costs (in Kwacha)*

Variable costs:	
Labor	14,000
Broodstock replacement	2,000
Feed	157,000
Transportation	7,000
Supplies	2,000
Miscellaneous	15,000
Fixed costs:	
Management	8,000
Heating Lamps	4,500
Depreciation	31,600
Interest (10%)	59,715
Total cost	300,815

3. *Investment costs (in Kwacha)*

Fixed capital costs	418,000
Additional capital costs	130,000
Working capital	49,150
Total	597,150

4. *Key economic indicators*

Profit = K 390,000 - 300,815 = K 89,185
Rate of return on investment = 14.94%

*Note: Selling price of fish is at Government rate. Commercial rate is 50% more at farm gates.

important candidates for active consideration in this respect and the investigations being conducted in Zambia have demonstrated the technical and economic feasibility of integrated fish farming using *O. andersonii*. While it is necessary to develop the techniques further in order to develop new systems and new species, especially polyculture, the applications made so far are suitable for adoption in different parts of Zambia. Considerable interest, especially from the private sector, has been generated in this country for development of fish farming. It is necessary to develop standardized management plans for ensuring regular supply of high quality tilapia seed and for the implementation of integrated fish farming systems suitable for different countries of the subregion.

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A Multivariate Model of Tilapia Growth, Applied to Seawater Tilapia Culture in Kuwait[a]

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Abstract

Traditional analyses of aquaculture growth experiments usually consider only the yield at the end of the experiments and ignore the growth data collected during intermediate samplings. A multivariate model based on an expansion of the "Gulland and Holt Plot" used in fisheries biology provides a methodology to extract growth information from the data from intermediate samplings. This model is applied to data from three tilapia yield experiments conducted in seawater in Kuwait. The effects of temperature, sex ratio and fish length on growth rate are quantified.

Introduction

Estimation of growth rates under different culture regimes is an essential part of aquaculture research. Three common expressions of growth rates were used with the following frequencies in Avault (1985).

absolute growth rates (g or g/day)	66 %
instantaneous rates of growth	28 %
von Bertalanffy growth function	6 %

Detailed presentations of these and other growth expressions can be found in Ricker (1979).

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Absolute growth rates are commonly used with analysis of variance (ANOVA) procedures to evaluate treatment effects in aquaculture yield trials. However, absolute rates are subject to severe restrictions. They can only be compared among groups of fish with the same starting size that have been observed for equal periods of time and under identical environmental conditions. Thus, valid generalizations from absolute rates are extremely difficult to make.

Instantaneous growth rates and growth functions such as the von Bertalanffy growth function (VBGF) are not subject to restrictions on initial size or period of time. The forms of growth assumed by these functions closely approximate many observed fish growth series (Ricker 1979).

This paper presents a detailed description of a multivariate method for analyzing fish growth in aquaculture experiments, which was presented in brief by Pauly and Hopkins (1983). The method uses multiple regression equations to evaluate the effects of environmental variables on growth. The parameters of VBGF can be estimated from those equations. Data from seawater tilapia culture experiments in Kuwait were analyzed to illustrate the method, which has also been used with integrated livestock-fish data by Hopkins and Cruz (1982) and Prein (1985). A similar method, without the ability to estimate VBGF coefficients, was used by Jones and Strawn (1985) in analyzing data from a cage culture experiment.

Derivation of the method

This method was first proposed by Pauly and Ingles (1981) for use in analyzing temperature effects on growth in a mark-recapture experiment with coral reef fish. The method was later named the "Extended Gulland and Holt Plot" by Pauly (1984) because the basic assumption was originally presented by Gulland and Holt (1959). The basic assumption is that the growth rate of fish (in length) decreases linearly as the fish grow larger and can be expressed as:

$$dL/dt = a + bL \qquad \ldots 1)$$

where L is length, t is time, and a and b are empirically determined constants. The differential equation (1) can be replaced by the following difference equation if the time interval is short:

$$\Delta L_t/\Delta t = a + b\bar{L}_t \qquad \ldots 2)$$

where ΔL_t is the increase in length during period t, t is the duration of period t, and $\bar{L}_t$ is the arithmetic mean of the lengths at the beginning and end of period t.

As weight is of more interest than length to the aquaculturist, an alternative equation (form) using weight is substituted.

$$\frac{\Delta \sqrt[3]{W_t}}{\Delta t} = a + b\,\overline{\sqrt[3]{W_t}} \qquad \ldots 3)$$

where $\Delta \sqrt[3]{W_t}$ is the change in the cube root of fish weight fish during period Δt and $\overline{\sqrt[3]{W_t}}$ is the arithmetic mean of the cube root of fish weights at the start and end of period t.

Equation (3) suggests that only weight affects growth rate. However, the regression equations can be expanded into multiple regressions of the form:

$$\frac{\Delta \sqrt[3]{W_t}}{\Delta t} = a + b_1\,\overline{\sqrt[3]{W_t}} + b_2 X_{2,t} + \ldots + b_n X_{n,t} \qquad \ldots 4)$$

where $X_{2,t}$, $X_{3,t}$ ... $X_{n,t}$ are variables conceived as affecting growth rate during period t. These variables can be continuous variables such as temperature, or qualitative variables such as the presence or absence of antibiotic in the feed. Qualitative variables are included by the use of dummy variables (e.g., 1 = presence of antibiotic while 0 = absence of antibiotic). Given measurements of these variables during each time interval t, multiple regression techniques can be used to identify which of the variables have a significant effect on growth (i.e., identify slopes, b, which vary significantly

from 0). The values of the slopes quantify the effects.

As the Extended Gulland and Holt Plot is based on multiple regression, it is constrained by the basic assumptions of the regression method being used. The Ordinary Least Squares method has these basic assumptions:

1. Homoscedasticity
2. Nonstochastic independent variables
3. No autocorrelation
4. Number of observations exceeds the number of coefficients to be estimated.
5. No multicollinearity

Homoscedasticity means that the error terms are not correlated with any of the independent variables. For example, the variation in growth must be the same whether the fish weight is 10 g, 100 g or any other number. Nonstochastic independent variables mean that the independent variables are fixed by the experimenter and do not vary randomly. No autocorrelation means that there is no relationship between the error terms in successive periods and implies there is no relationship between the independent variable, X_n, in period i and X_n in period i-1. Multicollinearity refers to correlations between the independent variables. Detailed presentations of these assumptions can be found in statistics texts such as Gujarati (1978) and Kmenta (1986).

In aquaculture experiments, the usual practice is to sample each pond a number of times throughout the experimental period. Clearly, the growth rate in period t is related to the growth rate in period t-1 and the assumption of no autocorrelation is violated. Although the Ordinary Least Squares method would provide unbiased estimates of the coefficients to Equation 4, the F and t statistics would be overestimated, precluding hypothesis testing (e.g., comparison of the effects of different independent variables). Therefore, a Generalized Least Squares procedure should be used. Assuming that only first-order autocorrelation exists, the following transformation will remove the autocorrelation (Gujarati 1978):

$$Y_t - pY_{t-1} = a(1-p) + b_n (X_{n,t} - pX_{n,t-1}) \quad \ldots 5)$$

where $p = 1 - 0.5d$ ... 6)

and d is the Durbin-Watson statistic. The transformation in Equation (5) leads to the loss of the first observation in each set of data. If the number of observations is small, alternative transformations can be used in order to include the first observation(s) (Kmenta 1986; Gujarati 1978).

In addition to the constraints imposed by the regression procedure, there are some other limitations to the Extended Gulland and Holt Plot. The fish samples should be unbiased and the sampling period should be short enough to ensure the validity of Equation (2) but not so short that growth is obscured by variation inherent in random sampling.

The VBGF can be expressed in terms of weight:

$$W_t = W_\infty (1 - e^{-K(t-t_0)})^3 \quad \ldots 7)$$

where W_t is the weight at time t, and W, K and t_0 are constants. W_∞ corresponds to the average maximum weight which could be attained by fish in the population. These VBGF parameters can be estimated from a Gulland and Holt Plot as follows:

$$K = - b_1 \quad \ldots 8)$$

$$W_\infty = (a/K)^3 \quad \ldots 9)$$

$$t_0 = t + \frac{1}{K} \ln \left(\frac{\sqrt[3]{W_\infty} - \sqrt[3]{W_t}}{\sqrt[3]{W_\infty}} \right) \quad \ldots 10)$$

If the Extended Gulland and Holt Plot is used, K and t_0 of the VBGF for weight are again estimated according to equations (8) and (10), respectively, whereas W_∞ for a given set of environmental conditions is estimated as follows:

$$W_\infty = ((a + b_2 X_{2t} \ldots + b_n X_n)/K)^3 \quad \ldots 11)$$

To estimate t_o using Equation (10), the average weight, W, of fish of a known age, t, is required. If absolute age data are not available, recursive forms of the VBGF can be used (Prein 1985).

AN EXAMPLE

Data Collection

The data for this example were collected during the course of three experiments conducted at the Mariculture and Fisheries Department (MFD), Kuwait Institute for Scientific Research, Salmiya, Kuwait. The growth and mortality rates of the tilapia in seawater were measured during these experiments. Only a summary of the experimental procedures is presented here. Additional details can be found in Hopkins et al. (1986).

The fish used in the experiments were produced in freshwater or brackishwater (3 ppt salinity) and were acclimated to seawater (38-41 ppt) before the experiments started. Loading rates were low (0.1 kg/liter/min) for small fish (<10 g) and were increased to 1 kg/liter/min as the fish grew larger (>50-100 g). The feeding regime approached satiation feeding. Supplemental aeration was provided with diffusers or airlifts.

The fish were anesthetized with a mixture of quinaldine:acetone:ethyl alcohol before handling. The fish were sampled 9-10 times during the experiments in addition to the initial and harvest samples. Bulk weights and counts were made during sampling. All fish were sexed externally at harvest using diluted ink to highlight the genital papillae.

In the first experiment, 1 g *Oreochromis aureus* and *O. spilurus* fingerlings of both sexes were stocked into 5-6 m^3 outdoor raceways in August 1982. The fish were harvested in April 1983 after 246-253 culture days.

In the second experiment, the male fish which were harvested at the end of Experiment 1 were restocked into the raceways in late April 1983, grown for an additional 175 days and harvested in October 1983.

The third experiment started in August 1983. Five groups of 1-2 g fingerlings were stocked into 2-m^3 circular tanks:

O. spilurus	both sexes
O. spilurus	testosterone treated
O. aureus	testosterone treated
O. aureus x *O. spilurus*	both sexes
"Red" tilapia	testosterone treated

The testosterone treated fish had been fed a diet containing 100 mg ethynyl testosterone per 1 kg feed for 6 weeks prior to this experiment in an attempt at sex reversal. The groups containing both sexes had been fed an identical diet without the testosterone. Any dead fish during the first two weeks were removed from the tanks and replaced with fish of similar size. Experiment 3 ended in March 1984 after 226 culture days. Details of fish size, per cent males, numbers and temperatures in the three experiments are summarized in Table 1.

Data Analysis

The first Extended Gulland and Holt Plot hypothesized for these seawater growth experiments was:

$$\frac{\Delta \sqrt[3]{W_t}}{\Delta t} = a + b_1 \sqrt[3]{W_t} + b_2 T_t + b_3 M_t + b_4 D_t \quad \ldots 12)$$

where $\Delta \sqrt[3]{W}$ is the change in the cube root of fish weight during period t, Δt is the duration of interval t, $\sqrt[3]{W_t}$ is the arithmetic mean of the cube root of fish weight at the start and end of period t, T_t is the average temperature (°C) during period t, M_t is the fraction (in decimals) of males in the fish population during period t, and D_t is the fish density (kg/m^3) during period t. Temperature effects on growth rate can be simply represented using a quadratic parabola (Ricker 1979). However, because the upper temperatures encountered in the experiments did not exceed the optimum temperatures for tilapia growth (Chervinski 1982), only the linear temperature term is used in Equation (12). Fish density refers only to

Table 1. Fish weights, numbers, percentage of males and culture temperatures.

Experiment number	Length (days)	Number of replicates	Number of sampling periods	Temperature range (°C)	Males (%)	Av. weight (g)	Stocking av. density (/m³)	Av. wt. (g)	Harvest av. survival (%)
Oreochromis aureus									
1	253	4	10	19-30	58	1	126	58	55
2	175	2	11	23-28	100	67	50	239	56
3	226	2	11	20-28	85[a]	1	154[b]	33	23
O. spilurus									
1	246	3[c]	10	19-30	39	1	243	59	66
2	175	2	11	23-28	100	106	50	371	83
3	226	2	11	20-28	50	2	102[b]	70	82
3	226	2	11	20-28	75[a]	3	101[b]	80	70
O. aureus (female) x *O. spilurus* (male) hybrid									
3	226	2	11	20-28	70	1	102[b]	67	95
Red tilapia from Taiwan									
3	226	2	11	20-28	80[a]	1	105[b]	132	38

[a]Testosterone-treated fish.
[b]Includes replacement of dead fish within 2 weeks of stocking.
[c]A fourth replicate was lost because of a water supply failure. Data are available for 3 sampling periods before the loss.

a space relationship between fish in these experiments and is not directly related to water quality because the water flows were increased as the fish grew larger.

The *O. spilurus* data from all three experiments were pooled and analyzed as follows:

1. A correlation matrix containing all of the variables was constructed in order to determine the degree of multicollinearity (Table 2). Density, D_t, showed a high degree of correlation with $\sqrt[3]{W_t}$. As the maximum densities (16 kg/m³) attained in the experiments were much lower than densities commonly attained in intensive tilapia culture systems (Balarin and Haller 1982), it can be assumed that density effects were relatively unimportant in these experiments. Therefore, density was eliminated as one of the independent variables. Equation (12) was thereby reduced to

$$\frac{\Delta \sqrt[3]{W_t}}{\Delta t} = a + b_1 \overline{\sqrt[3]{W_t}} + b_2 T_t + b_3 M_t \qquad \ldots 13)$$

2. An Ordinary Least Squares procedure was performed. As expected, the Durbin-Watson statistic indicated a significant degree of autocorrelation (d = 2.704 with 3 regressors and 99 observations).
3. The transformation in Equation (5) was then applied and the regression performed again. Results of that regression are presented in Table 3. The indication of autocorrelation by the Durbin-Watson statistic was inconclusive.
4. Steps 1 to 3 were then repeated for the *O. aureus* data. The coefficient of the percentage males had the wrong sign and was insignificant (T = -0.064, significance level of T = 0.95). Therefore, percentage of males was dropped from the model

Table 2. Correlation of independent variables within *Oreochromis spilurus* data in experiments 1-3.

Variables	$\sqrt[3]{W_t}$	Correlation coefficient (r) Temperature	% Males	Density
$\sqrt[3]{W_t}$	1.000	0.003	0.600	0.887
Temperature	—	1.000	0.556	−0.151
% Males	—	—	1.000	0.306
Density	—	—	—	1.000

Table 3. Results of regression of *O. spilurus* data which had been transformed to remove first degree autocorrelation.

	Variables $\sqrt[3]{W_t}$	Temperature	% Males	Constant
Coefficients, b	−0.00522	0.002012	0.01857	−0.03417[a]
Standard error of b	−0.00160	0.000389	0.00628	0.01296
Standardized beta	−0.54559	0.44598	0.33947	
T statistic	4.923	5.169	2.956	2.636
Significance level of T	0.0000	0.0000	0.004	0.01
R square		0.479		
Number of cases		87		
F statistic		26.09		
Significance level of F		0.0000		
Durbin-Watson statistic		2.365		

[a]The constant in this regression = a(1 − p) where p = −0.35. Therefore, the a value to be used in estimating VBGF coefficients is −0.02531.

and regression procedure was conducted using the remaining transformed data (Table 4).

5. Correlation matrices were then computed for the red tilapia and the *O. aureus* x *O. spilurus* hybrid data. Unfortunately, for these two groups, the $\sqrt[3]{W_t}$ and temperature data were highly correlated (r = -0.965 for red tilapia and -0.952 for the hybrid). This multicollinearity resulted because these fish were grown only in Experiment 3. Experiment 3 started in August as water temperatures were starting to drop and finished in April when temperatures were starting to rise again. Thus, the fish were growing larger as the temperature dropped. If the experiment had been continued with larger fish growing at higher temperatures, the multicollinearity would have been eliminated. As it is impossible to isolate the effects of size and temperature on growth based on the available data for the red tilapia and the hybrids, the Extended Gulland and Holt Plot could not be used to analyze the data for these fish.
6. The coefficients of the VBGF were then computed for *O. spilurus* and *O. aureus* for several different sets of conditions (Table 5). Example plots of growth curves prepared using these VBGF coefficients are presented in Figs. 1 and 2.

COMPARING THE EXTENDED GULLAND AND HOLT PLOT AND ANOVA

Using the Extended Gulland and Holt Plot to analyze growth through time is much more involved than using ANOVA to determine if there are differences in weight at harvest. Is the extra effort justified? A comparison of the results and conclusions based on an ANOVA of the

Table 4. Results of regression analysis of *Oreochromis aureus* data which had been transformed to remove first degree autocorrelation.

	$\sqrt[3]{W_t}$	Variables Temperature	Constant
Coefficients, b	−0.002596	0.002675	−0.05502[a]
Standard error of b	0.000811	0.000311	0.00989
Standardized beta	−0.2590	0.3972	
T statistic	3.200	8.614	5.563
Significance level of T	0.0020	0.0000	0.0000
R square		0.525	
Number of cases		76	
F statistic		40.3	
Significance level of F		0.0	
Durbin-Watson statistic		2.172	

[a]The constant in this regression = a (1 − p) where p = −0.26565. Therefore, the a value to be used in estimating VBGF coefficients is −0.04347.

Table 5. Estimated coefficients of the VBGF for two tilapia species cultured in seawater.

Species	Temperature (°C)	% Males	K (day^{-1})	W_∞ (g)	t_o[a] (days)
O. spilurus	22	50	0.00522	158	−4.15
	25	50	0.00522	283	3.35
	28	50	0.00522	461	8.43
	22	100	0.00522	371	6.30
	25	100	0.00522	581	10.55
	28	100	0.00522	858	13.70
O. aureus	22	[b]	0.00260	208	−36.21
	25	[b]	0.00260	733	−10.29
	28	[b]	0.00260	1,775	1.79

[a]Arbitrarily assumes that a 1 g fish is 35 days old.
[b]Percentage males had no measurable effect on growth of *O. aureus* in these experiments.

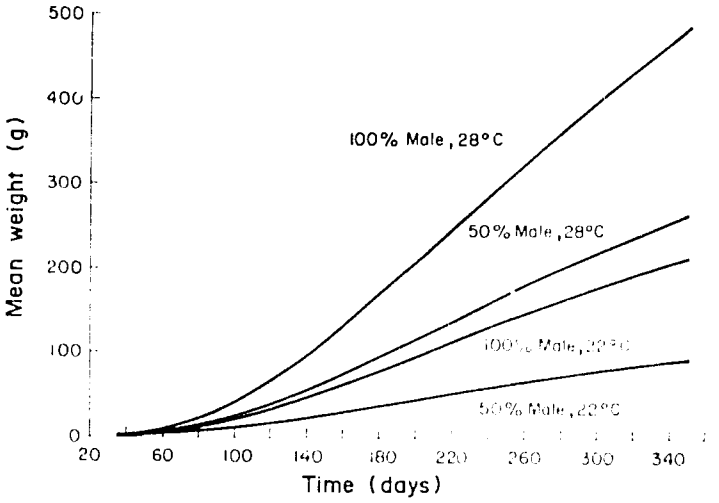

Fig. 1. *O. spilurus* growth curves at different temperatures and percentages of male fish.

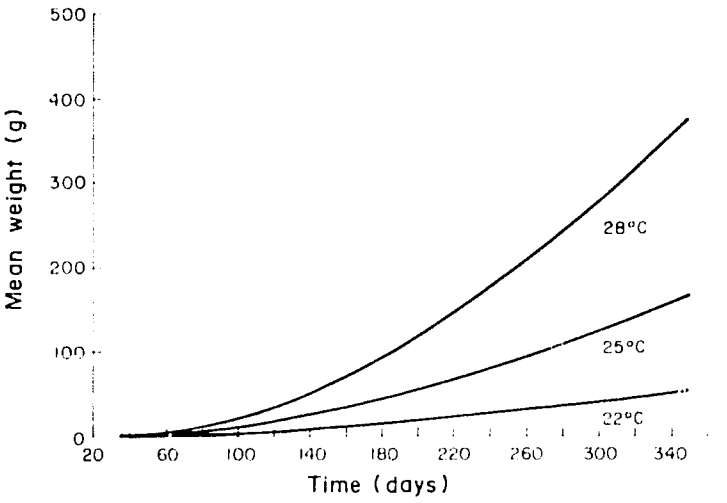

Fig. 2. *O. aureus* growth curves at different temperatures.

seawater tilapia data (Hopkins et al. 1986) and results and conclusions based on the Extended Gulland and Holt Plot shows that the Extended Gulland and Holt Plot can be used to extract additional valuable information from a typical aquaculture data set.

A summary of the results and conclusions based on ANOVA follows:

Experiment 1 (August 1982-April 1983). There was no significant difference in average harvest weight of *O. spilurus* and *O. aureus*. However, the *O. spilurus* males were larger than the *O. aureus* males. Complicating factors were a higher percentage of males for *O. aureus* than for *O. spilurus*, higher densities in the *O. spilurus* tanks than in the *O. aureus* tanks, and the loss of the fourth *O. spilurus* replicate because of a water failure.

Experiment 2 (April-October 1983). There was no significant difference in the instantaneous growth rates of 100%-male groups of *O. spilurus* and *O. aureus*.

Experiment 3 (August 1983-March 1984). The 85%-male groups of *O. aureus* were significantly smaller than the other groups of fish. There were no significant differences between the hybrids, the 50%-male *O. spilurus* and the 75%-male *O. spilurus* groups. The red tilapia were significantly larger than the other four groups of fish.

General Conclusions Based on Experiments 1 to 3. The *O. spilurus* males grow faster than *O. aureus* under winter conditions in Kuwait. Red tilapia grow fastest.

The Extended Gulland and Holt Plots and the VBGFs derived from those plots led to the following conclusions:

1. The most important factor affecting the growth of *O. spilurus* in seawater was fish weight, followed by temperature. Proportion of males had the least effect (based on an examination of the standardized beta coefficients).
2. The most important factor affecting the growth of *O. aureus* in seawater was temperature followed by fish weight. Proportion of males had no measurable effect on *O. aureus* growth. The probable reason for the difference in the effect of proportion of males between the *O. aureus* and *O. spilurus* was that *O. aureus* did not produce viable spawns in seawater while *O. spilurus* did.
3. The effects of fish size, water temperature and proportion of males on growth rate were quantified and the VBGF, a widely accepted mathematical representation of fish growth, was derived for *O. aureus* and *O. spilurus* at various combinations of temperature (19-30°C) and proportion of males (39-100%).
4. Plots of the VBGFs for *O. spilurus* and *O. aureus* indicated that 100% male groups of *O. spilurus* have a higher initial growth rate than *O. aureus*, particularly at lower temperatures. However, the W_∞ for *O. spilurus* are smaller than the W_∞ for *O. aureus*. Thus, the growth of *O. spilurus* will be superior unless a large harvest size (>700 g) is desired and the water temperature is high (≥ 28°C).
5. No conclusions about the hybrid and red tilapia were made using the Extended Gulland and Holt Plot as the data for these two groups of fish showed severe multicollinearity which could not be removed.

As can be seen from the preceding results, using the Extended Gulland and

Holt Plot can yield more information from a typical aquaculture data set than does ANOVA. This additional information quantifying the effects of fish size, proportion of males and environmental factors such as temperature are particularly useful in bioengineering and bioeconomic models. Also, the Extended Gulland and Holt Plot has the following attributes:

- the fish size does not have to be the same in each culture unit (pond, tank, etc);
- the culture periods for each replicate do not have to be the same length;
- the number of degrees of freedom in the statistical analyses is greatly increased because each sampling period is an observation thereby reducing the number of replicated culture units;
- Analysis of residuals can be used to test for linearity of response;
- the derived VBGFs provide a means of linking aquaculture data with growth models used in the general field of fishery biology and population modelling.

The first two attributes are particularly useful in that they allow the comparison of data collected by different researchers for different periods of time with different sizes of fish and to salvage data from replicates which are "lost" due to nonexperimental factors (e.g., water supply failure, fish theft, etc.). All the growth data collected before the "loss" can still be included in the analyses.

The Extended Gulland and Holt Plot does not replace ANOVA. Rather, it is an additional tool for analyzing aquaculture data sets. The Extended Gulland and Holt Plot is particularly suited for screening and quantifying the effects of a large number of variables with a minimum number of culture units. However, if the aquaculture conditions (e.g., initial stocking size, length of culture period, temperature regimes, etc.) are controlled or are relatively constant, ANOVA probably more efficient statistically than the Extended Gulland and Holt Plot.

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Artisanal Aquaculture of Tilapia in West Africa: Comparative Analysis of Different Culture Systems and Their Level of Development

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Abstract

Many attempts have been ma le over the last 40 years to develop aquaculture in Africa. Most were based on tilapia culture. Today, only very few development projects have successfully achieved their goal to make African rural populations aware of this new activity. This paper describes several small-scale tilapia aquaculture systems related to three very different socioeconomic and environmental conditions: ponds included in a hydro-agricultural scheme (Côte d'Ivoire); floating cages in a Sahelian river (Niger); and pens in a

brackishwater lagoon (Bénin). For each system, the following data are compared: description and cost of the rearing structure, as well as conditions and constraints for its implementation; main results of the different stages of tilapia production; production costs and profits. From the study of these three situations, conclusions are formulated and recommendations are suggested for the future of tilapia aquaculture development on the African continent.

Recent History of Aquaculture in Africa

The first recent attempts to develop African aquaculture began in the 1940s and were based primarily on raising tilapia in ponds with the rural population as the target group. The results can be considered to be still embryonic and have not yet warranted the high investment of effort expended on this activity.

Numerous reasons have been advanced to explain this situation:

- *Social factors*: fish farming was conceived as an activity for rural African populations who do not normally practice irrigated agriculture and/or intensive animal husbandry, two activities which have much in common with fish farming in ponds;
- *Technical factors*: up until a short time ago, the technical aspects of raising tilapia in ponds had not been mastered (e.g., control of proliferation, optimizing feeding/fertilization rates) leading to a mediocre quality of production (i.e., large numbers of *small* fish) and low yields;
- *Economic factors*: fish farming was done without concern for profitability as, in the spirit of the initiators, it was essentially a family subsistence activity. It has now been established that this approach does not produce sufficient incentive for fish farmers to pursue a new activity which requires at least a minimum of effort for the development of infrastructure, for maintenance and for financing the purchase of fry and feed.

At present, most fish farming development in Africa is carried out by government or by state-controlled organizations and nearly all existing aquaculture in the continent's rural environment is of a very low technical level. Also, there are very few private fish farms that are managed by Africans, the majority of the larger, more successful farms are run by foreigners. In 1980, FAO estimated that total aquaculture production in Africa was only 11,500 tonnes (FAO 1984).

The results set forth in the following paper were obtained from pilot projects, simulating full-scale production conditions, and from pilot fish farmers benefitting from close technical and economic supervision in three West African countries (Côte d'Ivoire, Niger and Bénin): very different environmental conditions and aquaculture systems. However, the three systems examined had one essential point in common: facilitation of small-scale operations in which fish culture is both a means of production and a development instrument, with the fish and socioeconomic benefits for the producers being equally important.

This type of small-scale fish farming differs from industrial schemes in that it is based on the farmer's capacity to exploit his production himself (with the help of his family) without employees. Industrial schemes on the other hand, involve wage earners (employees and laborers) hence the need for production units to be of a minimum size (and yield) to cover their expenses. Involved in such schemes are complex management practices that cannot be reproduced in a rural or fishing environment. Industrial schemes therefore are primarily reserved for individuals (or companies) who have the capacity to make large investments, while small-scale systems are tailored to families with the idea that, once techniques are mastered, they can evolve to larger dimensions.

Different Contexts and Types of Fish Culture

The three different types of fish culture described in the following sections correspond to three different physical, human and socioeconomic environments.

Fish culture in ponds in the northern region of Côte d'Ivoire

Fish farming in ponds is integrated into the overall hydro-agricultural programs in a region where numerous reservoirs were built in the 1970s for gravity-fed irrigation for rice growing. The region has secondary cities (e.g., Korhogo) where there is a high demand for fish, particularly for freshwater varieties. At present, fish supply to these cities consists of imported (frozen) marine fish. The distribution of imported canned fish is also set for expansion.

Farmers in the northern region of the Côte d'Ivoire have quickly adapted to irrigated rice growing techniques. Agricultural and agro-industrial by-products, e.g., rice bran from local sources, cotton cake, fish meal, etc. are available from sources within the country.

Fish culture in floating cages in Niger

Niger is a Sahelian country where the only permanent source of water is the Niger River.

The Niger River is 550 km long and constitutes the principal fishing and fish consumption area in the country, with the capital, Niamey, situated approximately at the midway point of the river. Fishermen are divided into two categories: professionals (generally foreigners) and occasional fishermen (local people who both farm and fish). Agricultural and agro-industrial by-products coming from Niger (e.g., rice and wheat bran, groundnut cake) or from regional sources (e.g., fish meal from Sénégal or Côte d'Ivoire) are the primary sources of feed or supplementary inputs available for aquaculture. The natural productivity of the Niger River is very low: average conductivity of 38 µmhos/cm and BOD 5 of 4 mg/l (Parrel et al. 1986), almost constant all year-round.

The Niger River is characterized by extreme variations in water flow during the year or from year to year. Throughout an average year the difference between flood and low water levels is 4 m and, with some exceptions, the river flows continuously. Under these conditions, the most suitable system for raising fish is probably the floating cage. For this system, fry and fingerlings are produced in ponds fed by water pumped in from the river, because gravity-fed supply is not possible.

With the droughts experienced since 1972 and the damming of the river for the development of hydro-agricultural systems, fish production from the Niger River decreased from a previous high of 6,000 to 2,000 t in 1984 and only 900 t in 1985.

Fish culture in pens in Bénin

In South Bénin, the major concentrations of inland waters are the lagoons which represent a total surface area of 320 km^2, principally Lake Nokoué and the Porto Novo Lagoon, where fish culture is developing. These lagoons have a uniform depth of about 1 m. The variations between high and low water levels are slight (approximately 0.50 m) and there is good water circulation. The most suitable fish culture system is the pen.

These two lagoons are close to two major urban centers, Cotonou and Porto Novo, where fish is consumed in quantity, as well as to Nigeria which imports large amounts of food products. The lagoons are inhabited by paludal populations and have numerous, highly efficient professional fishermen who practice a type of fishing, known as the acadjas system, which is

close to aquaculture. Agricultural and agro-industrial by-products, such as rice and wheat bran, groundnut and cotton cake and fish meal, are available from Cotonou. The level of the productivity of Lake Nokoué fluctuates roughly as follows:

- during flood, productivity is low due to high flow of freshwater through the lake and to high turbidity;
- during the dry season, the water of the lake is only partially renewed through the tide and maximum primary productivity occurs in April and May with levels of C fixation over 1 g $C/m^2/day$ which means quite high productivity during this period but with only poor influence on the high densities of fish used for pen rearing.

However, ecological changes in the area, resulting from the permanent opening of the Cotonou Channel, have disturbed wildlife in the lagoons and fish production has dropped from 12,000 to 6,000 t in the last few years.

Rearing Structures: Description and Investment Costs

Ponds

The ponds in Côte d'Ivoire are orthodox ponds supplied with water by gravity through a feeder canal with water flows regulated from a reservoir (Lazard 1980, 1986). Investments for the semi-intensive fish culture carried out in these ponds are principally for earthworks and a little for other construction work. The required earthworks can be done mechanically or manually. In either case the cost is approximately the same, if the workmen are paid at a standard rate. Manual construction of ponds done by rural populations has a satisfying intellectual appeal but is not always very realistic. The work is very laborious and the resulting ponds are often poorly constructed, with inadequate depth and weak banks, and require a large and dispersed supervisory workforce. One solution found has been to complete the major earthworks by machine and have the fish farmers do work, such as building of banks and canals, laying down grass, manually. In this case, the costs are divided: 67% for machines and 33% for manual labor. When ponds are planned and constructed at the same time as hydro-agricultural systems, their cost is marginal. The average total costs for pond infrastructure are 6 million F CFA/hectare (US$20,000/ha) for 0.1-ha ponds (for producing marketable fish) and 0.03-ha ponds (for producing fry and fingerlings). Lower investment costs can be found, locally, in Côte d'Ivoire, depending on the competition among contractors and on the local efficiency of manpower.

Floating cages

Each cage in Niger is made up of a floating superstructure supporting a submerged mesh cage section containing the fish (Parrel et al. 1986). The technology involved is simple and optimizes the use of locally available material. For example, the floating structure consists of a wooden pontoon and second hand 30-l plastic drums which ensure the buoyancy of the system and enable people to work around the cage. The submerged cage section is made of imported plastic mesh (NORTENE, a French registered trademark). This relatively light structure makes it easier to raise the cages for maintenance and harvesting.

Two types of 1.5-m deep cages are used. For growing 4 g fry to the fingerling stage of 30 g, 5-m^3 cages, of which 3.5 m^3 are under water, using 7 mm side-length mesh are used. For growing fingerlings up to a market size of approximately 250 g, 20-m^3 cages, of which 16 m^3 are under water, with 14 mm side-length mesh are used.

The cages can be linked together to create a modular system with a base consisting of one pontoon supporting two 20-m^3 cages or eight 5-m^3 cages.

Table 1 shows the investment costs for cages.

nets are used (210/48, 14 mm side-length mesh, 160 meshes deep). Upper bolt ropes are attached 0.80 m above the lake's low-water mark to palm stakes affixed horizontally to bamboo support pegs. Lower bolt-ropes are buried at a depth of

Table 1. Investment costs of a floating cage for raising tilapia (*Oreochromis niloticus*) in Niger in F CFA (1 US$ = 300 F CFA, January 1987).

Items	20-m^3 cage	5-m^3 cage
Floating structural materials	102,700	25,700
Plastic mesh for the cage	78,300	32,550
Labor[a]	15,700	3,750
Total	196,000	62,000
Cost/m^3	9,800	12,400

[a]For construction only.

Pens

Different pen-culture trials have led to the development of a simple and well-adapted technology in Benin (Morissens 1985, 1986; Morissens et al. 1986). The pens have an individual surface area of 500 m^2 (14 x 36 m), with the pens separated from each other by approximately 3 m allowing for easy access around the entire periphery. Heavy

0.50 m in a furrow dug in the bottom mud by means of a motorized pump. Finally, the pens are covered by a light net, and branches are installed in the pens identical to those used in the acadjas to encourage the development of plankton and periphyton and to reduce theft by preventing the use of cast-nets. Table 2 shows the investment costs for one of these pens.

Table 2. Investment costs of a 0.05 ha tilapia (*Oreochromis niloticus*)pen in Benin (in F CFA). (1 US$ = 300 F CFA, January 1987).

Items	Costs
Net	248,000
Net fixing materials[a]	66,000
Pump[b]	6,000
Labor[c]	28,000
Total	348,000
Cost/m^2 (or m^3)	7,000

[a]Bamboo pegs, palm stakes, bolt ropes, covering net.
[b]Operating cost of pump used for burying the lower bolt rope of the pen into the bottom of the lake.
[c]Labor for sewing and fixing the net.

Fish Farming Techniques

Each system described above, all of which raise *Oreochromis niloticus*, is adapted to the environment into which it must be integrated, and which involves the following principal constraints:

- Ponds need to economize on the water stocked in the reservoir upstream which also supplies nearby rice areas. Therefore, the flow reaching the ponds corresponds exactly to losses through evaporation and infiltration and does not allow for water circulation.
- Cages need to be adapted to the variations in the Niger River environment such as: flood and low-water conditions, the hot (24°C-32°C) and cold (15°C-24°C) seasons and the corresponding transitional periods.
- Pens need to be adapted to the environmental variations in Lake Nokoué and the Porto-Novo Lagoon; such as the water salinity (which varies from 0 to 18-24 ppt according to the site and year) and the flood and low-water levels of the river which flows into them.

Ponds

Culture technique. Lazard (1980, 1984) describes how 0.1-ha ponds are stocked with Bouaké strain *O. niloticus*, unsexed or all-male hand-sexed, fingerlings of 30 g, produced at a nursery or by the fish farmer himself. In both cases, the predator *Hemichromis fasciatus* is stocked with the tilapia at a rate of 13% of the biomass of unsexed tilapia, or 5-6% of the biomass in the case of manually sexed tilapia. This procedure allows a 5-10% margin of error in sexing (i.e., % of females).

There are no constraints as far as the pond stocking period is concerned since the physicochemical characteristics of the water permit this type of operation throughout the year. The stocking density varies from 1.2 to 2.2 fish/m^2. Two types of feed have been tested in full-scale conditions:

- Feed #1: pure rice bran (100 kg/ha/day throughout the rearing period).
- Feed #2: 75% rice bran + 25% cotton cake (feeding rate decreasing progressively and adjusted every month, according to the evaluated standing biomass in the pond, from 8% in the first month of rearing down to 1.5% during the last month).

Results. Results from this pond production system have been obtained on a large-scale (4.7-ha) pilot fish farm (Natio-Kobadara) and are shown in Table 3. Fish culture using crude rice bran (Feed #1) is very easy to set up and the presence of many rice mills in Côte d'Ivoire provides the fish farmer with an abundant regular supply of feed. The composite feed (Feed #2) gives a higher yield. Even better

Table 3. Pond (0.1-ha) production of *Oreochromis niloticus* to a marketable size (Côte d'Ivoire) using two different feed types (see text for details).

	Feed #1 (♂ + ♀)	Feed #2 (♂)
Stocking density (fish/m^2)	1.2	2.2
Growing-out period (days)	145	240
Av. initial weight (g)	30	31
Av. final weight (g)	215	270
Survival rate (%)	92	90
Daily growth (g/day) per individual	1.3	1.0
Feed conversion ratio	7.5:1	3.5:1
Yield (t/ha/year)	5.2	7.1

results have been obtained on the Bouaké research station where yields, extrapolated from trials carried out in 0.04-ha ponds, are up to 11 t/ha/year with a feed composed of 69% rice bran and 31% cotton cake and more than 15 t/ha/year with 75% rice bran, 15% cotton cake and 10% fish meal (Lazard 1984).

Cages

Culture technique. Parrel et al. (1986) describe cages (16-m^3 working volume) stocked with 30-g male hand-sexed *O. niloticus* fingerlings (Niger River strain). Stocking has to be done during the hot season between April and September while the water temperature is stable and above 24°C. Stocking densities vary between 85 and 135 fish/m^3. Feed composition is 45% groundnut cake, 50% rice or wheat bran and 5% fish meal (31.5% protein of which 10% is of animal origin) in the form of 4 mm diameter pellets. Tests done with 10% fish meal and with 2.5% premix did not reveal any significant improvement in growth performance.

The feeding rate is determined according to the size of the fish: from 3.0% of the biomass/day when individual weight is below 150 g to 2.0%/day when individual weight is above 200 g. These rates are further adjusted to temperature (from 100% normal feeding rate above 24°C to 15% normal feeding rate when temperature is between 16°C and 18°C) and to the River's hydrology (the ration is adjusted according to fish behavior during floods: it is reduced during high turbidity periods and feeding is even stopped when it is observed that fish do not come anymore to the surface to take the pellets).

Results. Results from the cage production system in Niger are shown in Table 4. A stocking density of 135 fish/m^3 gives the highest yields with only a small increase in the feed conversion ratio. This kind of farming only allows one growth cycle per year as cage stocking can only be done once a year and not in the cold season. As can be seen in Table 4, growth rates during the cold season are very slow. However, cage stocking can be spread out during the hot season which may allow the marketing of the fish to be spread throughout the year. No pathological problems have been noted as long as the feeding rate is adjusted to the hydroclimatic conditions. If not so, bacterial diseases *(Aeromonas hydrophila)* appear, spread very quickly throughout the cages and may cause high mortality, especially in case of overfeeding.

Table 4. Production of marketable-size *Oreochromis niloticus* in 20-m^3 floating cages (Niger) during hot season and cold season.

	Farming in HS + CS[a] (2/3 + 1/3)[b]		Hot season farming		Cold season farming
Stocking density (fish/m^3)	85	135	85	135	85
Growing-out period (days)	204	225	142	154	117
Av. initial weight (g)	35.5	35.7	55.2	52.6	31.0
Av. final weight (g)	218.5	217.9	229.9	249.4	67.4
Survival rate (%)	95.4	90.7	89.6	92.0	90.2
Daily growth (g/day) per individual	0.9	0.81	1.23	1.28	031
Feed conversion ratio	2.7:1	3.0:1	2.4:1	2.8:1	2.04:1
Yield (kg/m^3/cycle)	14.3	21.8	14.8	23.9	-

[a]HS = hot season (April to November); CS = cold season (November to March).
[b]2/3 of the total growing-out cycle (average: 215 days) takes place during hot season and 1/3 during cold season.

Pens

Culture technique. Morissens (1985, 1986) and Morissens et al. (1986) stocked pens with *O. niloticus* (Ivory Coast strain, Bouaké) nursery-reared unsexed fingerlings (15 g). Other species have been tested and results show that *O. mossambicus* has a slow growth rate and *O. urolepis hornorum* has a poor survival rate. The hybrid *O. mossambicus* × *O. niloticus* is being tested and is giving encouraging results.

Stocking is done when salinity is below 10 ppt, from August to January. Stocking densities vary between 15 and 25 fish/m². Two feeds have been tested:

- Feed #1 (35% protein of which 25% is of animal origin):
  30% groundnut or cotton cake, 23% wheat bran, 20% Brewer's grains, 15% fish meal, 10% chicken manure, 2% oyster shells and 0.25% micronutrient premix.
- Feed #2 (36% protein of which 20% is of animal origin):
  20% groundnut cake, 45% Brewer's grains, 25% wheat bran and 10% fish meal.

The feed is given as 4 mm diameter pellets or as powdered meal, and the feeding rate varies from 4.0% of the biomass/day at the beginning to 2.5%/day at the end of the growing period.

Results. Results from the pen production system in Benin are shown in Table 5. The higher stocking densities give the best yields; however, in certain cases pathological problems arise due to bacterial infections. The use of a powdered feed results in an increase in the feed conversion ratio, but may be justified by the fact that it is very simple to use where there is no pelleting equipment and the higher feed conversion ratio is offset by the cost of pelletization. However, as in the case of the cage system, this kind of culture only allows one production cycle per year due to environmental constraints (salinity).

Economic Analysis of the Culture Systems

Agricultural credit in many African countries has not yet been adapted to different types of agricultural production and its conditions vary widely among different countries; therefore, costs of such credit are not taken into account in the following analysis in order to present comparable data.

There are two ways of calculating labor input (or earnings): 1. observing real laboring time spent by a fish farmer on one production unit (1 pond, 1 cage, 1 pen) or 2. extrapolating laboring time from a larger-scale production farm (*x* ponds, *x* cages, *x* pens) to one production unit. The first method usually gives higher values. The second method is used here. Labor input for the same work fluctuates even within a single country from one locality to another and the data given here are only indicative.

Table 5. Pen (0.05-ha) production of marketable size *Oreochromis niloticus* in Benin.

	Feed no. 1		Feed no. 2	
	pellets	pellets	meal	pellets
Stocking density (fish/m²)	25	15	25	25
Growing-out period (days)	226	225	225	282
Av. initial weight (g)	12.8	16.0	17.8	18
Av. final weight (g)	229.6	200.5	168.0	200
Survival rate (%)	68.4	76.6	77.4	68
Daily growth (g/day) per individual	0.91	0.82	0.67	0.64
Feed conversion ratio	4.07:1	4.1:1	4.65:1	2.9:1
Yield (kg/m²/cycle)	3.62	2.15	2.87	3.1

Ponds

Table 6 shows the fixed and variable costs and income derived from the pond system in Cote d'Ivoire.

Based on the results in Table 6, the annual return to all owned inputs, including investment is 122,000/660,000 = 18.5%.

Earnings from one day's work, assuming that a 0.1-ha pond requires the equivalent of 30 days of 1 man's labor per year, are 122,000 F CFA/30 = 4,000 F CFA/day which must be compared with the salary level of an agricultural laborer in Côte d'Ivoire: 900 F CFA/day.

Cages

Based on Table 7, the annual return to all owned inputs, including investment is 97,250/200,500 = 48.5%.

Earnings from one day's work, assuming that one 20-m^3 cage requires the equivalent of 12.5 days of 1 man's labor per cycle, are 97,250 F CFA/12.5 = 7,780 F CFA/day which is to be compared with the salary of an agricultural worker in Niger: 900 F CFA/day.

Pens

Based on Table 8, the annual return on investment is 236,500/440,000 = 53.5%.

Earnings from one day's work, assuming that a 0.5-ha pen requires the equivalent of 30 days of 1 man's labor per cycle, are 236,500/30 = 7,880 F CFA/day which must be compared with the salary of an agricultural laborer in Bénin: 800 F CFA/day.

Table 6. Financial analysis for a 0.1-ha pond (Côte d'Ivoire).

Items (F CFA)	Per 10-are pond	Per kg of fish	Cost as a percentage (%) of total costs
Costs			
Fixed costs	50,000	62.0	25.0
• Amortization of pond (20 yrs)	30,000	37.0	15.0
• Amortization of small equipment (3 yrs)	20,000	25.0	10.0
Variable costs	152,000	187.0	75.0
• Maintenance[a]	-	-	-
• Fry: 2,200 x 2 x 365/240 x 10 F CFA[b]	67,000	82.5	33.0
• Feed: 3.5 x 710 x 26 F CFA[c]	65,000	80.0	32.0
• Transport (fry, feed, marketable fish)	20,000	24.5	10.0
Total costs	202,000	249.0	100.0
Gross sale: 810 kg x 400 F CFA[d]	324,000	400.0	
Net profit (return to owned inputs)	122,000	151.0	

[a]The cost for maintenance is assumed to be only labor; it is not taken into account in this financial analysis but is included in the labor time spent by the fish farmer for the fish culture activity (see text).

[b]Number of pieces required for a 0.1-ha pond x 2 (the males only are kept, assuming a 1:1 sex ratio) x number of possible growing-out cycles per year x price of one fingerling (♂ or ♀).

[c]Feed conversion ratio x net fish yield x price of 1 kg of feed.

[d]Gross yield x price of 1 kg of market-size fish.

Table 7. Financial analysis for a 20-m^3 floating cage (Niger).

Items (F CFA)	Per 20 m^3 cage	Per kg of fish	Costs (%)
Costs			
Fixed costs	29,500	67.5	11.0
• Amortization of cage (7 yrs)	28,000	64.0	10.5
• Amortization of small equipment (3 yrs)	1,500	3.5	0.5
Variable costs	243,000	558.5	89.0
• Cage maintenance[a]	5,000	11.5	2.0
• Fry: 2,200 x 45 F CFA[b]	99,000	227.5	36.0
• Feed: 363 x 3 x 100 F CFA[c]	109,000	250.5	40.0
• Transport (fry, feed, marketable fish)	30,000	69.0	11.0
Total costs	272,500	626.0	100.0
Gross sale: 435 kg x 850 F CFA[d]	369,750	850.0	
Net profit	97,250	224.0	

[a]The cost for maintenance takes only into account the materials needed for repairing the cages (plastic net and floating structure), excluding the labor.
[b]Number of pieces for stocking a grow-out cage x unit price of fingerling ♂.
[c]Net yield (kg) x feed conversion ratio x price of 1 kg of compounded feed.
[d]Gross yield x price of 1 kg of market-size fish.

Table 8. Financial analysis for a 0.05-ha pen (Bénin).

Items (F CFA)	Per 0.05 ha pen	Per kg of fish	Costs (%)
Costs			
Fixed costs	117,500	59	11.0
• Amortization of pen (4 yrs)	87,500	44	8.0
• Amortization of small equipment (3 yrs)	30,000	15	3.0
Variable costs	946,000	473	89.0
• Pen maintenance[a]	20,000	10	2.0
• Fry: 12,800 x 15 F CFA[b]	192,000	96	18.0
• Feed: 1,900 x 4 x 90 F CFA[c]	684,000	342	64.0
• Transport (fry, feed, marketable fish)	50,000	25	5.0
Total costs	1,063,500	532	100.0
Gross sale: 2,000 kg x 650 F CFA[d]	1,300,000	650	
Net profit	236,500	118	

[a]The cost for maintenance takes only into accoount the materials needed for repairing the pen (net, rope ...) excluding the labor.
[b]Number of pieces for stocking a grow-out pen x unit price of fingerling (♂ x ♀).
[c]Net yield of fish (kg) x feed conversion ratio x price of 1 kg of compounded feed.
[d]Gross yield x price of 1 kg of market-size fish.

Discussion

Of the three systems examined, pond culture seems to be the least flexible, from an investment point of view, for various reasons. The number of favorable sites is limited because of limited water availability. Installation of infrastructure is costly. Long-term amortization of loans, which would be necessary in some cases, creates problems where investment funding is concerned and therefore, credit problems. No costs of credit were included in the financial analysis. Pond culture gives the lowest annual return on investment (18% against 48% and 53% for cages and pens) and also has the highest proportion of fixed costs. Pond culture, however, is suitable in situations where it can be integrated with hydro-agricultural systems or animal husbandry. On the other hand, total variable (or operating) costs for cages and pens are both quite high in absolute terms and compared with net profits, due mainly to the high feed inputs. This points out the need for operating cash and the requirement for a system of annual credit. This kind of credit, usually granted by the national Agricultural Development Bank or commercial banks, is already working for other commodities such as cotton production (Côte d'Ivoire) or food crops such as rice, millet (Niger). Annual credit is actually extended to cage fish farmers in Niger (annual rate of interest on credit = 11%) returnable every year at fish harvest. As for pen culture, credit is free for the moment and is provided by the development project.

Production management. Of the three systems, pond culture is the most secure option, giving the greatest management flexibility, least risk and using the simplest techniques. Cage and pen culture systems are more demanding in terms of management, mainly due to feeding, and are riskier. There are pathological risks, theft is easier, and the feed conversion ratio may increase due to feed loss outside the rearing structure and misadjustment of the feeding rate.

Logistics. Apart from close supervision, which is absolutely necessary for new aquaculture activities in Africa no matter what the method used, cages and pens are dependent on external resources for the supply of fry and fingerlings, composite feed pellets and agricultural by-products of adequate quality and quantity (when these are not supplied from on-farm resources). In ponds, production is less dependent on external feed resources and fry and fingerlings can be produced on site. Financial calculations show that the greatest costs are related to feeds in pens and cages and to a lesser extent the same is true for ponds.

Development. Experience from different projects has shown that there is a clear preference on the part of potential farmers for pen and cage culture rather than pond farming. In addition to greater potential return on investment and earnings from labor, there is the fact that pens and cages generally concern fishermen populations (e.g., Benin and Niger) who are traditionally more dynamic than strictly rural farming populations. It also appears that yields must be above a certain fixed level. This therefore requires a certain technical ability of the producer, determined independently for each situation in order to guarantee a certain profitability for his activity. This factor is particularly important for ponds where a low yield even if it does not lead to financial loss may discourage the farmer in the long term.

Economics. The financial data presented above come from:

- a large-scale pilot fish farm for pond data, extrapolated using 1987 values of inputs and outputs;
- pilot cages run by a development operation prior to extension to fishermen (the first data of these latter show the validity of the pilot results); and
- real pilot pen farmers, closely supervised by a development project.

Pond culture, if one excludes costs of financing, and all other things being

equal, supplies fish at the lowest cost and is therefore most suitable for rural populations who are not as rich as urban populations who currently consume the majority of fish culture products.

On a strategic level for the future, it seems that for each specific environment, in the broad sense of the term, one (or more) suitable and well-adapted culture technique(s) must be developed. Also, one must be careful about the conditions under which techniques are transferred from one region to another. Initially, it is not systematically desirable for the farmer to depend solely on aquaculture as his only source of income if one considers the type of small-scale culture discussed here. Aquaculture should be an economic venture and therefore its profitability should be evaluated in each situation.

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Biological Nitrogen Fixation as a Source of Nitrogen Input in Fishponds

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Abstract

The potential input of nitrogen derived from natural biological fixation in water of fertilized tilapia grow-out ponds in Thailand was determined by the acetylene reduction technique for 12 ponds over a 15-month period. On average, nitrogen fixation ranged from undetectable levels of N to 105 µg/l/day in water column, compared with 200 µg/l/day input of N from chicken manure loading at a rate of 500 kg/ha/week. Estimated total nitrogen fixation in fishponds during a 5-month grow-out cycle ranged from 8.8 to 85.7 kg N/ha. The nitrogen fixation primarily occurred in daylight; it was inhibited in the dark and suppressed by elevated ammonium concentrations present in pond water. Nitrogen fixing blue-green algae commonly present in the pond water were *Anabaena*, *Cylindrospermum* and *Nodularia*.

Introduction

The contribution of nitrogen fixation by blue-green algae has long been recognized as an important source of nitrogen input in rice fields (Watanabe and Yamamoto 1971) and freshwater lakes (Dugdale and Dugdale 1962; Horne and Viner 1971; Granhall and Lundgren 1971). Blue-green algal blooms are common occurrences in fishponds. Some species provide significant amounts of nitrogen input from natural fixation providing a natural mechanism to compensate nitrogen fertility. Despite the speculated contribution of nitrogen input from natural fixation in

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fishponds few measurements have been made (Boyd 1982). This paper presents the quantity and variability of potential nitrogen fixation in extensive tilapia grow-out ponds in Thailand.

Materials and Methods

The nitrogen fixation rates were measured for three 5-month cycles during 1985-1986 at Ayutthaya Freshwater Fisheries Station located in the lower central plain of Thailand. The twelve ponds in this study were used in extensive culture of tilapia (*Oreochromis niloticus*), which were stocked at 1 fish/m^3. Each pond had a surface area of 250 m^2/pond and held 220 m^3 of water as the pond depth was maintained at 90 cm. Ponds received two types of fertilization treatments: (1) chicken manure (500 kg/ha/week) or commercial fertilizer (30 kg urea and 46 kg triple superphosphate per ha/week), with each treatment in four replicate ponds conducted during August-December 1985, and (2) chicken manure at four loading rates (125, 250, 500, 1,000 kg/ha/week), with each treatment in three replicate ponds conducted during mainly the dry (February-July) and rainy seasons (August-December) in 1986, respectively. The nitrogen fixation rates were determined monthly in the first and biweekly for the second fertilizer experiment.

The acetylene reduction technique of Stewart et al. (1967) was used to measure the nitrogen fixation capacity as indicated by the nitrogenase activity. Water samples were taken at three points in each pond using an integrated column sampler. An aliquot of 150-ml mixed water sample was dispensed into each of four 250-ml Erhlenmeyer flasks (two light and two dark) which were then covered tightly with serum stoppers. Using a syringe, 100 ml of air was withdrawn from each flask and injected with an equal volume of commercially prepared acetylene. All flasks were suspended 25 cm below the pond surface and incubated for 12 hours from 0600 to 1800 hours. At the end of each incubation period, a 10-ml gas sample was withdrawn from each bottle with a syringe and kept in a 15-ml blood-sample tube fitted with a serum stopper. To determine the effect of ammonium concentration on fixation, ammonium was added to a set of water samples at 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg NH_3-N/l.

The amounts of acetylene and ethylene in the gaseous samples were determined using a gas chromatograph (Perkin-Elmer), which was supplied with a Porapak nitrogen carrier and a hydrogen flame ionizing detector. The numbers of nitrogen molecules fixed was calculated based on 3:1 ratio for acetylene reduction to nitrogen reduction.

Ammonia and organic nitrogen concentrations in pond water were determined biweekly by standard methods (APHA 1981), and the composition of phytoplankton examined monthly.

Results and Discussion

Nitrogen fixation in inorganically an organically fertilized ponds

The nitrogen fixation rate, measured monthly from 16 September to 16 December 1985, varied from 0 to 643.8 µg/l/day in inorganically fertilized ponds and from 6.3 to 562.8 µg/l/day in organically fertilized ponds (Table 1). On average, the fixation activity in the former ponds was considerably lower than that in the latter. Large variations occurred within and between fertilizer treatments.

Fixation rates that occurred in the light incubation were 2 to 5 fold greater than those in the dark. However the dark fixation was not a linear function of the respective light activity. It has been well documented that the nitrogen fixation by blue-green algae is usually light dependent, with decreasing photosynthetic activity as light attenuates with increasing water depth (Horne and Fogg 1970; Horne and Viner 1971). Levine and Lewis (1984) reported that the nocturnal nitrogen fixation in a lake was less than 6% of the maximum daytime rate. The

Table 1. Comparison of nitrogen fixation rates (µg N/l/day) in pond water between inorganic (A) and organic (B) fertilizer treatments with three replicate ponds for each treatment. (L = 0600-1800-0600 hours).

Date	L/D	Ponds A-1	A-2	A-3	A-4	B-1	B-2	B-3	B-4
09/16	L	3.7	4.4	11.1	90.2	42.7	124.4	13.3	91.7
	D	17.0	0.8	3.9	14.8	13.6	41.3	8.1	18.1
	L+D	10.7	5.2	14.0	105.0	56.9	165.7	21.4	109.7
10/16	L	0.0	18.5	215.3	541.3	12.3	263.1	8.2	439.8
	D	0.0	4.1	45.1	102.5	4.1	26.6	4.1	123.1
	L+D	0.0	22.6	260.4	643.8	16.4	289.7	12.3	562.8
11/13	L	0.0	2.9	1.2	19.3	33.4	30.9	4.8	15.5
	D	0.0	1.6	0.4	7.1	13.1	14.0	1.5	7.1
	L+D	0.0	4.5	1.6	26.4	46.4	44.9	6.3	22.6
12/16	L	5.1	6.7	6.7	6.2	96.7	92.8	26.1	58.0
	D	0.0	0.0	2.6	2.0	37.2	38.4	13.1	18.6
	L+D	5.1	6.7	9.3	8.2	133.8	131.2	39.2	76.6
	Sum	20.9	39.0	285.3	783.4	253.5	631.4	79.2	771.7
	Ave.	5.2	9.8	71.3	195.9	63.4	157.9	19.8	192.9

dep[illegible] effect on nitrogen fixation in the fishponds was probably minimal because the physical data showed that the shallowness (90 cm) allowed extensive mixing in the water column.

Nitrogen fixation in ponds with various loading rates of chicken manure during dry and rainy cycles

Fig. 1 shows the biweekly values of nitrogen fixation in ponds of various fertilizer loading rates during the predominantly dry and rainy seasons. While the fixation was unmeasurable on many dates, particularly during the early weeks of each cycle, the upper limit often reached 200-300 µg N/l/day. Those values varied widely and did not correlate with fertilizer loading levels, but the average fixation rate measured during the more rainy period was persistently greater than that of the drier period. El Samra and Olah (1979) also showed that nitrogen fixation in temperate fishponds exhibited wide daily fluctuation and was particularly low following each fertilization. The estimated amount of nitrogen input through fixation during each grow-out cycle ranged from 8.8 to 33.9 kg/ha and from 31.6 to 85.7 kg/ha for the drier and wetter periods, respectively, contributing 1.5-40% of total nitrogen in the fishponds (Table 2). Although the total quantity of fixed nitrogen in the ponds was not correlated with chicken manure loading rate, the ratio of nitrogen input between those two sources increased with greater amounts of chicken manure loadings. In some cases, the ratio was less than 2, indicating that nitrogen fixation contributed greater than a third of total input of nitrogen fertility in those ponds. In contrast, El Samra and Olah (1979) reported that the amount of nitrogen fixed in their fertilized temperate fishponds was only 5.7 kg/ha/5 months, an insignificant quantity compared to regular fertilizer input. In natural lakes, relatively high nitrogen fixation rates were reported ranging from about 20 µg/l/day (Horne and Viner 1971) to 130 µg N/l/day (Dugdale and Dugdale 1962).

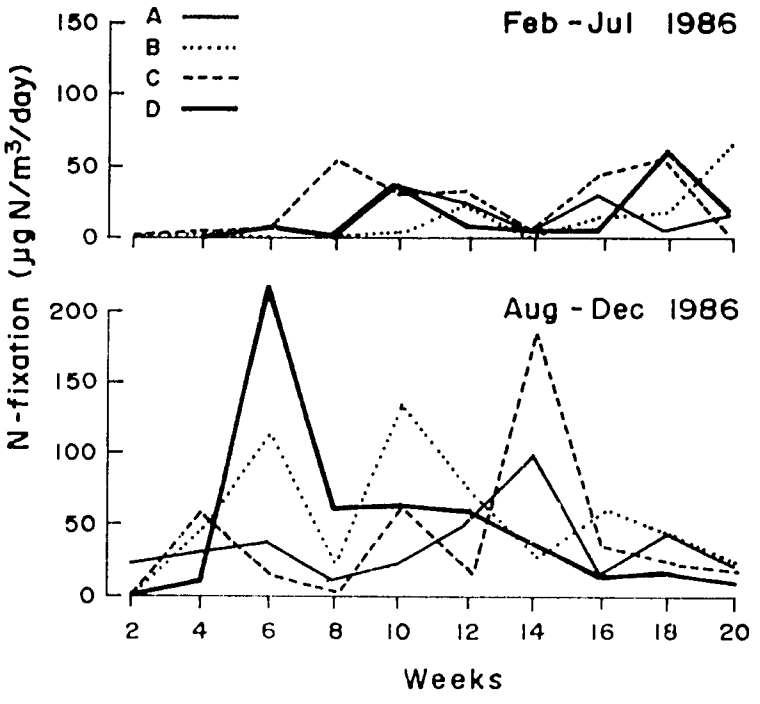

Fig. 1. Comparison of nitrogen fixation among ponds that received chicken manure at 125 (A), 250 (B), 500 (C) and 500 (D) kg/ha/week during the drier (Feb.-Jul.) and wetter (Aug.-Dec.) periods, 1986.

Table 2. Nitrogen fixation rate and number (filaments/ml) of nitrogen-fixing blue-green algae in inorganically (A) and organically (B) fertilized pond water (20 August 1985).

Genera	A-1	A-2	A-3	A-4	B-1	B-2	B-3	B-4
Anabaena	0	14	6	2,640	972	680	246	280
Cylindrospermum	0	80	0	132	200	15,812	6,874	146
Nodularia	0	0	0	52	28	1,228	14	0
Total filaments/ml	0	94	6	2,824	1,200	17,640	7,134	426
N-fixation (µg N/l/day)	45.0	57.4	46.1	147.3	146.0	172.8	170.6	198.1

Nitrogen fixation in relation to blue-green algae

Four blue-green algae that occurred commonly in the pond water were species of *Anabaena, Cylindrospermum, Nodularia* and *Oscillatoria*. The first three possess heterocysts, proven sites for nitrogen fixation. The blue-green algae were considerably more abundant in the chicken manured ponds (1,200-17,880 filaments/ml) than in inorganically fertilized ponds (0-2,624 filaments/ml). Nitrogen fixation rates in the former ponds were markedly greater and more persistent than in the latter (Table 3).

Table 3. Estimated nitrogen input (kg N/ha/5 months) in fish ponds from natural fixation (NF) and chicken manure (CM) fertilizer during dry and rainy cycles.

Pond no.	A-1	A-2	B-1	B-2	C-1	C-2	D-1	D-2
Drier period								
NF	20.0	19.4	24.4	12.2	33.9	31.9	33.6	8.8
CM	75.0	75.0	150.0	150.0	300.0	300.0	600.0	600.0
CM:NF	3.8	3.9	6.2	12.3	8.8	9.4	17.9	68.2
Wetter period								
NF	41.3	55.6	85.7	64.6	31.6	79.0	76.1	59.9
CM	75.0	75.0	150.0	150.0	300.0	300.0	600.0	600.0
CM:NF	1.8	1.3	1.8	2.3	9.5	3.8	7.9	10.0

Nitrogen fixation rate has been reported from various habitats to be correlated with the abundance of heterocystous algae (Stewart 1965; Ogawa and Carr 1969; Horne and Fogg 1970). The fact that relatively low numbers of nitrogen fixing algae occurred in inorganically fertilized ponds probably resulted from the inhibitory effect of higher ammonium concentrations in those ponds.

Effect of ammonia concentration on nitrogen fixation

The nitrogen fixation rate was inversely related to the added ammonium concentrations in pond water (Fig. 2). An increase in the NH_3-N concentration to 1 mg/l reduced the nitrogen fixation to about 64% of the control. It has been shown that the presence of ammonium or nitrate salts is suppressive to heterocyst formation and nitrogen fixation in blue-green algae (Fogg 1974). Nitrogen fixation in lakes generally occurs when the concentration of inorganic nitrogen in lake water is low (Dugdale and Dugdale 1962; Horne and Fogg 1970). Considerably higher ammonia concentrations were recorded in inorganically fertilized ponds than in chicken manured ponds (Fig. 3), which was inversely related to nitrogen fixation rate in those ponds.

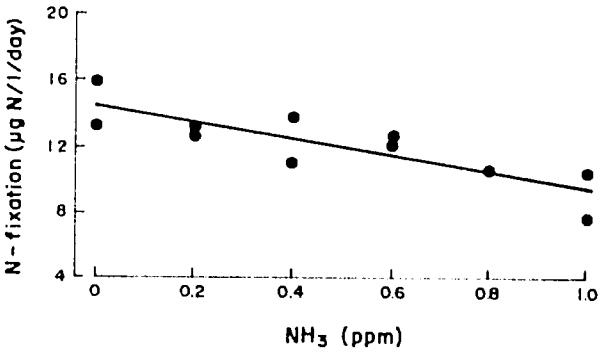

Fig. 2. Effect of ammonium concentration on nitrogen fixation.

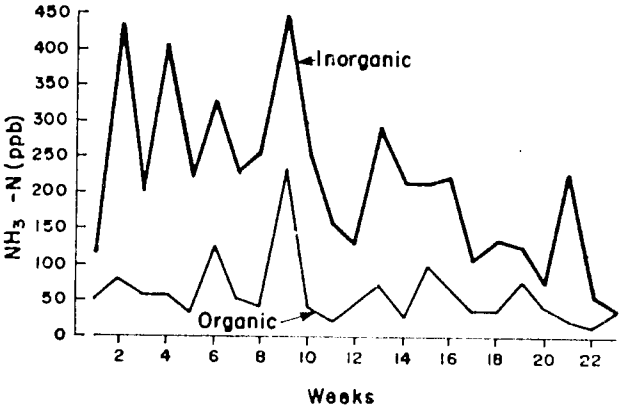

Fig. 3. Comparison of ammonia concentration present in ponds receiving fertilization treatments: chicken manure (org.) and inorganic fertilizers.

Conclusions

A significantly large amount of nitrogen fixation occurred in these fertilized fishponds. However, the nitrogen fixation rate fluctuated over a wide range, with little correlation among various loading rates of chicken manure. Nitrogen fixation occurred mainly during the day, but was suppressed by high ammonium concentrations in pond water. Blue-green algal standing crops play a major role in nitrogen fixation in fishponds. However, the nitrogen input from natural fixation as measured by the indirect acetylene reduction technique is considered as an estimated potential.

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Culture of Nile Tilapia *(Oreochromis niloticus)* in a Rice-Fish Culture System Using Chemical and Commercial Organic Fertilizers

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Abstract

A study was conducted to determine the performance of Nile tilapia *(Oreochromis niloticus)* in rice-fish culture using IR-64 rice and four different fertilizer treatments, i.e., different N-P-K levels derived from inorganic, commercial organic (18-22-28) or combined inorganic plus organic fertilizers: I) inorganic alone (60-40-45 kg/ha); II) 50% inorganic (30-20-22 kg/ha); III) 25% inorganic (15-10-11 kg/ha) plus 75% commercial inorganic (14-16-21 kg/ha); and IV) commercial organic alone (18-22-28 kg/ha). However, there were no statistically significant differences for fish production (95-119 kg/ha) or for daily weight gain (0.34-0.39 g) among treatments. The highest rice production (4,617 kg/ha) was obtained in Treatment I and was significantly different ($P = 0.01$) from that of Treatments III (4,033 kg/ha) and IV (3,900 kg/ha). Treatment II gave 4,517. Water quality was similar in all treatments and within the range favorable for fish culture. The highest net return per crop (P8,794.35/ha) was obtained from Treatment II followed by Treatments I, IV and III.

Introduction

With high-yielding rice varieties, more fertilizers are needed. Chemical fertilizers are important ingredients of modern farming. However, due to economic difficulties in many Asian third-world countries, rice farmers are unable to purchase the required inorganic fertilizers because of their prohibitive price. They can replace some of these with equivalent organic fertilizers. Commercial organic fertilizer

(compost) is relatively cheap and could make a considerable contribution to rice-field fertility and availability of natural food in the water for rice-fish culture. This study compares the use of four different treatments with inorganic and commercial organic fertilizers, alone and combined, in experimental rice-fish culture.

Methods

The experiment was conducted at the Freshwater Aquaculture Center (FAC), Central Luzon State University (CLSU), Muñoz, Nueva Ecija, Philippines. The study was conducted from 15 June to 25 October 1986 and lasted 120 days for rice cropping and 75 days for fish culture. A Randomized Complete Block Design (RCBD) was used with three replications per treatment.

Ammonium phosphate (16-20-0), urea (46-0-0) and muriate of potash (0-0-60) were used to achieve different N-P-K loadings, following Boyd (1979). The required fertilizer mixture was given as a basal application two days before rice transplanting: applying the total amount of muriate of potash and ammonium phosphate and two-thirds of the urea. The remaining amount of urea was utilized for top dressing after one month of rice planting. The total amount of commercial organic fertilizer (18-22-28) was applied as basal application. Treatments are summarized in Table 1.

Management practices prescribed at FAC for rice-fish culture were generally employed. The high-yielding rice variety (IR-64) was planted. Nile tilapia *(Oreochromis niloticus)* fingerlings were used from the FAC stock (predominantly Ghana strain, introduced via Israel). They were stocked at about 8.5 g individual weight at 5,000/ha, 12 days after rice transplanting (Table 1).

The effects of the different treatments on fish growth rate, survival, estimated total production, water quality parameters, rice yields and economic returns were determined. Ten per cent of the fish were sampled per replicate for each treatment. All of the data gathered were tabulated and analyzed statistically using ANOVA in a RCBD (Gomez and Gomez 1984).

Results and Discussion

Table 1 presents data on fish growth, recovery and estimated total production. There were no statistically significant differences between the treatments. The low percentage recoveries (50 to 60%, Table 1) were due to predators such as mudfish *(Channa striata)*.

Table 1. Mean growth rate, recovery and estimated total production of Nile tilapia *(Oreochromis niloticus)* during 75 days rice-fish culture applied with commercial organic fertilizers (CoF), inorganic fertilizers (IF) and combinations of both. The daily weight gains, estimated total production and per cent recovery data are not significantly different between any treatments (P = 0.05).

			Initial		Final				
	Treatment*	Density (per/ha)	Length (mm)	Weight (g)	Length (mm)	Weight (g)	Daily gain (in wt. (g))	Estimated total production (kg/ha)	Per cent recovery
I	IF only (60-40-45)	5,000	78	8.30	129	33.78	0.34	95	56
II	50% IF (30-20-22) + 50% CoF (5-11-14)	5,000	80	8.50	134	36.09	0.37	104	51
III	25% IF (15-10-11) + 75% CoF (14-16-21)	5,000	80	8.41	125	35.60	0.37	108	60
IV	CoF only (18-22-28)	5,000	81	8.59	[illegible]9	37.60	0.39	119	63

*N-P-K loadings are given in brackets in kg/ha.

Water quality for fish is affected by the fertilizer nutrients applied for rice culture because of its direct effect on the fish and its role in plankton production for fish feed. Data on various water quality parameters are presented in Table 2. No significant differences were observed among treatments. The data are all within the range favorable for fish culture.

Table 3 indicates groups of planktonic organisms according to their abundance. Plankton densities were rather low for all treatments; possibly due to low orthophosphate concentrations in the water. According to Round (1984), euglenoids and rotifers are dominant in waters high in organic matter. Organic fertilizers have been found to affect and increase the

Table 2. Means[1] of selected water quality parameters taken from rice-fish culture (*Oreochromis niloticus* stocked at 5,000/ha with IR 64 rice) using commercial organic fertilizer (CoF), inorganic fertilizer (IF) and combinations of both during the period 5 August 1986 to 16 October 1986; for details of treatments in terms of N-P-K loadings, see Table 1.

Treatment	Water quality parameters									
	Temperature (°C)		pH		Dissolved oxygen (mg/l)		Ammonia (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	Ortho-PO_4 (mg/l)
	Min.[2]	Max.[2]	Min.	Max.	Min.	Max.				
I IF only	23.2	27.4	7.2	9.0	3.1	7.1	0.06	0.007	0.005	0.010
II 50% IF + 50% CoF	23.2	27.6	7.6	8.9	2.8	7.3	0.08	0.009	0.006	0.011
III 25% IF + 75% CoF	23.1	27.8	7.7	9.0	2.	7.7	0.08	0.009	0.006	0.012
IV CoF only	23.1	27.5	7.8	9.1	2.6	7.9	0.09	0.010	0.007	0.011

[1] Mean of three replicates.
[2] Minimum and maximum water quality values taken in the morning and afternoon.

Table 3. Mean[1] plankton abundance sampled from 5 August 1986 to 16 October 1986 in rice-fish culture (*Oreochromis niloticus* stocked at 5,000/ha with IR 64 rice) using commercial organic fertilizer (CoF), inorganic fertilizer (IF) and combinations of both: for details of treatments in terms of N-P-K loadings, see Table 1.

Treatment[2]	Phytoplankton (organisms/ml)					
	Chlorophyta	Cyanophyta	Chrysophyta	Euglenophyta	Pyrrophyta	Total
I	190	85	47	888	39	1,249
II	118	148	38	827	51	1,182
III	102	105	60	931	28	1,226
IV	88	56	56	991	86	1,277
Total	498	394	201	3,637	204	

Treatment	Zooplankton (organisms/l)				
	Rotifera	Cladocera	Copepoda	Ostracoda	Total
I	276	10	44	17	347
II	280	40	60	—	380
III	334	50	67	27	478
IV	319	50	89	16	474
Total	1,209	150	260	60	

[1] Mean of three replicates.
[2] There were no statistically significant differences among treatments for any of the data presented (P = 0.05).

abundance of plankton and benthic organisms in ponds (Boyd 1979). In this study, the highest density of plankton was obtained in Treatment IV followed by Treatments III, II and I. However, no statistically significant differences were found. *Euglena* sp, *Phacus* sp. and *Tracheolomonas* sp. were the dominant phytoplankters and *Polyartha* sp., *Brachionus* sp. and *Trichocerca* sp. were the dominant zooplankters.

Table 4 presents the mean rice production. The treatments with the greatest use of inorganic fertilizer gave the best yields: Treatments I and II were highly significantly different from Treatments III and IV (P = 0.01).

Table 5 shows that Treatment I gave the highest total return per hectare followed in descending order by Treatments II, III and Treatment IV. However, Treatment II gave the highest level of net return followed in descending order by Treatments I, III and IV. Moreover, Treatment II had the highest rate of percentage rate of return on total costs followed in order by Treatments I, IV and III.

Table 4. Mean[1] rice (IR-64) production[2] in rice-fish culture (*Oreochromis niloticus* stocked at 5,000 ha) using commercial organic fertilizer (CoF), inorganic fertilizer (IF) and combinations of both: for details of treatments in terms of N-P-K loadings, see Table 1.

Treatment	Extrapolated mean rice production kg/ha[3]/120 days
I IF only	4,617[a]
II 50% IF + 50% CoF	4,517[a]
III 25% IF + 75% CoF	4,033[b]
IV CoF only	3,900[b]

[1] Mean of three replicates.
[2] Rice (IR-64) culture period: 19 June to 17 October 1986.
[3] Data with different suffixed letters are significantly different (P = 0.01); data with the same suffixed letters are not significantly different (P = 0.05)

Table 5. Simple cash costs and return analysis (₱/ha) for rice-fish culture using various fertilizer treatments: for details, see text and Table 1.

Items	Treatments I	II	III	IV
Total return	14,822	14,728	13,446	13,292
Total costs	6,114	5,933	5,842	5,751
Total net return	8,708	8,794	7,604	7,540
Rate of return on total costs, %	142	148	130	131

Note : Based on 1986 prices
US$1 = ₱20.35
Total net return = Total return − Total costs
Percentage rate of return on total costs = $\frac{\text{Total net return} \times 100}{\text{Total costs}}$

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Preliminary Studies on the Performance of *Oreochromis shiranus chilwae* in Ponds with Respect to Water Quality and Temperature

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MSISKA, O.V. 1988. Preliminary studies on the performance of *Oreochromis shiranus chilwae* in ponds with respect to water quality and temperature, p. 63-68. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623 p. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Abstract

Oreochromis shiranus chilwae were stocked in 0.05- to 1-ha ponds at 10,000 to 12,100/ha and fed once daily with madeya (coarse maize bran) six days a week. Fish averaging 15 to 25 g were stocked at three altitudes (Chingale, 450 m; Domasi, 750 m and Satemwa, 1,000 m above sea level). There were poor fish yields at Domasi (193 to 545 kg/ha/180 days) but superior yields at Satemwa (787 to 2,019 kg/ha/180 days). Water quality and nutrient parameters were measured (conductivity, orthophosphate, total nitrogen, total hardness, total alkalinity, pH, sodium, potassium, calcium and magnesium). Site differences in water quality probably exerted a stronger influence on fish yields than temperature within the range of the altitudes studied. The establishment of a successful fish farming system for *O. shiranus chilwae* at an altitude higher than its natural habitat (Lake Chilwa, 620 m) suggests that upland fish culture can succeed in Malaŵi if suitable species or strains are chosen. These results are discussed in relation to future upland aquaculture development in Malaŵi.

Introduction

In Malaŵi, recent population growth has led to increased exploitation of natural fisheries and a reexamination of the potential of aquaculture in rural development. Among the native tilapias suitable for aquaculture, *Oreochromis shiranus chilwae* is particularly important. *O. shiranus chilwae* is fully described by Trewavas (1983, p. 348-356). It consumes a wide range of foods including higher plants, diatoms, crustaceans, aquatic insects and fish remains (Bourn 1974). Maturity is attained at 15.0 cm total length in males and 11.5 cm in females. Fifty per cent of either sex mature after the first year; at 13 to 14 months for females and about 18 months for males (Kirk 1970).

This species was first introduced into ponds at Domasi to conserve the stock after Lake Chilwa, its natural habitat, dried up. Initial experiments were conducted by Morgan (1971) who showed that production of about 709 kg/ha/year was possible using inorganic fertilization. This species has since assumed prominence in ponds and dams in Malawi, but like most tilapias its precocious breeding results in a high proportion of small fish. Recent monosex trials have raised yields (Msiska 1982). Landell Mills Associates (1983) observed an inverse relationship between *O. shiranus chilwae, Tilapia rendalli* and *Clarias gariepinus* pond yields and altitude/temperature between 100 and 1,300 m (Fig. 1). Balarin (1987) used the same data to emphasize that fish production is related to temperature. While the influence of temperature on tilapia growth cannot be ignored, other factors may play a significant role. This study investigated the effect of water quality factors on yields of *O. shiranus chilwae* between 450 and 1,000 m.

Materials and Methods

Oreochromis shiranus chilwae fingerlings (15-25 g), obtained from the government Domasi Experimental Fish Farm, were stocked at 10,000-12,100/ha in ponds at three locations: Chingale, Domasi and Satemwa. Table 1 describes their characteristics. The fish were fed with madeya (coarse maize bran) at 4% fish biomass/day for 6 days a week. This feeding rate was adjusted every 2 weeks after sampling the population. The ponds were also fertilized with 400 kg/ha dry chicken manure every 2 weeks. The culture period was 180 days.

Prior to this study, there were no data on the water chemistry of these ponds except for Domasi (Msiska 1981). The high expense involved did not permit regular analysis to be conducted but samples of pond water were taken from each pond 20 cm below the surface during the first and last weeks of the experiments. The samples were then pooled for each site and analyzed using standard methods:

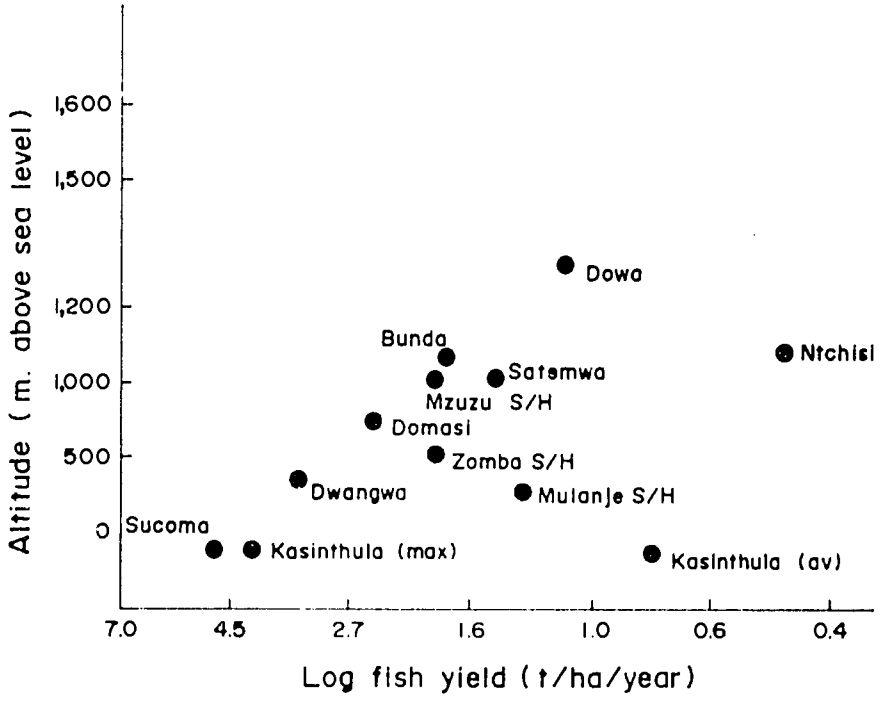

Fig. 1. The relationship between fishpond tilapia yields and altitude in Malawi; redrawn from Landell Mills Associates (1983). S/H = smallholder farms.

Table 1. Characteristics of three locations in Malawi used for comparative yield trials with *Oreochromis shiranus chilwae* in pond culture.

Location	Altitude (m above sea level)	Air temperatures (oC) Min.	Air temperatures (oC) Annual Mean	Air temperatures (oC) Max.	Number/size (ha)/ ages (yr) of pond used
Chingale Fish Farm	450	13.4	24.0	33.5	4/0.6/8
Domasi Experimental Fish Farm	750	12.2	22.4	31.4	6/0.30/7
Thyolo (Satemwa Tea Estate Company)	1,000	11.1	20.9	29.2	6/1.8/6

[1]Calculated from Van der Velden (1980). According to Landell Mills Associates (1983) pond water temperatures equal or slightly exceed air temperatures.

conductivity with a portable conductivity bridge (Evershed's meter); [pH was measured with a portable meter]; total alkalinity and hardness, titrimetrically (Golterman 1971) and total nitrogen and orthophosphate using a Hach DR EL/2 portable kit.

Results and Discussion

Table 2 summarizes the yields and feed conversion ratios obtained at the three sites. The net yields were highest for Chingale, followed by those of Satemwa and Domasi.

Water temperatures at Domasi and Satemwa fall below 22°C [a threshold for acceptable tilapia growth; Landell Mills Associates (1983); Balarin (1987)] during May to August and April to October. At Domasi, such seasonal low temperatures are restricted to a few hours during mornings and probably nights. Chingale is definitely the warmest site. Had temperature been the most important parameter

Table 2. Summary of results of the 180-day pond culture trials with *Oreochromis shiranus chilwae* at three sites in Malawi in 1979-1980. For details of feeding and pond fertilization, see text.

Site	Stocking rate/ha for each pond	Average stocking wt (g)	Average final wt (g)	Net yield (kg/ha)	Food conversion ration (based on added madeya)
	10,100	17.0	167.7	846	1.4
	10,500	30.1	131.3	871	2.7
Chingale	12 000	20.0	115.9	1,278	3.0
	12,000	27.3	104.5	970	3.0
Average	11,150	23.6	124.9	991	2.5
	10,000	20.0	80.0	409	3.4
	10,200	15.0	50.0	194	4.0
Domasi	12,000	25.0	70.0	259	2.5
	12,100	30.0	70.0	319	3.0
	10,000	20.0	90.0	545	4.0
	11,000	25.0	70.0	317	3.0
Average	10,883	22.5	71.7	340	3.3
	11,100	22.3	166.7	2,019	2.8
	11,000	20.0	200.0	1,597	2.6
	10,000	25.0	176.5	1,058	3.0
Satemwa	12,000	20.0	150.4	921	2.0
	12,000	20.0	187.0	1,247	2.5
	11,200	25.0	150.0	788	3.5
Average	11,050	22.0	165.1	1,272	2.7

controlling yields, the decreasing order would have been Chingale > Domasi > Satemwa. Fish yields were instead higher at Satemwa than at either sites (Table 1) and this was further indicated by fish growth data (Fig. 2). Reduced growth was experienced in July and August at Chingale; July to September at Satemwa and not at all at Domasi (although it was low here anyway). Thus up to 3 months in a year are unsuitable for adequate growth. In general, the yields compare favorably with those reported by Lovshin (1982) of 1,648 kg/ha/180 days using a similar feeding regime for *Oreochromis niloticus* in Brazil.

Table 3 summarizes the water quality parameters at the three sites. The yield results suggest that problems with low fish production in upland areas (up to 1,000 m) could be mitigated by raising water quality to levels similar to those of the Satemwa ponds. However, it should be noted that no estimates of natural feed availability were made during this study. The parameters measured included those shown to be main water quality factors affecting tilapia production in warmwater ponds (Boyd 1982). While the concentration of free orthophosphate was considered satisfactory at all sites, total nitrogen was too low at Domasi. It is generally considered that total alkalinity should exceed 20 mg/L for satisfactory utilization of nutrients in ponds. Domasi water was again marginal. Alkalinity was almost twice as high as hardness at Chingale indicating some bicarbonate and carbonate association with sodium, which keeps higher concentrations of anions in solution than association with magnesium (Mandal and Boyd 1980). Thus the high amounts of magnesium at Satemwa may precipitate carbonates at higher pH and thereby maintain a moderating influence on alkalinity.

Although not conclusive, this study does suggest that factors other than altitude/water temperature play a decisive role in fishpond yield in Malaŵi. A detailed study of the influences of water quality and temperature could allow

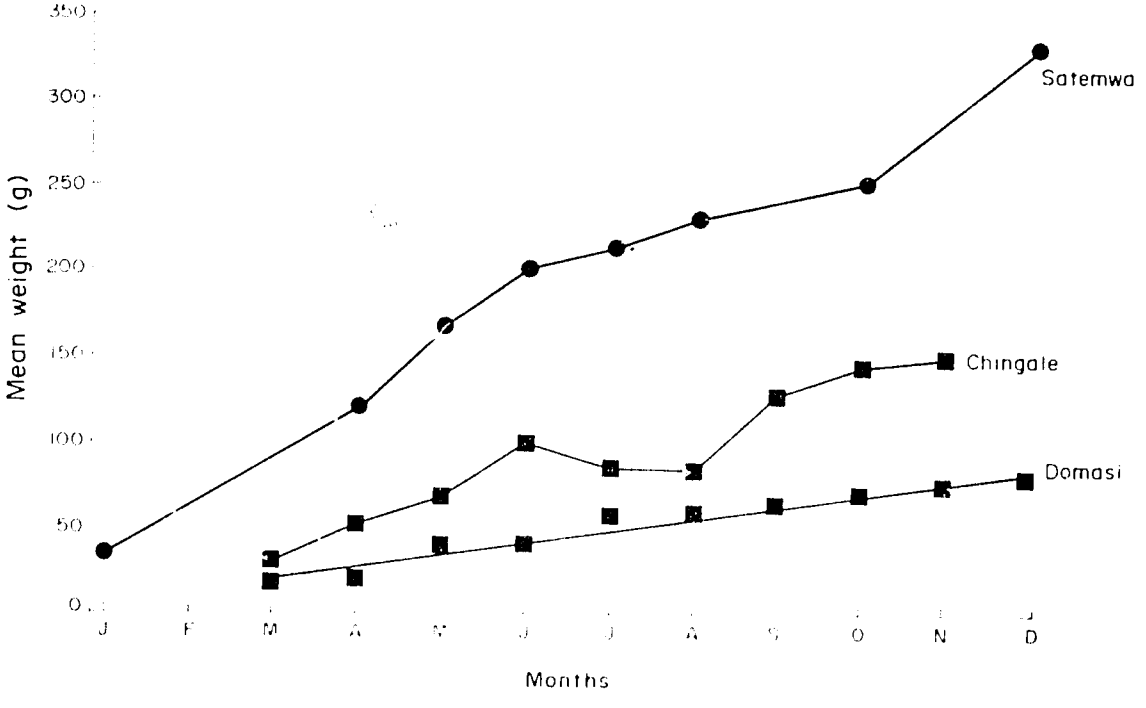

Fig. 2. Growth of *Oreochromis shiranus chilwae* at three sites in Malaŵi.

Table 3. Summary of selected water quality parameters in tilapia *(Oreochromis shiranus chilwae)* ponds at three sites in Malawi. Unless otherwise stated the data from pooled samples taken during the first and last weeks of a 180-day culture period. For details, see text.

Parameter	Chingale	Satemwa	Domasi
pH (measured at 0800)	7.7	7.0	6.5
Filtrable orthophosphate (mg/L)	1.0	0.5	0.8
Total nitrogen (mg/L)	4.5	2.0	1.3
Total hardness (mg/L)	60.0	72.1	29.6
Total alkalinity (mg/L)	107.0	85.0	20.0
Electrical conductivity (μmho/cm)	140.0	180.0	94.6
Sodium (mg/L)	43.5	17.0	52.2
Potassium (mg/L)	6.6	4.4	17.9
Calcium (mg/L)	22.5	6.5	34.3
Magnesium (mg/L)	7.8	66.5	36.6

Pond water temperature (°C)	Chingale	Domasi[1]	Satemwa[2]
Minimum	N/A	19.0	17.0
Maximum	N/A	33.0	29.0
Average	N/A	24.5	22.1

[1]Estimated from Msiska (1984).
[2]Periodic measurements between 0700-0800 and 1400-1600, this study.

correlations with yields to be computed. This would greatly assist choice of locations and suitable fish species and strains for upland aquaculture.

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The Effect of Predation by *Lates niloticus* on Overpopulation and Stunting in Mixed Sex Culture of Tilapia Species in Ponds

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OFORI, J.K. 1988. The effect of predation by *Lates niloticus* on overpopulation and stunting in mixed sex culture of tilapia species in ponds, p. 69-73. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623 p. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Abstract

Pond studies were carried out on the use of the predator, *Lates niloticus*, to control overpopulation and stunting in a mixed sexed culture of three tilapias: *Oreochromis niloticus*, *Sarotherodon galilaeus* and *Tilapia zillii* in ponds.

Four ponds each measuring 0.2 ha were initially stocked with the mixed tilapia fingerlings at 1 fish/m2 and reared for 3 months. After this period, three different batches of 8, 13 and 25 *Lates niloticus* were introduced into three of the ponds (termed B, C, D), whilst the fourth (A) served as a control. The tilapias were cultured together with *Lates niloticus* for an additional 5 months. The ponds were fertilized with chicken manure at a rate of 60 kg/ha/day and the fish fed a mixture of rice or wheat bran at 4% body weight/day for 5 days/week for 4 months.

The average weight of tilapia and production under the different unreplicated treatments in ponds were compared. The percentage composition of large-size tilapia from the different ponds were in the following order (predator: prey ratio in brackets): B (1:250) > D (1:80) > C (1:154) > A (0). In ponds B, C and D, large tilapia attained weights ranging from 160 to 320 g. The weight range for large tilapia in pond A was 90 to 100 g. Pond A gave the highest total production of 190 kg equivalent to 1,427 kg/ha/year. The predation pressure of *Lates* on tilapia, determined in aquaria, hapa and cage systems ranged from 2.0 to 3.2 fish/day. The economic implications of a *Lates*-tilapia polyculture system are discussed.

Introduction

In Africa most tilapia culture is mixed sex culture. Ponds soon become overcrowded and small, stunted fish are produced due to prolific breeding. To control overcrowding and its associated effects, polyculture with predators has been suggested (Wohlfarth and Hulata 1983). Bardach et al. (1972) mentioned

catfish *(Clarias),* large mouth bass *(Micropterus salmoides)* and *Hemichromis* spp. as possible predators to control tilapia recruitment. Guerrero (1982) has also summarized a list of possible predators and predator-prey stocking ratios. Hopkins et al. (1982) have discussed an alternative method to predator-prey ratios in predicting recruitment. Dunseth and Bayne (1978) have reported on the use of the predator, *Cichlasoma managuense,* to control recruitment and production of *Oreochromis aureus.*

Although the technique of culturing tilapia with predators is not new in Africa (Shehadeh 1976), available literature on work with *Lates niloticus* as a predator is limited (Planquette 1974; Huet 1986). In Africa, where the skill of most fish farmers is low and there are financial constraints to employing modern methods to control tilapia breeding, the adeption of a non-genetic method such as the use of predators could be appropriate until farmers can adopt alternative methods. The main problems with the use of predators appear to be the choice of an efficient predator and determining the size and numbers to be stocked in order to obtain high production of large-size tilapia without loss of stocked fish to the predator.

The study describes predation by *Lates niloticus,* a voracious predator, on tilapia in fishponds. The predation pressure of *L. niloticus* on tilapia was also investigated. The economic implications of the results are also discussed.

Materials and Methods

Four 0.02-ha ponds of mean depth of 1.2 m were filled with freshwater from the Volta River and stocked initially with fingerlings of three tilapia species *(Oreochromis niloticus, Sarotherodon galilaeus* and *Tilapia zillii)* between 24 January and 6 February 1986. This initial stocking was with an unsexed mixture of the three species and the relative quantities of the different species were not known. This is common practice in fish culture in Ghana at present. Therefore this account of *L. niloticus* predation on tilapia, in unreplicated ponds, is merely a preliminary attempt to indicate its possible use as a system for adoption by present farmers, given their mixed seed supply and the difficulties in separating species at the fingerling stage. The ponds were fertilized with dry chicken manure (60 kg/ha/day) and the fish fed a mixture of rice or wheat bran at 4% body weight/day for 5 days/week. The stocked fish were cultured for 90 days after which three batches of 8, 13 and 25 *L. niloticus* were introduced, respectively, into three ponds while the fourth served as control. Feeding and manuring was then continued at the same rates for a further 150 days. The experiment was terminated in the second week of October when the ponds were drained, all survivors were sorted and counted and representative samples weighed. Numbers stocked and initial weights are shown in Table 1.

Predation of *L. niloticus* on tilapia fingerlings was observed in glass aquaria (90 x 38 x 30 cm), a cage (90 x 60 x 76 cm) and a net hapa (2.5 x 1.7 x 1 cm). Again a mixed species population of tilapia fingerlings (mainly *O. niloticus* and *S. galilaeus)* was stocked. The fish were categorized as three size groups: small (S), 0.5-5.0 g; medium (M), 5.5-10.0 g and large (L), 15.0-25.0 g. Ten tilapias of each size group were stocked in each container, totalling 30 per container. A single *L. niloticus* (200-240 g; total length 234-300 mm) was added to each container.

The numbers and sizes of prey consumed daily were determined by noting the difference between the initial prey stocked and that remaining every 24 hours. Any dead or consumed fish were then replaced with fish of similar size. The experiment was run for 20-28 days after which the daily predation pressure was determined using the following formula:

$$\text{Daily predation pressure} = \frac{\text{Total no. of prey eaten by one predator} \times 100}{\text{Total no. of prey supplied} \times \text{no. of days}}$$

Table 1. Numbers of fish stocked and mean weights at stocking for a predator-prey polyculture system of tilapias (stocked as a mixture of *Oreochromis niloticus, Sarotherodon galilaeus* and *Tilapia zillii* fingerlings) and *Lates niloticus* in 0.2-ha nonreplicated earthen ponds receiving rice bran and dry chicken manure as inputs. For further details see text.

Pond/ Treatment	Fish species	No. stocked per pond	Predator: prey ratio	Mean weight (g) (± S.D.)
A control	Tilapias only	2,000	-	15.4 ± 0.4
B	Tilapias	2,000	1:250	15.5 ± 0.3
	Lates niloticus	8		350 ± 15
C	Tilapias	2,000	1:154	15.5 ± 0.3
	Lates niloticus	13		350 ± 20
D	Tilapias	2,000	1:80	15.7 ± 0.5
	Lates niloticus	25		350 ± 17

The average predation pressure was then used to estimate the likely prey requirements of *L. niloticus* over the culture period and the economic consequences of this for the farmer.

Results

Details of the fish harvested are presented in Table 2. The control pond (A) gave the highest production: 190.3 kg, equivalent to 1,427 kg/ha/year. The highest production from ponds containing *L. niloticus* came from pond B: 120 kg, equivalent to 900 kg/ha/year. Tilapia production in ponds with *L. niloticus* was also highest in pond B: 106.6 kg. *S. galilaeus* was the most abundant tilapia harvested from each pond: over 50% by number in all ponds except Pond D where it comprised 45%. *Tilapia zillii* was next in relative abundance whilst *O. niloticus* was the least abundant.

Large-size tilapia were relatively more abundant in ponds with predators where they constituted over 40% by number. The control pond (A) produced lesser numbers of bigger fish, only 10%. Small and medium-size tilapia, however, constituted over 50% and 30%, respectively.

The predation pressure results are shown in Table 3. The daily predation pressure ranged from 2.0 to 3.2 fish/day.

Discussion

The differences observed in fish production especially tilapia production from ponds clearly reflect predation by *L. niloticus*. Pond B with the lowest predator-prey ratio of 1:250 had the highest fish production apart from the control whereas Pond D with a predator-prey ratio of 1:80 produced the least. Bigger tilapias were more abundant in ponds with predators. Pond D produced tilapia with the highest average weight of 321 g. At the same stocking rate of the prey species, in ponds with more predators more tilapias were consumed. This reduced competition amongst tilapias for available food and space, enabling the remaining fish to grow bigger.

In a summary of the use of predators for the effective control of tilapia recruitment, Guerrero (1982) cited Planquette (1974) as giving a predator-prey ratio of 1:20 to 1:84 for *L. niloticus* with tilapia. The highest predator-prey ratio used here was 1:80. The low fish production achieved at this ratio suggests that 1:80 is too high and that ratio between 1:150 and 1:250 may be better. However more investigations with replicated treatments and consideration of interaction between different prey species are needed.

Table 2. Harvesting results from predator-prey polyculture of mixed tilapia fingerlings *(Oreochromis niloticus, Sarotherodon galilaeus* and *Tilapia zillii)* with *Lates niloticus* in 0.2-ha earthen ponds after growout of 240 days (tilapias) and 150 days *(L. niloticus).* For further details of stocking and treatments, see Table 1 and text. For the tilapia harvest details - L = large (> 150 g); M = medium (50-100 g) and S = small (< 50 g).

Pond/treatment	Size	No./biomass (kg) of fish harvested	% size composition	Remarks
A (tilapias only)	L	1,205/77.5	10.2	Predominantly *S. galilaeus.* Average large-size fish was less than 100 g. Total biomass represented a total production of 1,427.1 kg/ha/year.
	M	4,577/69.6	38.9	
	S	6,007/43.2	50.9	
	Total	11,782/190.3		
B Tilapias	L	499/90.6	67.4	Predominantly *S. galilaeus.* Largest species *T. zillii* and *O. niloticus.* Total production 899.6 kg/ha/year. No. of large *L. niloticus* harvested. 6 - increased numbers due to reproduction in pond.
	M	235/15.8	31.7	
	S	6/0.3	0.8	
L. niloticus		53/13.4		
	Total	740/120.1		
C Tilapias	L	406/69.3	47.7	Predominantly *S. galilaeus.* Largest species *O. niloticus.* Total production is 886.3 kg/ha/year. Number of big *Lates* harvested = 12. Other due to reproduction
	M	323/27.1	38.0	
	S	122/3.3	14.3	
L. niloticus		55/18.6		
	Total	906/118.3		
D Tilapias	L	152/43.6	62.7	Predominantly *S. galilaeus.* Numbers of big *Lates* harvested are 17 and 10 medium fish. Increment due to reproduction. Total production is 538 kg/ha/year.
	M	77/1.6	31.8	
	S	13/0.1	5.4	
L. niloticus		38/26.7		
	Total	280/71.9		

Table 3. Daily predation pressure of *Lates niloticus* on tilapia fingerlings (mixed population, mainly *Oreochromis niloticus* and *Sarotherodon galilaeus)* stocked in different containers at 30 tilapias per container (10 large (L), 15.0-25.0 g; 10 medium (M), 5.5-10.0 g and 10 small (S), 0.5-5.0 g; with one *L. niloticus* (200-240 g). For details of tilapia prey replenishment, see text.

Container		Total no. of tilapia supplied during the trial	Duration	Total no. of tilapia consumed	Size composition of tilapia consumed (% of total)	Daily predation pressure (No. of fish consumed x 100/ no. of supplied x no. of days)
Aquaria	a	151	25	81	S 85.2 M 14.8	2.3
	b	81	20	52	S 64 M 36	3.2
Hapa		108	24	52	S 86 M 14	2.0
Cage		138	28	80	S 65.5 M 34.5	2.1

The predation pressure experiment pointed to heavy predation of small tilapia by *L. niloticus.* Only after a reduction in the number of small fish were the medium-size tilapia taken. In the Volta lake, Vanderpuye and Ocancey (1972) reported that sub-adults and juveniles of *Lates niloticus* feed mainly on the small clupeid *Pellonula afzeluizii* and tilapia. Werner (1972) reported that for all species, bigger individuals normally take bigger prey. These observations suggest close management of *L. niloticus*/tilapia cultures lest the predator should consume so many of the unwanted recruits that it then turns to eat the stocked fish.

This might explain the relatively lower numbers of stocked tilapia survivors in ponds with *Lates*. In the predation pressure experiment, tilapia beyond a total length of 118 mm and 27 g weight were not consumed by the *L. niloticus* used here (234-300 mm total length). They could probably be taken by larger fish.

The daily predation pressure (2.0-3.2/day) was low compared to the observation of ten prey species in individual stomachs of *L. niloticus* of length 294 mm TL from the Volta Lake (Vanderpuye and Ocancey 1972).

Based on results from these one-test trials and the current market prices (per kg) of ¢300 for *Lates* [¢229 (cedi) = US$1]; ¢200 and ¢100 for large and small tilapia, respectively, a *L. niloticus*/tilapia predator-prey polyculture has cultural and economic implications for the fish farmer in Ghana. The fish farmer not only produces a higher yield of large tilapia, but also generates a higher gross income. For example, computed gross income of the production from the different treatments show that income from treatment B with a predator-prey stocking ratio of 1:250 was 3.5% higher than the control. The great appetite of *Lates* for tilapias has to be appreciated lest overstocking the predator would result in a drastic reduction of harvest and income. A loss in income of 26% was observed in treatment D compared to the control. For this culture system to be adopted as a viable alternative for rural aquaculture in Ghana, further work is needed especially on the effect of interaction between the different tilapia species under culture and on economic evaluation.

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Intensive Tilapia Farming in Brackishwater from an Israeli Desert Aquifer

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Abstract

Israeli is situated within the semi-arid geographical belt, in which droughts are predictable and freshwater is in constant over-demand. This has forced the direction of development of fish culture into the Rift Valley (Arava) of Israel, where there are vast areas of non-agrarian land and brackishwater aquifers. A farm was established there, incorporating several small ponds, lined with PVC and with modified aeration and programmed water flow. The fish chosen for comparative trials were F1 hybrids of *Oreochromis niloticus* x *O. aureus;* red hybrids of a Taiwanese line *(O. mossambicus/O. niloticus);* red tilapia of a Singapore line *(O. mossambicus)* and red hybrids of a Philippine line (possibly *O. mossambicus/O. niloticus).* These were compared for growth, fecundity and inheritance of red color by their progeny. The different lines investigated showed very different growth and fecundity responses, the lowest being the Taiwanese red and the highest the Philippine red. At densities of 30 fish/m^2, the yearly production was 80-120 t/ha, with food conversion ratios of 1.8-2.1 and daily weight increases of 1.3-3.2 g. The red color inheritance was very different in the different tilapia lines.

Introduction

The importance of aquaculture for the supply of animal protein to humans is constantly increasing, being particularly valuable in regions in which other types of husbandry are expensive or impossible; for example, arid and semi-arid zones, where conventional agriculture is strongly limited by habitat and climate, especially rainfall. The idea of desert fish farming (DFF) was formulated in 1963-65 and tested experimentally (Fishelson and Loya 1969). This showed that desert saltwater can be successfully used for fish culture. Moreover, the high mineral content of these waters, the high temperatures and solar radiation enable very high primary productivity, forming a food base for the fish. There has been commercial production of fish in ponds close to the Dead Sea.

Some tilapias are euryhaline and eurythermal (Lotan 1960; Hepher et al. 1983). They can withstand situations of very low oxygen availability (Becker and Fishelson 1986) and are therefore excellent subjects for DFF.

The present study shows the results obtained from DFF in the Ein Yahav farm located in the Arava Valley in Israel, the most extreme Israeli desert region with almost no rainfall, extremely high temperatures during the summer and mild temperatures in winter (Fig. 1). The Ein Yahav settlement is situated over a very large aquifer that extends 1,200-1,500 m below the surface. The water is brackish. It has a temperature of almost 40°C, and contains a variety of minerals (Table 1). Its use for agriculture is very limited and it was therefore decided to test the possibility of warm water aquaculture. Climatically, this region has almost 365 days of sunshine and this, with the high mineral content of the water, provides a very productive aquatic habitat. Because of the high cost of labor and electricity, the fish farm was designed to yield cash crops, worth as much as the other agricultural products of the settlement. Because of this, we did not use the conventional Israeli large earth ponds, that on average produce 3,500/kg fish/ha/year and use 10,000-40,000 m^3 water per tonne of fish. We decided instead to build small high production units as used in Taiwan, but modified for our special needs. For the same reason we abandoned the idea of conventional polyculture and decided to focus on tilapia monoculture.

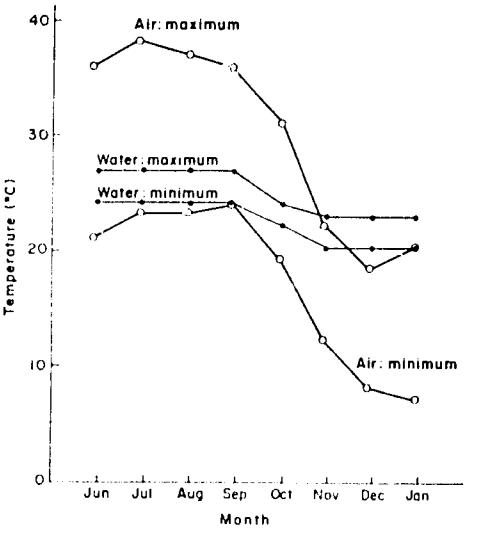

Fig. 1. Monthly average maximum and minimum air and water temperatures at the Ein Yahav fish farm from June 1985 to January 1986.

Table 1. Mineral content of desert water used in the Ein Yahav fishponds.

Ion	$SO_4^{=}$	HCO_3^{-}	Cl^-	Mg^{++}	Ca^{++}	Na^+ and K^+	TDS*
mg/l	382	255	514	74	125	283	1,683

*Total dissolved solids

The major goal of this study was to choose a fish line that would give good growth and food conversion, uniform red coloration, high fingerling production and good adaptability to intensive monoculture in small, PVC-lined ponds.

Materials and Methods

Pond and water management

The pond system established included three types of ponds lined with PVC (Fig. 2). A: Four ponds of 25 x 10 m and 1 m deep for reproduction, fingerling acclimation and early growth. B: Four ponds of 22.5 x 22.5 m and 1 m deep for growout to market size. C: Two ponds of 10 x 10 m and 1 m deep for storage before marketing and for growth experiments.

irrigation with the eutrophic pond water was an important side benefit in this project, especially for salt-resistant crops, i.e., date palms.

For experimental growth and observation of young fish, a greenhouse of igloo type was erected near the ponds and PVC containers provided with water and aeration were placed inside. In these containers the fry were treated with testosterone to produce an all-male population (Rothbard et al. 1983).

For aeration of ponds, propeller aspirator pumps (PAPA) were used (Boyd and Martison 1984). In the A and B ponds, two PAPA of 1 hp each were installed. One 0.5-hp PAPA was sufficient for the C ponds. The PAPA ensured homogenous mixing of the water (without any stratification) and a strong circular flow of water around the walls that produced a central

Fig. 2. The general area of the Ein Yahav ponds.

In the Type B ponds, the bottom was constructed gently sloping towards the center, where a 20.5-cm outlet pipe was inserted. Through this pipe the water from the pond could be directed into a settling pond and then redirected for irrigation of the nearby fields. In view of the very poor organic content of the desert fields around,

vortex. This enhanced settlement and concentration of organic matter on the center of the pond bottom. Once or twice a day this organic matter could easily be extracted from the pond via the outflow pipe. In this way 1-10% of the pond water was changed daily. This removal of wastes enabled a very high stocking density.

The oxygen content in the pond water (Table 2) was correlated with fish biomass and ambient temperature. During the winter, when temperatures were low (Fig. 1), we used more hot water from the aquifer, attempting to maintain the pond temperature at a constant 26°C. This water change frequently caused a drop in phytoplankton, thus lowering the food biomass in the pond. During summer, as the air temperature was very high, the hot aquifer was used less or not at all. At such times the water was changed less frequently, consequently creating a build-up of the organic load and a decrease in oxygen content (Table 2). This also had a detrimental effect on fish growth. The transparency of the water was measured by a 12-cm Secchi disc. The optimal depth for organic biomass and oxygen content was judged to be 35 cm.

The main production data were obtained from a period of 160-220 days of growth, at which time selective harvesting of 350-500 g fish was started.

Fish and husbandry methods

Four different tilapias were used: 1. Taiwanese red tilapia - a line selected from an F1 hybrid group of *Oreochromis mossambicus* and *O. niloticus* (Liao and Chang 1983). 2. Philippine red tilapia - possibly of the same origin (Gabman and Avtalion 1983). 3. Singapore red tilapia - a line presumably derived from *O. mossambicus* and isolated in Singapore. 4. F1 hybrids of the normal *O. niloticus* female x *O. aureus* male cross. These fish, produced in Israel, served for comparison of growth with the red tilapia lines.

The fish were fed three times daily with pellets of various dimensions, containing 28% protein. The amount fed in the ponds was calculated according to 5-8% of the body weight for fish up to 50 g, 4% body weight for fish of 50-200 g and 2% body weight for fish of 250-500 g. All commercial growout was at a density of 30 fish/m^2.

For experiments on reproduction and survival, groups of males and females of the various lines were kept separately in aquaria of 200-350 l, well fed and at a temperature of 26-27°C. After spawning the fry were isolated from the adults and their survival and growth were studied in aquaria, and at a later stage in the ponds of Ein Yahav. The offspring of these fish served for commercial growth and marketing.

Fingerling production

At the beginning, the fingerlings were produced in aquaria at Tel Aviv University and later transferred to the desert ponds. Later all production was concentrated in one of the small ponds as well as in larger containers within the igloo-type greenhouse situated near the ponds. By taking eggs or larvae from brooding females 4-5 days after commencement of incubation, the females were liberated for repeat spawnings. The separated eggs and larvae were raised for 8-10 days in Zuger jars with permanent water flow and aeration. Although keeping the adult fish of all lines in temperatures optimal for reproduction (26-28°C) enabled the production of fry all year-round, the highest production was observed from

Table 2. Dissolved oxygen concentrations in the Ein Yahav ponds. The values given in ppm are means of daily measures ± standard deviation.

Time	Spring-summer		Winter	
	ppm.	%sat.	ppm.	%sat.
morning (0600)	5 ± 1.5	60- 85	2 ± 0.5	35- 50
noon (1200)	10 ± 2.0	100-120	8 ± 2.0	60-100

April to August. This seems to be due to the increase in day length.

The normal F1 hybrids of *O. niloticus* x *O. aureus* were produced by introducing adult fishes of both species into a separate 250-m^2 pond. From this pond, the fry were transferred to containers in a greenhouse, where they underwent testosterone treatment in order to produce 100% males (Rothbard et al. 1983).

Growth

Groups of fingerlings, both morph-siblings and from various lines were introduced into common commercial ponds, thus enabling the comparison of their growth performance under identical conditions. These fish were checked once a month for a total of over 6 months.

Results and Discussion

From adult fish of the Philippine line, fingerlings of four color morphs were produced. Grown together, the surviving fish of such siblings showed a more or less equal growth performance (Fig. 3), the white morph showing the highest deviations from the averages.

Fig. 4 summarizes the comparative growth of the four morphs of the Philippine red line, males and females separately. As shown, the best performance was by the red males, that in 160 days attained a body weight of over 200 g. The growth of males of the pigmented and white morphs, as well as the females of all morphs, was much lower. The lowest growth was shown by the males and females of the blotched morph.

Fig. 5 presents the commercial growth in a common pond of a mixture of Philippine red, Singapore red and F1 hybrids of

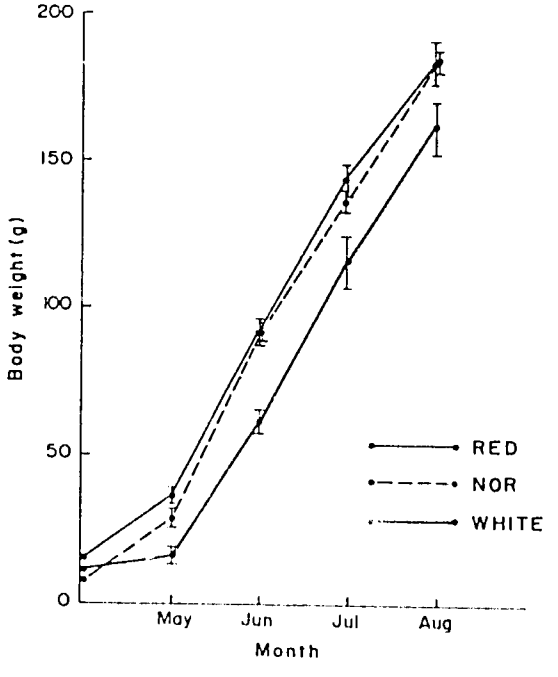

Fig. 3. Growth of pigmented (NOR), red (RED) and white tilapia siblings in a common pond (n = 465); bars represent standard error.

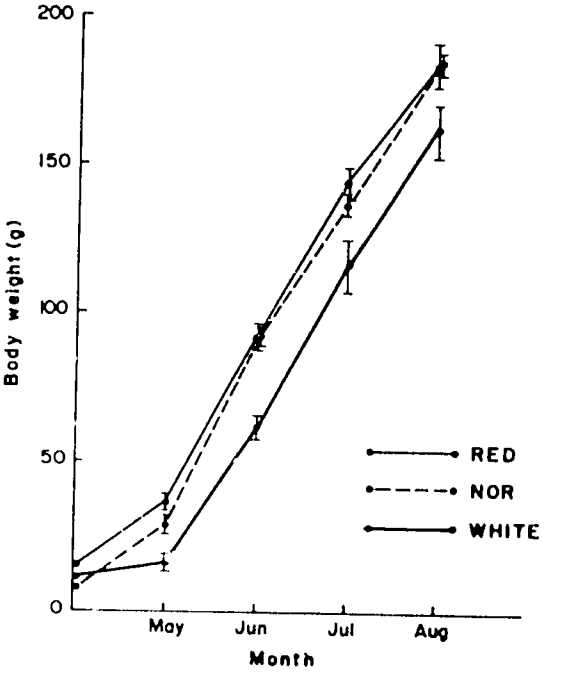

Fig. 4. Growth performance of males and females as well as blotched sibling tilapias compared to normal hybrids. NOR - F1 of hybrids of *O. niloticus* x *O. aureus*; BL - blotched fish.

O. aureus x *O. niloticus* at densities of 30 fish/m^2. Here again the excellent performance of the Philippine red morph was demonstrated. These fish attained 240 g in 160 days, with an SGR of 3.22%. The closest to these were the F1 hybrids of *niloticus* x *aureus* with SGR of 2.56%. The *O. mossambicus* Singapore red remained behind with SGR of 1.43%. This clearly points to the advantage of the Philippine red morph over other fish in the pond.

It was calculated that these ponds produced 65 to 100 t/ha/year. If stocking was with fish of 50 g rather than of 10-14 g, the yield would range between 90 and 140 t/ha. From these ponds the fish were marketed at an average weight of 350-500 g per fish.

The fingerlings produced by the Singapore red *O. mossambicus* had almost 100% survival and the developing fish were 100% monochromic red. Six thousand of these were transported to the Ein Yahav farm for growth experiments and, under all conditions, fell behind the growth rates of other lines (Fig. 6). Following this, further commercial studies on this line have been abandoned.

The Taiwanese red hybrids were brought for spawning in aquaria at various times and the fertility rate of their spawnings varied from 14% to a maximum of 72%. In all progeny the development of four color morphs was observed: 1) black; 2) dark blotched; 3) red; and 4) white. In the pigmented fish the peritoneum was found to be black, whereas in the latter two it was white. In all progeny, the percentage of each of the morphs varied:

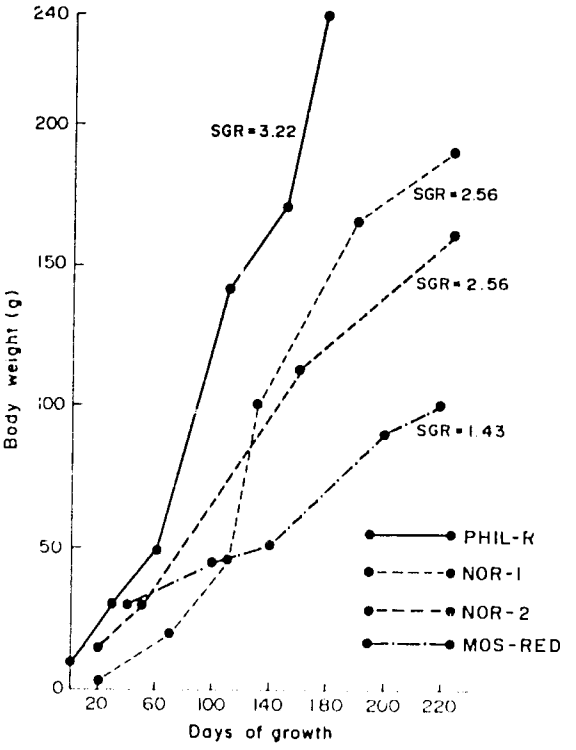

Fig. 5. Comparison of average growth rate of various lines of tilapia in a 220-day period; PHIL-red - Philippine red; NOR-1 and NOR-2 - F1 hybrid of *O. niloticus* x *O. aureus;* MOS-RED - *O. mossambicus* red; SGR - specific growth rate in % per day.

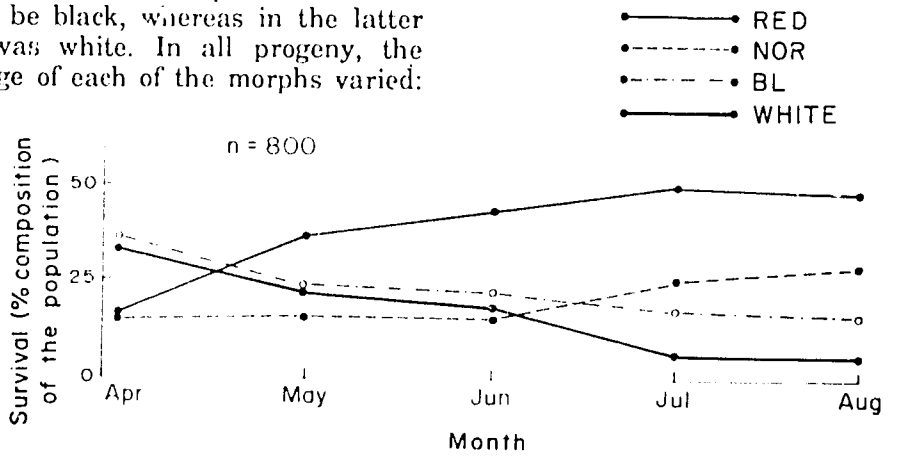

Fig. 6. The survival rate of sibling Philippine tilapias; NOR - pigmented; BL - blotched (as percentage of total).

on average 37% black and blotched, 52% red and 11% white.

Crossing of Taiwanese and Singapore red tilapias produced hybrid progeny that were 50% normal *O. mossambicus* color and 50% red. The Philippine red tilapias also produced progeny with grey and red morphs. Observations showed that red fry were vigorously preyed upon by their parents and grey siblings, possibly due to their conspicuous coloration. Introduction of bundles of twigs serving as refuges for the red juveniles greatly increased their survival rate, enabling the accumulation of a large stock for commercial growth.

Although these various morphs did not differ strongly in growth performance, they did differ in survival (Fig. 5). Therefore, for production of red fingerlings for commercial ponds the production of fry must be 40-50% higher than standard crosses.

Adaptability to intensive culture

Provided sources of brackishwater are at hand, DFF of various tilapia lines can be a successful enterprise, able to provide food and income for the producers.

The high primary productivity of desert fishponds and the long growth season enable high yields of tilapias. The red tilapia morphs are particularly desirable and suitable for cash crop production in Israel.

As the high summer temperatures increase primary production and warm up the ponds, there is an increase in organic load and more frequent water replacement is advised. This of course decreases the biomass of algae available to the fish, and brings a decline in growth and an increase in food conversion ratio. Therefore, for each pond and farm a model for management should be selected that will optimize temperature, algal biomass matched to fish biomass and oxygen concentration. The high price of water in Israel demands its re-use in irrigation. Therefore, the replacement fishpond water should be integrated with the general use of water resources.

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Energy Budgets for Cultured Tilapias

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Abstract

The culture of fishes depends upon manipulation and management of a series of energy-dependent events. In this paper, the major components of a typical energy budget for tilapias have been compiled from a wide-ranging survey of the experimental literature and using other original unpublished data. The budget has been structured into an interactive microcomputer model, allowing full investigation and manipulation of important variables in intensive culture of tilapias. This approach has proved useful in quantifying the effects of different approaches to husbandry and management. The ability of culturists to manipulate and control various aspects of the budget is considered in an effort to improve our understanding of, and output from, the overall intensive culture process. The feasibility of extending this approach to more extensive culture is discussed.

Introduction

The concepts of energy partitioning in animal production systems are well established (Petrusewicz and MacFadyen 1970). There are various ways of considering energy use, either in simple terms (energy in feeds, energy of swimming) or in gross terms where all inputs to and outputs from the system are considered as energy terms. An alternative to this is the concept of an energy budget in which all aspects of the biology and physiology of the animal are integrated into a model describing inputs and outputs from the animal. The simplest way of expressing this model is in the form:

$$C = P + R + F + U \qquad \ldots 1)$$

where C = consumption, P = production, R = respiration, F = feces and U = nonfecal losses. This budget equation can be expressed in terms of energy per unit time (hours, days or years) or as a percentage of the total energy consumed, each type having its uses. Since a large number of biotic and abiotic factors affect most aspects of the physiology of animals, the

production of a comprehensive energy budget-based model depends on being able to quantify the effects of these factors on each of the budget variables. Much of this information can be derived from laboratory studies on respiration, nutrition, etc., while other factors must be evaluated in the field, or in the case of aquaculture, in commercial-scale production systems. Laboratory-based studies are necessary in order to give reliable data and these can contribute directly to both short and long-term budgets. Longer-term field studies are, however, necessary in considering energetic strategy particularly in the natural environment, e.g., in ponds. In this context it is inadvisable to consider the various aspects in isolation and consequently a good appreciation of the natural history of the species is essential. Short-term, usually daily, budgets can be used to evaluate the immediate effects of diet, temperature, stress, etc. whereas cumulative long-term budgets, usually annual, are of value in considering strategy in the natural environment and monthly/yearly production planning and management of both farms and fisheries.

An excellent review of fish energetics is provided by Brett and Groves (1979) who give a number of examples of more or less complete energy budgets for a range of fish species. These authors have compared general energy budgets for fishes with different life styles and it is interesting to note the difference between broadly carnivorous species (Equation 2) and broadly herbivorous species (Equation 3). In the latter case the lower protein diet, containing a large proportion of non-metabolizable material, results in a lower fraction of the ingested energy being utilized for growth, and a high energy loss in the feces.

$$100C = 29P + 44R + 20F = 7U \quad \ldots 2)$$

$$100C = 20P + 37R + 41F + 2U \quad \ldots 3)$$

Few authors have quantified tilapia energetics in a complete form. Mironova (1976) estimated C, P and R in *Oreochromis mossambicus* and showed that assimilation efficiency increased with fish age and water temperature in fish fed to satiation. When fish were fed a restricted diet, an increase in temperature resulted in a decrease in production due to an increase in R. Caulton (1982) working with *Tilapia rendalli* derived an energy budget, based on much of his previous work, and suggested that the overall balance was very sensitive to environmental temperature. Musisi (1984) reported by Brafield (1985) constructed a series of full energy budgets over 30-day periods for *O. mossambicus*. All budget variables were measured simultaneously, and fish were fed a variety of natural and artificial diets at a rate of 12% body weight per day, in three meals. Generally, values of F and U were similar to those shown in equation (2), but values of R were higher and values of P were lower than equation (2). Further studies have been conducted on carbon budgets in *Oreochromis aureus* by McDonald (1985) and an energy budget for a lake population of *O. mossambicus* (Hofer and Schiemer 1983), both studies involving algae as the sole food source. It is clear in reviewing these limited studies that much remains to be done and that a well-designed model of the energetic budget would be a useful tool in evaluating current knowledge and in pinpointing areas of weakness.

In the last few years various more or less useful models have begun to appear for application to aquaculture systems, and very recently models incorporating energetics have been published for some species. Much is known of the physiology and energetics of tilapias, but the data are often incomplete and disparate. However, in view of the significance of tilapias in world aquaculture it is timely to attempt to construct an energy model for both research and management evaluation.

Derivation of the Tilapia Model

For convenience, all equations are written in BASIC notation for straightforward implementation on microcomputers.

Energy input; consumption (C)

The energy content of the diet is usually measured by calorimetry and if such data are available this is the best way to calculate the input to the fish. An alternative technique is to calculate the energy value of the diet from a proximate analysis and using the generally accepted conversion factors for protein, lipid and carbohydrate of 23.6, 39.5 and 17.2 kJ/g, respectively. In view of the fact that calorimetric determinations are the exception, this model allows both routes of input. It should be noted, however, that proximate analysis of the food is still required in order to calculate some of the terms of R (below). The daily food intake (RW; g) is calculated from the ration level (RL; %) and the body weight of the fish (WT; g).

$$RW = WT/100 * RL \quad \ldots 4)$$

The daily protein energy intake (PE; kJ/d) can then be calculated from the protein content of the diet (PC; %) using the expression:

$$PE = RW/100 * PC * 23.6 \quad \ldots 5)$$

The energy intake from lipid (LE; kJ/d) carbohydrate (CE; kJ/d) and fiber (FE; kJ/d) can be similarly calculated from the lipid, carbohydrate and fiber content of the diet (LC, CC, FC; %) and using the appropriate conversion factors for these materials.

The total energy consumed (C; kJ/d) is then given by the sum of these individual inputs:

$$C = PE + LE + CE + FE \quad \ldots 6)$$

Energy output; respiratory energy losses (R)

The most usual way to measure energy losses (heat losses) due to metabolism is by measurement of oxygen consumption and subsequent conversion of the daily oxygen consumed to energy using the oxycalorific coefficient (Qox). The value for Qox varies according to the respiratory substrate (protein = 13.36 J/mg o2; lipid = 13.72 J/mg o2; carbohydrate = 14.77 J/mg o2) and so a weighted Qox should be calculated, depending on the substrate being respired (Brafield 1985). This may not always be in the proportions expected from dietary analysis, due to variations in the digestibility of the source materials used and a more applicable weighted Qox will result if these individual digestibilities are known. The coefficient derived for this model is based on dietary analysis and incorporates digestibilities of the individual components where available. First, the total energy assimilated (A; kJ/d) is calculated using the expression:

$$A = (PE + LE + CE) * DIG \quad \ldots 7)$$

DIG (a fraction) represents the overall digestibility of the diet, but if the digestibility of the individual components is known then both equations (7) and (8) can be rewritten to more accurately allow for these. Finally, the corrected Qox is given by:

$$Qox = ((PE * DIG)/A * 13.36) + ((LE * DIG)/A * 13.72) + ((CE * DIG)/A * 14.77) \quad \ldots 8)$$

The resting respiratory rate, corrected for body weight (WT) and temperature (T), can be calculated from a multiple regression recalculated from the data of Ross and Ross (1983) and can be further corrected for salinity using a factor (FR) derived from the work of Farmer and Beamish (1969). The daily energy loss due to resting metabolism (RS; kJ/d) is then given by:

$$RS = (10^{(-0.64 - (\log WT * 0.397) + (\log T * 2.49))}) * (WT/1000) * FR * 24 * (Qox/1000) \quad \ldots 9)$$

The respiratory rate during routine motor activity is difficult to define due to the range of possible activity levels. It is known that during general foraging

activity and social interactions tilapias will swim at a specific swimming speed which will be lower than their maximum sustainable swimming speed. From the data of Farmer and Beamish (1969) a scale-up factor (RRF) of limited accuracy was calculated based on absolute swimming velocity (VEL; cm/s) and body weight (WT). The total length (TL; cm) of *O. niloticus* of known body weight can be calculated by:

$$TL = 10\,(0.493 + (0.365 * \log WT)) \quad \ldots 10)$$

By specifying a specific swimming speed (BLS; body lengths/s) the absolute velocity can then be calculated using the expression:

$$VEL = TL * BLS \quad \ldots 11)$$

The scale-up factor is then given by:

$$RRF = 0.877 + (0.0233 * VEL) + (0.000434 * WT) \quad \ldots 12)$$

Using this approximate correction factor, the additional energy loss due to routine activity (RR; kJ/d) can thus be calculated, allowing for the photoperiod (PH; h) and the fraction of this time spent in routine swimming (RT) using the expression:

$$RR = ((RS/24) * RRF) - (RS/24) * (PH * RT) \quad \ldots 13)$$

For a limited period each day tilapias indulge in even higher levels of activity, probably also involving periods of burst swimming activity. This is, again, difficult to quantify but regression equations of the maximum sustainable aerobic swimming velocity (VMAX; cm/s) for *O. mossambicus* of a given size have been derived at 26 and 31°C using the data of Ross, Ross and Dunn (unpublished).

$$VMAX = (10\ (0.934\text{-}0.0273TL)) * TL \quad (@\ 26^{o}C) \quad \ldots 14)$$

$$VMAX = (10\ (1.04\text{-}0.0312TL)) * TL \quad (@\ 31^{o}C) \quad \ldots 15)$$

The regressions of Farmer and Beamish (1969) can then be used to estimate active respiration based on body weight and salinity. By selecting the appropriate regression constants (a & b), based on VMAX, the energy loss incurred during the period of intense motor activity (RA; kJ/d) adjusted for the fraction of the time active (AT) can be calculated, and in this model it is scaled up by an arbitrary factor of two to allow for periods of anaerobic burst swimming and subsequent recovery. RA is then given by:

$$RA = (((10\ (a + b \log WT)) * (Qox/1000)) - (RS/24)) * (PH * AT) * 2 \quad \ldots 16)$$

Metabolic energy losses due to specific dynamic action (SDA) are affected by a number of factors, but principally ration size and protein content of the diet. Based on data for *O. spilurus* (Spencer and Ross, unpublished data) the SDA coefficient appears to vary from about 3% to 14% of the total ingested energy. SDA increases in magnitude with increasing food intake, and the duration of the effect increases with the dietary protein level. Thus, an estimate of energy losses due to SDA (RF; kJ/d) based on ration size and protein level can be made, and the total daily energy losses of metabolism are then given by the equation:

$$R = RS + RR + RA + RF \quad \ldots 17)$$

Energy output; fecal energy losses (F)

Ideally, fecal energy loss should be estimated by collecting feces and then using bomb calorimetry on the samples, and if such data are available they should be used. As the energy assimilated (A) = P + R + U, then if A is known (equation 7), fecal energy (F; kJ/d) can be derived from the equation:

$$F = C - A \quad \ldots 18)$$

This model was designed to allow both for direct and indirect estimation of F.

Energy output; nonfecal energy losses (U)

The principal nitrogenous excretory end product in fish is ammonia, although small but significant amounts of a range of other compounds (urea, uric acid, amino acids) may be produced. Ammonia production is small and difficult to measure in experimental systems but Brafield (1985) suggests that 2.7J are lost in ammonia per milligram of oxygen consumed in respiring protein. Brett and Groves (1979) suggest that U is only 7% of C for a generalized carnivorous fish (equation 2).

In this model U can be input directly or can be calculated from the amount of protein metabolized, using the equation:

$$U = ((PE * DIG/A) * (R/13.36) * 2.7)/(C * 100) \quad \ldots 19)$$

Net energy gain; production energy (P)

The principal aim of the model, as currently implemented, is to examine the effects of varying parameters on the value of P. In this model P is finally calculated by difference, and is given by the equation:

$$P = C - (R + F + U) \quad \ldots 20)$$

Discussion and Summary

The main starting point in any energetic model is evaluation of the energy input, C. Clearly, if the diet is well defined and controlled, and its energy content has been measured, then this value should be used. It should be noted, however, that the combusted material is completely dry and an allowance for moisture content in the ingested food must therefore be made. In practice, calculating the energy content of the diet relies on a complete proximate analysis and not a compilation of literature values. It is particularly important to know the fiber content of the diet as this may be high in practical diets and it is known that although fiber energy is not utilized by the fish it does contribute to total dietary energy, if measured by combustion. Henken et al. (1986) point out that the calculated energy content of feeds is consistently lower than that derived by combustion. Using analyses from a number of diets of known energy content it can be shown that our model may underestimate C by up to 5%.

It is necessary to know, as a minimum, the digestibility of the diet, but the model is improved if individual digestibilities of the dietary components are available. The values are of importance in calculating the weighted Qox (equation 8), which has further implications in calculating U, (equation 19). These detailed digestibilities are also of value in deriving the value of A (equation 7).

The principal energy loss is in R and in compiling this value a number of assumptions must be made. It is generally assumed that tilapias are only active during daylight, with virtually no movement at night. As the data for resting respiration rate are substantially correct, it then becomes important to know the fraction of time spent in routine and active behavior during daylight. From our own observations on tilapia in tanks, fish of 50 to 100 g may spend 50% of the time in "routine" activity and 5 to 10% in "active" swimming. This will clearly be greatly affected by system design and stocking density and much further work is required to refine these inputs. Ultimately this could be clarified by construction of a series of system- and density-related time budgets at different activity levels. The subsequent calculation of routine rate is, however, also a problem in that it is based on equation (13) which is derived from a very limited database. It should be noted that currently this correction is probably only valid between approximately 50 and 150 g body weight. Calculation of R in this model appears to result in a value which is somewhat lower than values of R quoted by other authors, in a range of species. This is, at least in part, due to the fact

that routine respiration and active respiratory losses are, at present, difficult to predict accurately.

The techniques used to calculate both F and U produce values which are broadly in agreement with those measured by other authors. Fiber has a large influence upon F and thus it is important to have an estimate of fiber independent from NFE. Furthermore, since F is calculated using A, fecal energy will be underestimated if digestibility is overoptimistic (see above). The calculation of U will be strongly influenced if R is underestimated, and is further dependent on reasonable estimates of C and protein digestibility.

On the basis of previously published energy budgets, our model at first appears to underestimate R and overestimate P. However, it is possible to approximately verify the results by calculation of specific growth rate (SGR), on an energy basis, and by comparison of this value with those expected from typical growth trials. An approximation of SGR can be calculated based on the body weight and water content and using a factor for the dry matter energy content of approximately 26.05 kJ/g. This is then given by:

$$SGR = (P/((WT * ((1-WC)/100)) * 26.05)) * 100 \quad \ldots 21)$$

Using this technique in conjunction with data for trials using practical diets SGR falls within the range of expected values and it would appear that our estimate of P is correct. This may follow because our compiled budget is based on data for cultured fish, and it is known that production in culture normally greatly exceeds that in the natural environment (c.f. data on growth rates in natural waters (Lowe-McConnell 1982)).

Production can, of course, be subdivided into somatic growth (Ps) and reproductive growth (Pg) and it must be borne in mind that, at a given stage in the life of the fish, some of the energy available as P will be diverted into gonad and gamete development. The stage at which this occurs varies widely, and so no attempt has been made at this stage to incorporate this dichotomy of effort into the model.

Clearly, temperature, salinity and body weight have a large influence within the model. It is interesting to note that although a single brief stress event causes a massive increase in oxygen consumption (Ross and Ross 1983) the calculated energetic effect of this is to add only about 3% to the overall daily cost of respiration. It may be possible to control photoperiod, and hence activity levels, which could reduce the value of R resulting in higher production. Extension of the model to pond culture will only be feasible if C and R can be accurately determined. Although pond values for R could be resolved by amassing field data on activity, the estimation of real values for C may well be impossible.

The selection of optimal management strategies depends on the availability of at least a minimal description of the system of an input/output form and such simulation studies will usually preceed the attempted application of optimization techniques (Brockington 1979). Any mathematical model designed for use in aquaculture or agriculture should be as simple as possible to operate and it is also useful if the number of variables employed in calculations can be minimized. Sensitivity analysis allows exploration of the effects of all variables in the model and gives an appreciation of those which have a major effect. From this can arise insights which fuel further research or which may suggest management strategies. The model described here is a synthesis of the current reliable data available for tilapias and should prove a valuable framework for both research and production studies.

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Border Method and Fish Culture: Synergistic Effects on the Yield of Rice Grain

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Abstract

A rice-fish culture system is under trial for use by resource poor farmers that reduces labor and resources input by up to 25% while maintaining rice production and supplying dietary fish-protein supplements. Pond-reared *Oreochromis niloticus, Cyprinus carpio* and *Puntius gonionotus* fingerlings at 5-7 cm were stocked into 1 rai (0.16 ha) rice fields at 800 fish/rai (5,000 fish/ha) prepared with a refuge pond or trench, mean depth 1 m. Culture temperature was 24° ± 2°C in the trench with the field temperature normally 5°C higher during the afternoon.

Rice was planted conventionally or according to the 'Border Method', i.e., leaving every fourth row vacant (rice variety: RD6; spacing: 30 cm x 30 cm), in two fields of different soil fertility and normal cultural practices followed. After a growth period of 120 days the rice and fish were harvested together.

In 'Border Method' and 'Cultured Fish' plots, rice production was not significantly decreased while tillering increased (16.7%) and panicle weight increased (32.7%) compared with control plots (conventionally planted rice and no fish). Fish production was 193 kg/ha including 63.6% naturally occurring organisms. No supplemental feed was given. Bovine manure was incorporated into the soil as fertilizer (3.1 t/ha) and probably contributed to the fish diet.

Rice production is not increased, but this is compensated for by the production of fish and the reduction of cultivation inputs (seed, fertilizer and pesticide, and the labor of planting, weeding and harvesting) by up to 25%. For resource-poor farmers, this type of culture system would allow them to maintain rice production, while reducing inputs and harvesting a dietary protein and/or cash crop simultaneously.

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Introduction

Border effect is defined as the difference between the performance of the plants adjacent to the border compared with that in the center of the field (Gomez and DeDatta 1971). It is the basis for all crop spacing recommendations presently in practice worldwide. Roots excrete toxins (aliphatic acids, phenolic acids) that inhibit the growth of other plants, particularly weeds. Above a threshold concentration self inhibition occurs (McClelland 1929).

In the "Border Method", the roots of the plants adjacent to the vacant rows get twice the area for horizontal root expansion and can exploit new soil and nutrients. When rice is planted leaving every fourth row vacant in whole field, the grain yield is not significantly reduced compared to rice planted according to DOA recommendations (Taylor et al., unpubl. data) and may be actually increased in large verification trials over a period of six years (Rathi 1982).

When fish are introduced into a field planted in the border method style, it is hypothesized and supported by observations that the vacant spaces become pathways for the fish to enter and exploit the natural food sources. Ardiwinata (1957) suggested a 40 cm or wider spacing of rice plants to give the fish greater freedom of movement; since in 25 cm or 30 cm spaced rows, limited space is available for fish to exploit the weed and insect food resources. Weed diversity when fish are present is higher in conventionally planted fields and overall weed infestation is lower (Taylor et al., unpublished data). Although weed infestation is higher under the border method, when fish are present they are consuming the plants or are causing a change in the plant habitat. Chapman et al. (unpublished data) found little evidence to support the theory that fish, particularly *Cyprinus carpio*, actively consume plants. They are more likely to uproot small weeds and increase water turbidity in their search for food. *Cyprinus carpio* appear to adapt to rice fields by exploiting a wider range of foodstuffs, including surface plankton (Chapman et al., unpublished data).

This study investigates the effects on rice and fish production of combining an appropriate method of low-cost rice cultivation with a low-cost form of fish culture. By definition, the border method results in a 25% decrease in rice production cost by decreasing the quantities of seedlings, fertilizer and pesticide required while also decreasing the labor input.

Materials and Methods

The study was conducted on-station in a fertile fluvial plain, 30 km southwest of the northern Thai city of Chiang Mai, elevation 300 m, tropical and irrigable all year-round.

The randomized complete block design (RCBD - 6 blocks) was chosen using two experimental fields (A and B, 1 rai (0.16 ha) each). Each field contained three blocks, each block with four 100-m^2 treatment plots arrayed in random order. The four treatments were:

T-1	:	border method and cultured fish
T-2	:	border method alone
T-3	:	conventional method and cultured fish
Control	:	conventional method alone.

Light sensitive, glutinous, improved variety RD6 rice was planted conventionally (30 cm x 30 cm hill centers) in the twelve T-3 and Control plots. In the twelve T-1 and T-2 plots, the border method was used leaving one row in four vacant.

A trench or pond was dug around each of the two fields to provide the fish refuge from predators. Cultured fish were excluded from plots by using #16 blue plastic netting as a fence around the 10 m x 10 m plots, pushed well down into the mud. If a few fish happen to enter, the effect was considered negligible; under conventional on-farm rice cultivation wild fish are an important component of the paddy ecosystem. Fish species used were,

Oreochromis niloticus (Nile tilapia), *Puntius gonionotus* (common silver barb) and *C. carpio* at a ratio of 2:3:4 stocked at 800/rai (5,000/ha). Bovine manure was the only fertilizer used, incorporated into the soil at 500 kg/rai (3.1 t/ha). No form of pesticide or herbicide was used. The experiment duration was 120 days after which fish and rice yields were sampled over a 3-day period.

Rice yield performance, i.e., grain yield (t/ha), number of tillers (shoots of original stalk) and height (cm) were collected from four quadrats (8 m^2) in each of the 24 treatment plots (96 samples). Estimates within each plot followed standard agricultural methodology (De Datta et al. 1978).

A pooled sample of panicles (seed heads) was collected from 12 hills, and six individual panicles were taken from three rows (outer, middle, outer) in border method plots and from all four rows in conventional plots. Only two blocks (III, VI) were sampled in conventional plots. Field calculations indicated that a low standard deviation could be expected between rows of conventionally planted rice. Two sample blocks were thus considered sufficient. Total number of samples was 336.

Rice height, tillering and number of panicles per hill were taken for each hill sampled. Polar orientation of the sampled rows were randomly assigned to avoid the bias caused by the advantage of anti-auxinic effects of light on southern and eastern exposure rows.

Rice samples were sun dried for 16 hours, weighed and corrected for 14% moisture using a hand held Kett grain moisture tester. Sample weights were corrected for missing rice hills in the 8-m^2 quadrats (De Datta et al. 1978). During rice harvesting, time and motion studies of laborers cutting-tying and carrying were conducted to the compare relative speeds of border method harvesting and conventional methods.

Differences in mean rice performance were analyzed for significance at the 5% level ($P < 0.05$) by ANOVA and mean separation by Duncan's Multiple Range Test (DMRT) (Steel and Torrie 1980).

Results

All fish species were observed actively searching for food throughout the day, particularly during early morning and late afternoon. Plastic netting was a satisfactory method of excluding fish from the no-fish treatments. Harvesting of field A yielded 30.9 kg/rai (193 kg/ha) of: stocked fish, 11.3 kg (36.4%) and other naturally occurring organisms, 19.6 kg (63.6%). *Oreochromis niloticus* grew fastest, but was the least common cultured species remaining at harvest; they are easily shot by poachers using crossbows. *Cyprinus carpio* performed the best, yielding 6.3 kg of the 11.3 kg total. Poachers drained Field B of water and captured all but a few fish.

Other captured species, in order of frequency were: epibenthic snails, *Anabas testudineus*, freshwater prawns *(Macrobrachium lanchesteri), Trichogaster trichopterus*, freshwater crabs *(Somanniathelphusa sinensis dugasti), Channa striata, Clarias batrachus, Betta splendens, Notopterus* sp. and *Mastacembelus erythrotaenia.*

Field A was chronically congested with aquatic weeds, particularly water hyacinth *(Eichhornia crassipes),* joyweed *(Alternanthea sessilis)* and bulrush *(Scirpus juncoides)* (Taylor et al., unpubl. data). Fish regularly penetrated the field center during the early days of the trial, but were prevented from doing so when weeds became too thick and congested. At the end of the experiment it was clear that the fish were penetrating only 80-110 cm from the edge of the perimeter trench. Weeds were controlled within this strip and it was concluded that the fish were responsible. Weeds were particularly thick in the vacant rows of border method plots. Weed infestation appeared more uniform in conventional plots but fish access was still poorer than in border method plots. Field B had considerably fewer weeds overall and weeds were clearly absent from fish (T-1 and T-3) treatment plots.

Field A was strongly acidic with medium to high fertility (pH = 4.6; organic matter (O.M.)= 2.51%; P = 17 ppm; K = 63 ppm). Field B was slightly less acidic but

considerably lower in fertility (pH = 5.1; O.M. = 1.39%; P = 15 ppm; K = 61 ppm). Rice paddy fields are known for high rates of organic matter decomposition because of the lack of an efficient carbon dioxide - bicarbonate - carbonate buffering system which favors rapid fluctuations in pH making a theoretically poor medium for fish culture.

No significant difference ($P < 0.05$) was found between the mean rice yields of treatments as expected, although a slight (10%) decrease in production was noted from border method plots compared with conventional plots (Table 1). 'Blocks' were found significant at the 5% level. There was significant difference between mean yields of rice from field A which was heavily weed infested, of high fertility and acidic, from those of field B which was relatively free of weeds, of poor soil quality but producing more rice grain.

Tillering activity under border method and fish culture was significantly different ($P < 0.01$) from that in other treatments. Rice yield analysis for the conventional method indicates that fish presence is not significant, but with the border method, when planting on low fertility soils, fish must be included to maintain rice yields.

The differences in pooled panicle weight between border method rows were highly significant ($P < 0.01$) when fish are present and not significant when no fish are present (Table 2). No significant interactions between rows and fish were evident for pooled panicle weights, but weights of outer rows were increased by 13.8% over middle rows when comparing fish/no-fish treatments and 32.7% when fish-only treatments were considered (Fig. 1).

Total number of tillers was increased similarly by 13.3% over pooled fish/no-fish treatments although this was not significant (Table 2) as in the all-plots analysis (Table 1). Partitioning treatments into tillering activity with and without fish mirrors the results of the analysis of pooled panicle weight. With-fish treatments gave significantly better tillering (16.7%) than without fish when considering border method alone (Table 2).

Table 3 demonstrates the grain yield per row resulting from the treatments. Values were scaled up from the pooled panicle data in Table 2. Compare with the results of Rathi (1982): Percentage of total yield from border method; 35.4% (outer row), 30.6% (middle), 34.0% (outer).

No significant difference was found between rows or treatments on rice plant height. Incidence of plant lodging [grain plants falling over if panicles are too heavy which increases harvesting labor time] did not appear affected by border method plantings.

Total number of panicles per hill is closely related to tillering activity and as such is highly significant ($P<0.01$)

Table 1. Mean rice yield (t/ha) and number of tillers/hill of two fields comparing border method and cultured fish effects with the performance of conventional methods in an RCBD experiment.

Treatment	Field A	Field B	Mean	# Tillers/hill
Border and fish	3.14 a	4.21 a	3.67 ns	11.4 a
Border and no fish	3.54 ab	3.73 b	3.63 ns	10.2 c
Conventional and fish	3.77 b	4.28 a	4.03 ns	10.6 abc
Conventional and no fish	3.71 b	4.29 a	4.00 ns	9.9 c
Coefficient of variation	5.75%	4.10%	7.21%	11.77%

Data bearing different suffix letters (a, b, c) are significantly different (Duncan's Multiple Range Test, P = 0.05, ns = not significant).

Table 2. Row performance of RD6 improved variety rice planted according to the border method with or without cultured fish.

Treatment	Rows	Panicles pooled wt. (g)	# Panicle/ hill[1]	# Tiller/ hill[1]	Height (cm)[1]	# Seeds/ panicle[2]	Wt. single panicle (g)[2]
With fish	Outer	300**	12.3**	12.7*	159.9	223.5*	3.62*
	Mid.	231.6	10.4	10.8	158.7	158.7	2.59
	Outer	318.1**	12.2**	12.5*	159.6	159.6*	3.23*
No fish	Outer	292.9	11.0*	11.3	158.0	□	□
	Mid.	244.6	10.0	10.3	157.3	□	□
	Outer	276.2	11.2*	11.5	157.8	□	□

**Significant (P = 0.01); *Significant (P = 0.05); Others (not significant).

[1] Values calculated from r = 12 rice hills/plot.
[2] Values from r = 6 hills/plot.

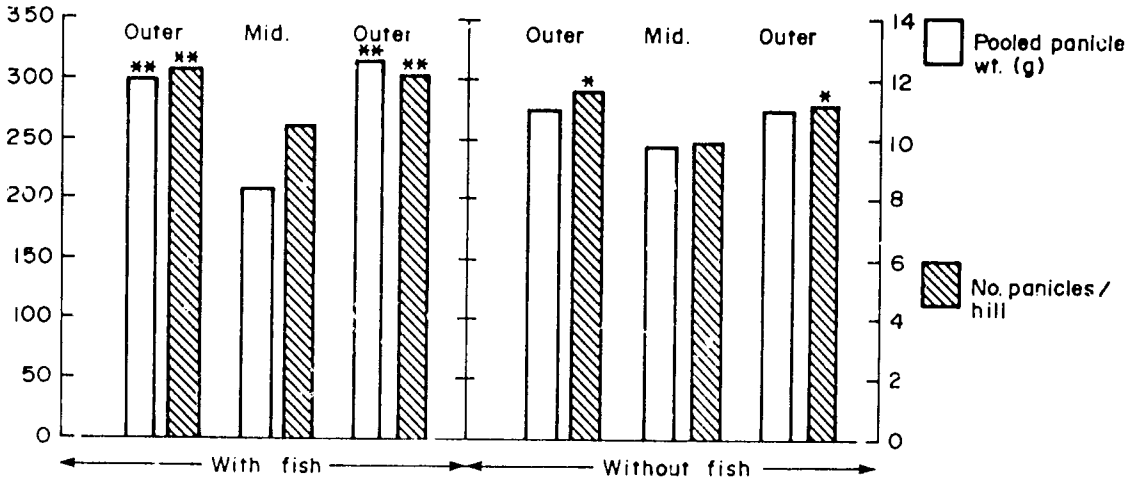

Fig. 1. Mean values of pooled panicle weights (g) and panicle number per hill demonstrating interactions of three rows of border-method affected rice with fish as primary treatment in a RCBD experiment in Chiang Mai, Thailand. ** = significance at 1% level; * = significance at 5% level. Replicates = 12.

between rows with fish treatments and is significant ($P < 0.05$) between rows without fish (Table 2, Fig. 1). This trend of mean significant differences continues to hold true for both the number of seeds/single panicle and weight of single panicles. For these characteristics, only the with-fish border method treatments can be considered because of destroyed samples.

In control plots, performance components showed no significant difference between rows but did demonstrate slightly better performance when under treatment by fish.

Through simple observations on frequency of rice plant *whiteheads*, the insect pest "stemborer" was enumerated. In contrast to Chapman et al. (unpubl. data) who found increased incidence of

Table 3. Mean grain yield of different rows and their relative contribution to the total yield per unit area.

Treatment	Grain yield (t/ha)*	Row	Grain yield component (t/ha)*	Proportion of total yield (%)
Border method and fish	2.12	outer	0.79	37.3
		mid	0.58	27.3
		outer	0.75	35.4
		Total	2.12	
Border method and no fish	2.03	outer	0.73	36.0
		mid	0.61	30.0
		outer	0.69	34.0
		Total	2.03	
Conventional and fish	2.33	4 X regular	0.58	25.0
Control	2.31	4 X regular	0.58	25.0

*Yields calculated from pooled panicle weight of 12 hills; 24 total plots (from Table 2).

stemborers in rice-fish fields over control fields, although very low in total incidence, we found twice as many stemborers in no-fish treated fields as in with-fish treated fields. Further, we found twice the incidence on bordering rows than middle rows (Table 4). Overall higher incidence than Chapman et al. (unpublished data) was observed but the study areas are widely separated and cannot be compared on a 'total incidence' basis.

A simple time and motion study indicated that plots prepared by border method planting were 16% faster to harvest than conventionally planted plots.

Discussion

When inputs to rice cultivation are reduced by 25% using the border method and fish are cultured concurrently, gross effects on the yield of rice grain are not significantly affected. Bordering rows compensate for vacant rows by increasing the production of tillers, panicles and number of seeds per panicle.

When fish are present and searching for food in and around the rice plants, they disturb weed growth in the vacant spaces between the rows. To a limited extent, depending on weed species, soil

Table 4. Total incidence from 12 plots per treatment of *whiteheads* indicating stem borer infestation.

Treatment	Row		
	Outer	Middle	Outer
Border and fish	8	4	7
Border and no fish	15	10	22

fertility and fish species, the fish will control weeds by direct consumption *(P. gonionotus)* but more likely by disruption of plant habitat (increased turbidity, uprooting) in the case of *C. carpio*. This aids the expansion of rice roots by limiting competition for nutrients and ultimately aiding rice grain production. In heavily weeded areas dominated by large plant species (water hyacinth) or species difficult to ingest (bulrush, sedges), fish cannot effectively control weeds. Small, soft stemmed plants (horsetail, water clover) appear to be easily controlled. Heckman (1979) concluded that the density of emergent vegetation (including rice) limits the movement of most adult fish.

With the border method and fish culture we found an increase in tillering, panicle weight, number of seeds/panicle and number of panicles/hill. This demonstrates quantitatively the close interrelationship between fish and rice plant growth. Tillering and panicle number increased under border method alone, but only with fish was a significant increase in panicle weight seen. We found the performance of bordering rows did compensate for the missing 25% of plants associated with border method.

Our results (36.0% outer; 30.0% mid.; 34.0% outer) are similar to Rathi (1982) for the border method without fish, but if fish are considered, then there is a relative increase in contribution to performance of the two bordering rows (37.3%; 27.3%; 35.4%) and a sustained performance of the middle row (Table 3).

The improved access to the plants by the vacant rows allows the fish to exploit food sources not normally available. Particularly *C. carpio* improves the growth of the bordering plants by: aerating the soil thereby increasing the rate of decomposition and reduction (Heckman 1979); disturbing competing weeds (Chapman et al., unpubl. data); eating root pests--nematodes, insect larvae, algae; recycling and redistributing inorganic nutrients back into the soil fraction (Heckman 1979).

Analysis of gut contents has shown that in rice paddies, *C. carpio* prefer zooplankton, insect larvae and some detritus, similar to pond-reared carp (Chapman et al., unpublished data). The paddy ecosystem has a much higher biotic diversity than that of a pond; this can be exploited by cultured fish, providing high fish yields if managed properly. *Puntius gonionotus*, a herbivorous carp, has a negligible effect on pest control but may contribute to weed control by direct consumption of plant material or by alteration of the weed habitats. *Oreochromis niloticus* appears to consume blue-green algae, diatoms, phytoplankton and some plant materials directly (Chapman et al., unpublished data; Philippart and Ruwet 1982). The digestion of plants with high fiber content such as water hyacinth, joyweed and bulrushes is unlikely, due to a lack of cellulase in the gut of *O. niloticus*. They may be able to obtain nutrition through gut acid hydrolysis of detritus and some water vascular plants (Edwards et al. 1985). *O. niloticus* is important for the recycling of inorganic nutrients from the detritus and food chain back into the soil as well as an important human food source.

Feeding fish have been observed in the paddy field in less than 10 cm of water where the dorsal fin was well above the water surface. Deep water may afford the fish more protection from predators and sunburn and may contribute to limiting weed growth. High temperature seems to be of little relevance to rice-fish culture. We recorded afternoon water temperatures as high as 44.5°C in the paddy, well above the lethal tolerance level of *C. carpio*, which could be seen at this time feeding vigorously and with no visible ill effects. Cool water (< 20°C) does appear to slow growth of *O. niloticus* and *P. gonionotus* but not *C. carpio* until extremely cold temperatures are reached.

Acknowledgements

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Chandrapanya, Director, and Mr. Supachai Bangliang, Supervisor. Rice fish culture work at FSRI is funded by the International Development Research Centre (IDRC) - Canada. Thanks to Dr. V.R. Carangal of the International Rice Research Institute (IRRI) and Dr. K.S. Rathi of Kanpur, India, for the inspiration and advice to complete this work. Mr. Oothai Tanapanyo, San Pa Tong Rice Experiment Station kindly provided staff and study fields for our use. Technical services were expedited by Mr. Prasert Natengum. Mr. Shawn Taylor is seconded by CUSO, the Canadian Volunteer Development Agency; Mr. Gerald Van Koeverden, Director. Also thanks to Mr. Greg Chapman (CUSO), John Sollows (CUSO), William Bourne (VSO) and Dr. Ken MacKay (IDRC) for their words of support. The manuscript was initially edited by Dr. Dorothy Jackson.

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SESSION II: PATHOLOGY

Hematological and Histopathological Changes in *Oreochromis mossambicus* After Exposure to the Molluscicides Aquatin and Brestan

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CRUZ, E.R., M.C. DE LA CRUZ and N.A. SUÑAZ. 1988. Hematological and histopathological changes in *Oreochromis mossambicus* after exposure to the molluscicides Aquatin and Brestan, p. 99-110. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (ed.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623 p. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Abstract

Duplicate static 96-hour bioassays were conducted to determine the median lethal concentration for *Oreochromis mossambicus* (LC_{50}) of two organostannous molluscicides, commonly used in fishponds: Aquatin and Brestan. *O. mossambicus* was more sensitive to Brestan. The acute toxicity of both Aquatin and Brestan ceased towards the end of 96 hours. The 24, 48, 72 and 96 hour LC_{50}'s were 4.91, 3.97, 2.95 and 2.58 ppm formulated product for Aquatin and 0.35, 0.18, 0.10 and 0.09 ppm for Brestan, respectively. The computed safe concentrations for Aquatin and Brestan are 0.30 and 0.01 ppm, respectively.

Exposure to lethal concentrations of Aquatin resulted in an immediate reduction in hemoglobin and hematocrit levels. Hemoglobin content was likewise lower in Brestan-exposed fish, whereas their hematocrit level was higher than that of the control fish.

Histological analyses of gills, intestine, liver and kidney showed pathological changes even in sublethal levels tested. Damage became severe with increasing concentration of the pesticide. The behavior and symptoms exhibited by the fish and the physiology of hematological and histopathological changes are discussed.

Introduction

The use of pesticides such as insecticides, herbicides, molluscicides, piscicides is common among rice and fish farmers in controlling pests and predators. Pesticide pollution in the aquatic environment comes mainly from agricultural run-off, increased use of pesticides and improved irrigation. Indiscriminate use of pesticides poses a great risk to aquatic organisms, especially food fishes, and consequently to humans.

This danger may be reduced through effective and efficient screening procedures of various pesticides and use of pesticides that have low toxicity to fishes. However, information on the tolerance levels of fish to pesticides is limited. Important considerations in the use of pesticides are knowledge of the tolerance levels of fish species and effects on fish physiology.

Bioassays of some organochlorine and organophosphate pesticides have been conducted in the Philippines by the National Pollution Control Commission, the Freshwater Aquaculture Center (FAC) of Central Luzon State University and the Brackishwater Aquaculture Center (BAC) of the University of the Philippines in the Visayas (de la Cruz and Cagauan 1981). However no studies have been made on the toxic effects of Aquatin and Brestan on *O. mossambicus*.

This study was conducted to determine the 96-hour median lethal concentrations (LC_{50}) for *Oreochromis mossambicus* of two organostannous molluscicides, Aquatin (AQTN) and Brestan (BTN), commonly used to control snails in fishponds, and evaluate their effects on fish hematology and histopathology.

Materials and Methods

O. mossambicus (average weight, 30 g) were obtained from a private freshwater pond and acclimatized to laboratory conditions for at least one week in 300-l fiberglass tanks with continuous aeration and daily feeding with fish pellets. They were starved for 48 hours prior to and during the experiments.

Two separate static bioassay tests with aeration were conducted for 96 hours each as described by APHA (1975). A complete randomized design was used in the experiment with 5 fish/40-l freshwater.

Aquatin (AQTN) (20% organostannous compound; Planters Products, Philippines) and Brestan (BTN) (60% triphenyl tin acetate, Hoechst AG) were obtained from a local agricultural shop and dissolved separately in distilled water to form stock solutions which were added to the aquaria to yield the desired final concentrations. The concentration ranges used (formulated product) with three replicates per concentration were 1.0 to 6.0 ppm for AQTN and 0.1 to 0.5 ppm for BTN plus a control. The median lethal concentration (LC_{50}) values and its corresponding 95% confidence limits were calculated by Probit Analysis (Finney 1982).

Water was analyzed at the start and termination of the experiment for temperature, pH, ammonia-nitrogen (NH_3-N) and hardness.

Fish were sampled after 12, 24 and 96 hours of exposure for histopathological analyses of selected tissues. Gills, intestine and kidney were preserved in 10% buffered formalin and liver in Bouin's fixative. Tissues were processed, sectioned at 4 µm and stained with hematoxylin and eosin, using standard histological techniques.

In a separate experiment, hematocrit and hemoglobin levels were determined on moribund fish from each treatment after 6, 12, 24, 48, 72 and 96 hours of exposure. Blood was sampled by severing the caudal peduncle. Three fish were sampled for each treatment and blood analysis was done in duplicate. A corresponding control fish was likewise sampled. Hematocrit was determined by the capillary method and hemoglobin content per 100 ml of blood by the Sahli-Hellige method. Statistical analysis was done using ANOVA, Duncan Multiple Range Test (DMRT) and Student's t-test.

Results

Behavioral changes

Upon addition of higher concentrations of the test solution, fish showed initial disturbed swimming movements, rapid opercular movements and surfacing behavior indicative of avoidance response. This was followed by blackening of the

whole body, unusual lethargy and tendency of the fish to settle at the bottom motionless with slow opercular movements. Lower levels of pesticide did not produce any obvious change in fish behavior for the entire duration of the experiment.

Toxicity

Table 1 summarizes the range of the physicochemical parameters of control and treated fish observed during the experimental period. There was no difference between control vs. treated nor between treatments. The NH_3-N levels significantly increased at the termination of the experiment.

The LC_{50} values of AQTN and BTN at different time intervals and the 95% confident intervals are presented in Table 2. Note that the acute toxicity of both AQTN and BTN ceased towards the end of 96 hours.

Hematological changes

Changes in hemoglobin content and hematocrit values for AQTN-exposed fish are presented in Figs. 1 and 2. Exposure to AQTN decreased the values of hemoglobin and hematocrit, resulting in hypochromic anemia. Higher levels of AQTN caused an immediate reduction of both blood parameters after 6 hours of exposure. The effect was highly significant ($P < 0.05$) when fish were exposed to 5.0 and 6.0 ppm AQTN. The reduction was observed in all levels except 2.0 ppm as exposure time increased.

Changes in hemoglobin content and hematocrit values for BTN-exposed fish are presented in Figs. 3 and 4. Unlike in AQTN-exposed fish, the highest level of BTN (0.5 ppm) resulted in an increased hematocrit value after 12 hours of exposure ($P < 0.05$). The increase, however, did not parallel that of hemoglobin content. At the end of the experiment, hematocrit was significantly lower in 0.1 ppm exposed fish when

Table 1. Ranges of physicochemical parameters during experiments on the toxicity of Aquatin and Brestan to *Oreochromis mossambicus*.

Parameter	Control	Aquatin	Brestan
Temperature (°C)	28 — 28.5	28 — 29	28 — 29
pH	8.4 — 8.9	8.2 — 9.0	8.3 — 9.0
NH_3-N (ppm)	0.08 — 4.38	0.07 — 4.62	0.07 — 4.76
Hardness (ppm $CaCO_3$)	202 — 230	217 — 252	224 — 256

Table 2. Aquatin and Brestan median lethal concentrations (LC_{50}) and 95% confidence intervals (in ppm) for *Oreochromis mossambicus*.

Time (hours)	Aquatin LC_{50}	Aquatin 95% confidence interval Lower	Aquatin 95% confidence interval Upper	Brestan LC_{50}	Brestan 95% confidence interval Lower	Brestan 95% confidence interval Upper
24	4.01	3.83	4.19	0.345	0.229	0.523
48	3.97	3.57	4.44	0.185	0.091	0.376
72	2.95	2.32	4.01	0.104	0.082	0.131
96	2.58	1.95	3.67	0.092	0.064	0.135

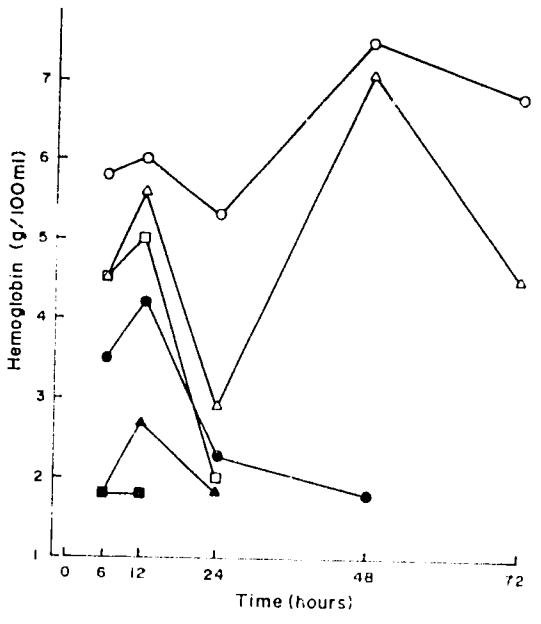

Fig. 1. Graph showing the effect of different levels of Aquatin on the hemoglobin content in the blood of *Oreochromis mossambicus*. ○——○, Control; △——△, 2 ppm; □——□, 3 ppm; ●——●, 4 ppm; ▲——▲, 5 ppm; ■——■, 6 ppm.

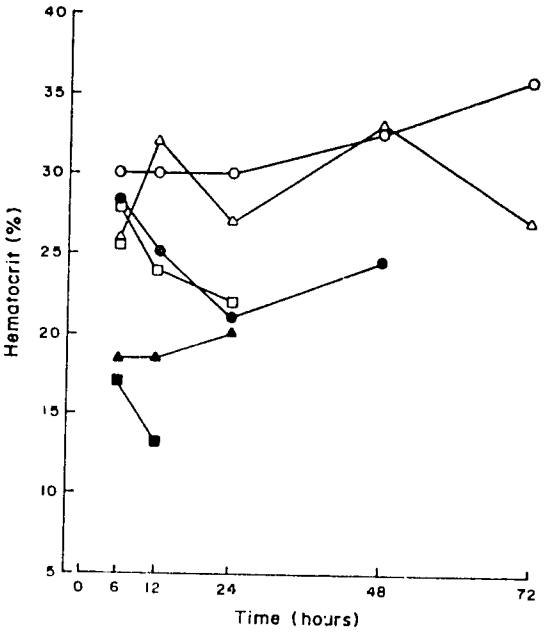

Fig. 2. Graph showing the effect of different levels of Aquatin on the hematocrit value in the blood of *O. mossambicus*. ○——○, Control; △——△, 2 ppm; □——□, 3 ppm; ●——●, 4 ppm; ▲——▲, 5 ppm; ■——■, 6 ppm.

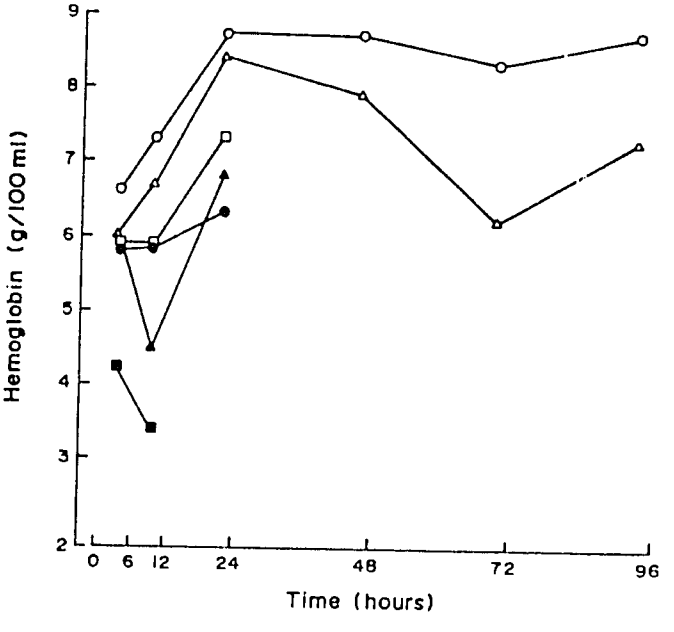

Fig. 3. Graph showing the effect of different levels of Brestan on the hemoglobin content in the blood of *O. mossambicus*. ○——○, Control; △——△, 0.1 ppm; □——□, 0.2 ppm; ●——●, 0.3 ppm; ▲——▲, 0.4 ppm; ■——■, 0.5 ppm.

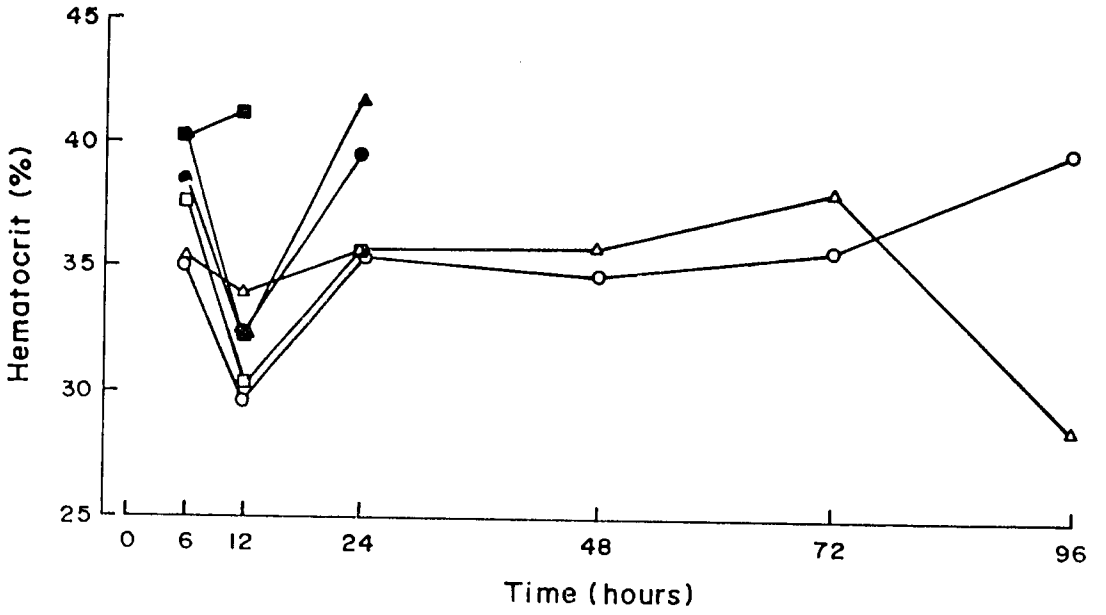

Fig. 4. Graph showing the effect of the different levels of Brestan on the hematocrit value in the blood of *O. mossambicus*. ○——○, Control; △——△, 0.1 ppm; ◐——◐, 0.2 ppm; ●——●, 0.3 ppm; ▲——▲, 0.4 ppm; ■——■, 0.5 ppm.

compared with the control fish. Hemoglobin content was consistently lower in all treatments.

Histopathological changes

Gills of *O. mossambicus* exposed to AQTN exhibited varying degrees of epithelial hyperplasia among filaments and among treatments. Normal gill filaments (controls) are shown in Fig. 5. Hyperplasia was generally more pronounced toward the distal tip of the filament near the base (Fig. 6) in fish exposed to 5.0 ppm AQTN for 12 hours. After 96 hours of exposure in 1.0 ppm AQTN, hyperplasia resulted in fusion of many lamellae markedly reducing the respiratory surface area of some filaments (Fig. 7).

Gills of *O. mossambicus* exposed to BTN showed varying degrees of damage. Fish exposed to all concentrations showed separation of respiratory epithelium from underlying supportive tissue. Slight epithelial lifting occurred in fish exposed to 0.1 ppm BTN (Fig. 8) for 96 hours. Generalized separation of the epithelial layer from capillaries was observed in fish exposed to 0.5 ppm BTN (Fig. 9) for 12 hours. Occasionally, hyperplastic and club-shaped lamellae were seen in a few lamellae of fish exposed to 0.1 ppm BTN.

Intestine

The intestinal mucosa of control fish had no significant pathological change (Fig. 10). Fish exposed to 5.0 ppm AQTN for 12 hours had necrotic intestinal mucosa (Fig. 11). Sloughing of the epithelial cells into the lumen occurred in fish exposed to 1.0 ppm AQTN for 96 hours (Fig. 12) whereas 4.0 ppm AQTN-exposed fish had atrophied intestinal mucosa (Fig. 13).

Severe necrosis of epithelial cells of intestinal mucosa (Fig. 14) was observed in fish exposed to 0.5 ppm BTN for 12 hours. Sloughing of epithelial cells and infiltration of lymphocytes into the lumen was also noted (Fig. 15). Severe necrosis of intestinal mucosa (Fig. 16) occurred in fish exposed to 0.1 ppm BTN for 96 hours.

Liver

Fig. 17 shows the histostructure of liver from control fish. Livers of fish exposed to 5.0 ppm AQTN for 24 hours had extensive necrotic hepatocytes whereas fish exposed to 4.0 ppm AQTN showed fibrosis and congestion of sinusoids (Fig. 18) with necrotic pancreatic acinar tissue. Fish that survived after 96 hours of exposure in 1.0 ppm AQTN exhibited vacuolation of hepatocytes and pancreatic acinar tissues were congested with red blood corpuscles (RBCs) presumably demonstrating stasis (Fig. 19). The nuclei of hepatocytes were conspicuously located near the sinusoids.

Livers in fish exposed to 0.5 ppm and 0.3 ppm BTN showed necrosis of hepatic and pancreatic acinar cells, respectively (Fig. 20). Several arteries, veins and pancreatic nodules exhibited an abundance of RBCs. Loss of cellular outline was evident at this stage. Pyknotic nuclei and fibrotic tissue were abundant. Furthermore, acinar cells had less zymogen granules. Pancreatic acinar tissue of fish exposed to 0.1 ppm BTN for 96 hours had atrophied and lacked zymogen granules (Fig. 21).

Kidney

The histostructure of kidney from control fish is shown in Fig. 22. Fish exposed to 5.0 AQTN for 24 hours had necrotic renal tubules (Fig. 23). Depletion of hematopoietic tissue was evident. Disintegration of renal tubules was observed in fish exposed to 4.0 AQTN for 96 hours (Fig. 24). Tubular necrosis was also observed even in the lowest concentration tested (Fig. 25).

Renal tubules were necrotic (Fig. 26) in fish exposed to 0.5 ppm BTN for 12 hours. Eosinophilic casts were present on the lumen of many tubules. Depletion of the hematopoietic tissue was evident. Necrotic renal tubules, depletion of hematopoietic tissue and lymphocyte infiltration also occurred in fish exposed to 0.1 ppm BTN for 96 hours (Fig. 27).

Discussion

Changes in opercular rate as shown in this experiment have been demonstrated to be a sensitive indicator of physiological stress in fish subjected to sublethal concentrations of pollutants (Davis 1973). Thomas and Rice (1975) suggested that increased opercular movement may be caused by decreased efficiency in oxygen uptake or transport.

The manufacturer's recommended rate of application for AQTN and BTN are "1-1/2 to 3 quarts" ha and 330 g/ha, respectively, with a water depth of 10 cm or just enough to submerge the snails. It has been reported that both pesticides reduced the abundance of zooplankton and other animals in *lablab* (plant/animal complex in ponds) at concentrations of 2.0 ppm AQTN and 0.3 ppm BTN (Anon. 1974).

The results of the present study showed that the 96-hour LC_{50} values are within the levels used in fishponds. *O. mossambicus* was more tolerant of AQTN and less tolerant of BTN.

Forty eight-hour and 96-hour LC_{50}'s are useful measures of relative acute lethal toxicity to organisms under certain experimental conditions. However, these values do not represent safe concentrations in natural habitats. The 'safe level' of a compound is derived by multiplying the 96-hour LC_{50} with an application factor of 0.1 to 0.01 for the less persistent organophosphate pesticides (FAO 1969 in Koesoemadinata 1980). Such application factors are applied to acute toxicity test data to estimate the concentrations that are safe for chronic exposure.

The importance of hematology in the diagnosis of fish diseases and for the assessment of the effects of pollution has been widely accepted. Changes in the hematology of fishes in response to stress agents are indicators of stress.

Hematocrit and hemoglobin values in AQTN-exposed *O. mossambicus* were lower than control fish resulting in hypochromic anemia. Similar hemodilution was observed in chinook salmon infected with bacterial kidney disease

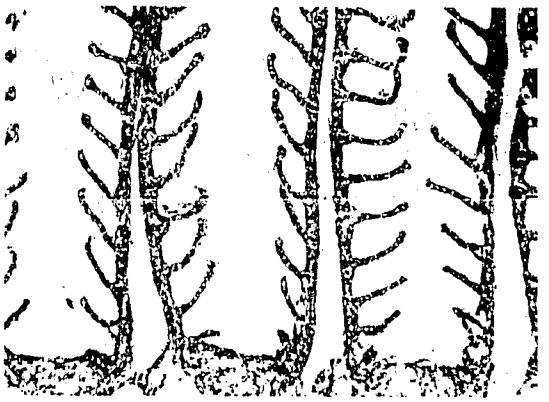

Fig. 5. Normal gill filaments of *Oreochromis mossambicus:* control (x 68).

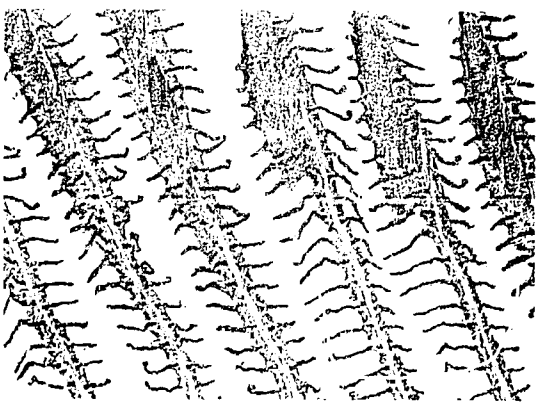

Fig. 6. Gill filaments of *Oreochromis mossambicus* exposed to 5.0 ppm Aquatin for 12 hours showing hyperplasia of distal tip of the filaments (x 34).

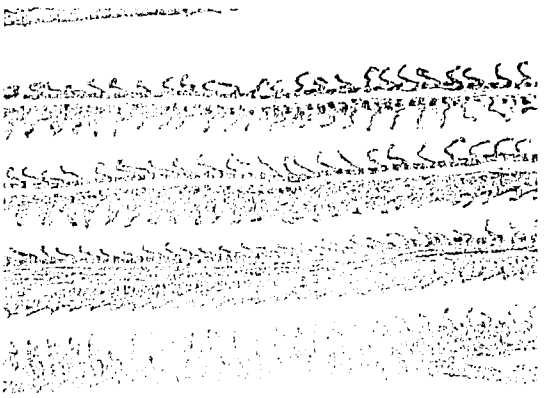

Fig. 7. Gill filaments of *Oreochromis mossambicus* exposed to 1.0 ppm Aquatin for 96 hours showing severe hyperplasia which resulted in fusion of adjacent lamellae (x 34).

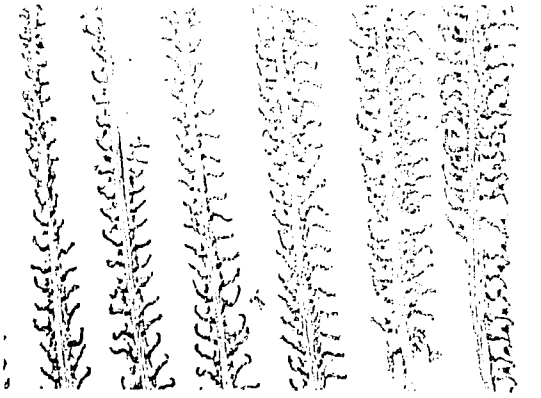

Fig. 8. Gill filaments of *Oreochromis mossambicus* exposed to 0.1 ppm Brestan for 96 hours showing slight epithelial separation (x 34).

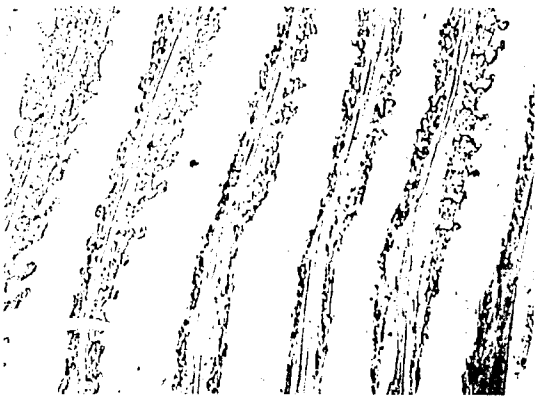

Fig. 9. Gill filaments of *Oreochromis mossambicus* exposed to 0.5 ppm Brestan for 12 hours showing extensive epithelial separation (x 34).

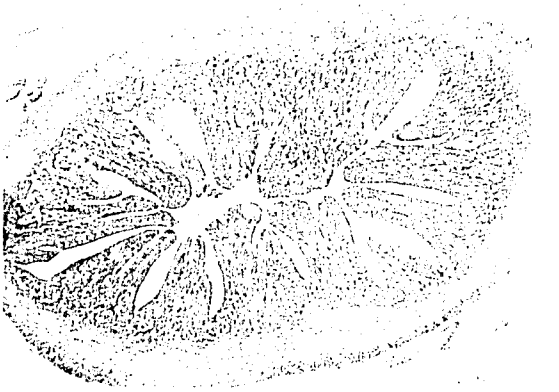

Fig. 10. Normal intestinal mucosa of *Oreochromis mossambicus:* control (x 34).

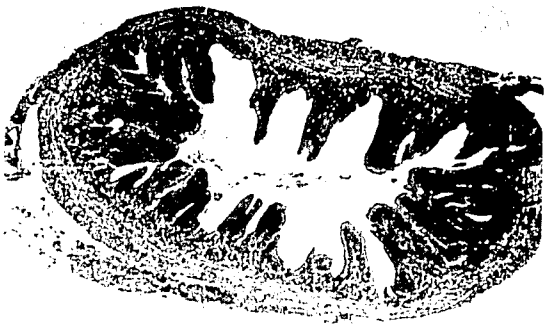

Fig. 11. Necrotic intestinal mucosa of *Oreochromis mossambicus* after exposure to 5.0 ppm Aquatin for 12 hours (x 34).

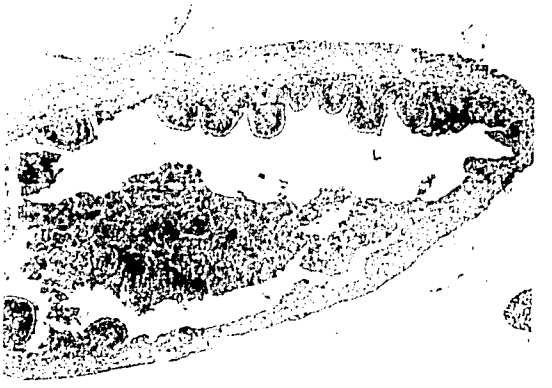

Fig. 12. Sloughing of epithelial cells with necrotic intestinal mucosa of *Oreochromis mossambicus* after exposure to 1.0 ppm Aquatin for 96 hours (x 34).

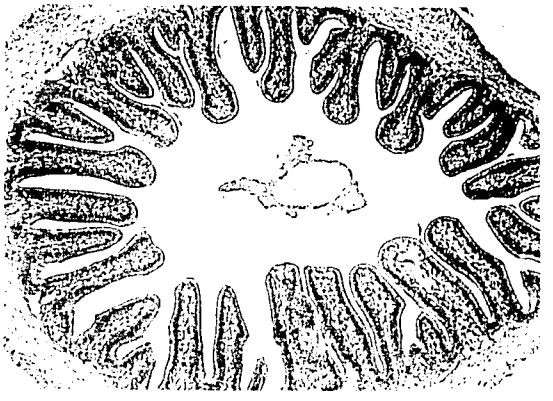

Fig. 13. Atrophied intestinal mucosa of *Oreochromis mossambicus* after exposure to 4.0 ppm Aquatin for 96 hours (x 34).

Fig. 14. Necrotic intestinal mucosa of *Oreochromis mossambicus* after exposure to 0.5 ppm Brestan for 12 hours (x 34).

Fig. 15. Sloughing of epithelial cells in the intestinal lumen of *Oreochromis mossambicus* with lymphocyte infiltration after 12 hours of exposure to 0.5 ppm Brestan (x 34).

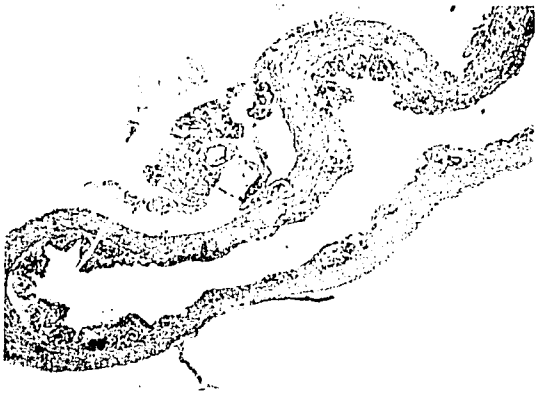

Fig. 16. Severe necrosis of intestinal mucosa of *Oreochromis mossambicus* after exposure to 0.1 ppm Brestan for 96 hours (x 34).

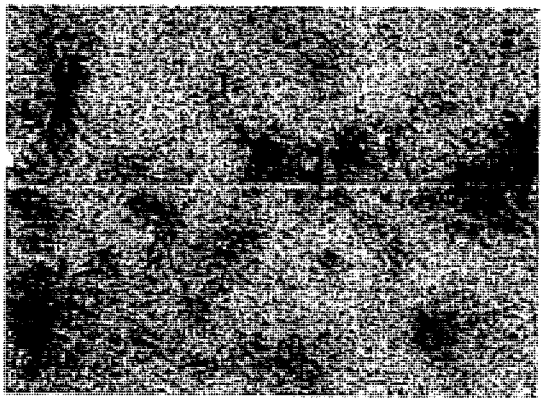

Fig. 17. Normal hepatocytes of *Oreochromis mossambicus:* control (x 34).

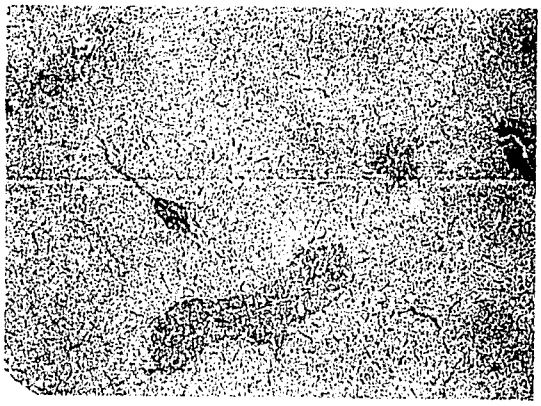

Fig. 18. Fibrosis and congestion of sinusoids with necrotic pancreatic acinar tissue in liver of *Oreochromis mossambicus* exposed to 4.0 ppm Aquatin for 24 hours (x 68).

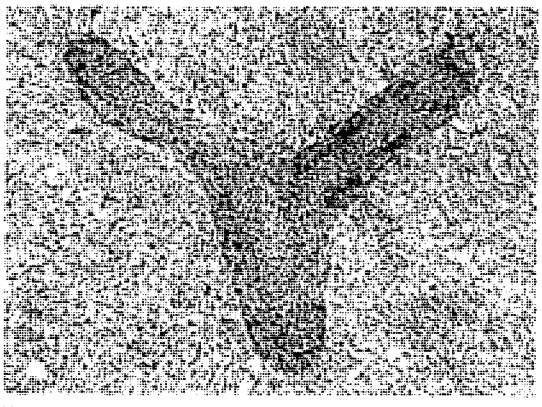

Fig. 19. Liver parencyhma and pancreatic acinar tissue of *Oreochromis mossambicus* exposed to 1.0 ppm Aquatin for 96 hours. Note vacuolation of some hepatocytes and congestion of RBCs in pancreatic acinar tissue. Nuclei of some hepatocytes are located near sinusoids (x 68).

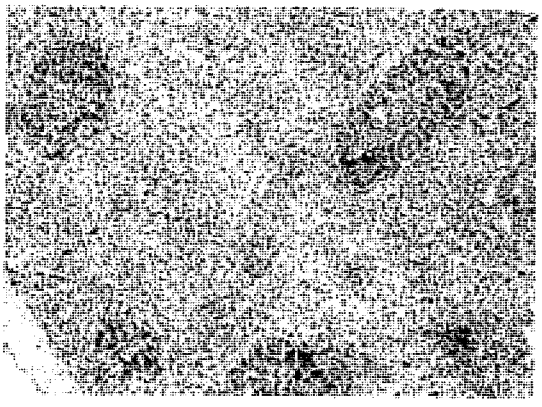

Fig. 20. Necrosis of liver parenchyma of *Oreochromis mossambicus* after exposure to 0.5 ppm Brestan for 12 hours (x 34).

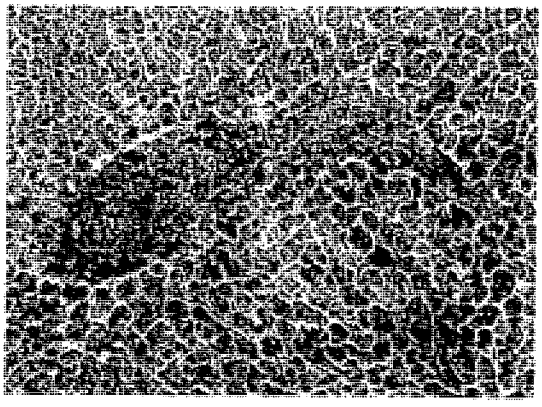

Fig. 21. Atrophy of pancreatic acinar tissue of *Oreochromis mossambicus* after exposure to 0.1 ppm Brestan for 96 hours (x 34).

Fig. 22. Normal renal tubules of *Oreochromis mossambicus:* control (x 34).

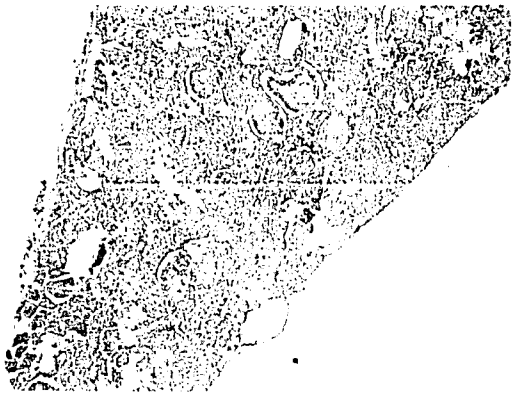

Fig. 23. Necrotic renal tubules of *Oreochromis mossambicus* with depletion of hematopoietic tissue after exposure to 5.0 ppm Aquatin for 24 hours (x 68).

Fig. 24. Disintegration of renal tubules of *Oreochromis mossambicus* after exposure to 4.0 ppm Aquatin for 96 hours (x 34).

Fig. 25. Tubular necrosis in kidney of *Oreochromis mossambicus* exposed to 1.0 ppm Aquatin for 96 hours (x 68).

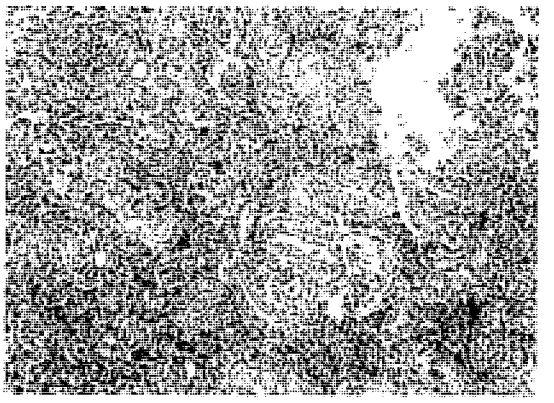

Fig. 26. Necrotic renal tubules with depletion of hematopoietic tissue in *Oreochromis mossambicus* exposed to 0.5 ppm Brestan for 12 hours. Eosinophilic casts are present in the lumen of tubules (x 68).

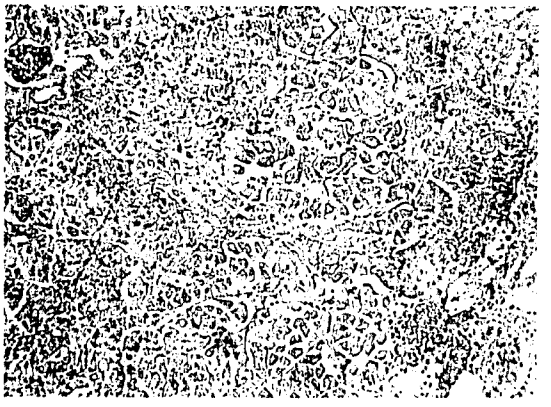

Fig. 27. Necrosis of tubules, depletion of hematopoietic tissue and infiltration of lymphocytes in *Oreochromis mossambicus* exposed to 0.1 ppm Brestan for 96 hours (x 34).

(Iwama et al. 1986), *Channa punctata* subjected to NaCl-stress (Dheer et al. 1986), and fishes with nutritional abnormalities (Herman 1970; Bills and Hunn 1976; Hilge 1979). The reduction in hematocrit and hemoglobin levels can be attributed possibly to progressive damage to the kidney by the pesticide and/or the lysis of the red blood cells.

Hematocrit values in BTN-exposed *O. mossambicus* were higher than control levels. The blood became viscous and difficult to sample. An increase in hematocrit was observed in *Cyprinus carpio* anesthetized with benzocaine hydrochloride (Ferreira et al. 1981) and cells subjected to starvation (Sano 1962). An increase in hematocrit can be ascribed to an increase in numbers of red blood cells (Ferreira et al. 1981; Ross et al. 1986). The lowering of hematocrit in the lowest concentrations tested after 96 hours indicates that the hematological response to stress requires time to develop. The increase of hematocrit, however, did not parallel the hemoglobin content in the present experiment. Hemoglobin was consistently lower in all treatments. The reasons for this difference from other experiments are not known.

Heaviest mortalities occurred in fish showing severe gill epithelial hyperplasia, separation of the gill epithelial layer from supportive tissue, necrosis of intestinal mucosa, necrosis of liver hepatocytes, and necrosis of renal tubules. Both AQTN- and BTN-exposed tilapia had similar histopathological alterations except in the gills.

Gill alterations such as epithelial hyperplasia and separation of the epithelial layer from supportive tissues are usually directly related to gill function disorders, which may affect the physiology or cause the death of fish (Eller 1975; Gardner 1975; Smart 1976). Hyperplasia and separation of the epithelium are associated with asphyxiation; partial or complete loss of secretory or excretory function; impairment of oxygen-carbon dioxide exchange; and loss of plasma electrolytes or proteins (Burrows 1964; Gardner and Yevich 1970; Smith and Piper 1972; Mitchell et al. 1978).

Atrophied and necrotic intestinal mucosa, accompanied by lymphocyte infiltration, have been found in fish exposed to pesticides such as DDT (Walsh and Ribelin 1975; Yokote 1982).

Liver parenchymal necrosis, blood cell congestion, loss of hepatic muralia, and fibroses are non-specific liver lesions associated with pesticide toxicity (Johnson 1968; Eller 1971; Cahn 1975; Couch 1975; Pierce et al. 1980; Solangi and Overstreet 1982).

Necrosis of renal tubules have been reported to affect the metabolic activities and promote metabolic abnormalities in fish (Yokote 1982).

The histopathological alterations found here in the gills, intestine, liver and kidney of *O. mossambicus* seem to be all caused by the pesticide.

Based on these results, *O. mossambicus* can tolerate the levels of AQTN and BTN being used in fish ponds. However, histological analyses of gills, intestine, liver and kidney showed pathological changes even at sublethal levels. Thus, an application factor of 0.1 is recommended to be multiplied with the 96-hour LC_{50} value to estimate the safe concentrations of AQTN and BTN for *O. mossambicus:* 0.30 and 0.01 ppm, respectively.

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Major Diseases Encountered in Controlled Environment Culture of Tilapias in Fresh- and Brackishwater Over a Three-Year Period in Arizona

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Abstract

A number of diseases adversely affected cultured populations of tilapias reared in high density recirculating systems at the University of Arizona's experimental controlled environment fish culture station. The tilapias cultured were *Oreochromis mossambicus*, *O. aureus*, a golden hybrid of *O. mossambicus* x *O. urolepis hornorum*, a Taiwanese red strain of *O. mossambicus* and *Tilapia zillii*.

Gill hyperplasia, presumably due principally to chronic ammonia and nitrite toxicity, was common in recirculated fresh- and brackishwater culture systems. Skin and gill parasitism by the protozoa *Trichodina* sp., *Epistylis* sp., *Ichthyobodo* sp., *Apiosoma* sp. and *Ambiphrya* sp. were also common and were occasionally associated with mortalities. Bacterial septicemias were observed on several occasions. The principal bacterial isolates from affected fish reared in freshwater were *Aeromonas hydrophila* and *Edwardsiella tarda*, while a *Vibrio* sp., *A. hydrophila*, and an *Aeromonas* sp. were the most important organisms isolated from infected fish reared in brackishwater. Fungal infections observed included superficial *Saprolegnia* infections on eggs and fry and a granulomatosis renal infection by an imperfect fungus of the genus *Paecilomyces*. Of interest was the occurrence of tumor-like lesions of the kidneys in two senescent adult golden tilapias.

Introduction

Various tilapia species have been experimentally cultured in high densities (20 to 115 kg/m3) in fresh and saline water (5 to 40 ppt) recirculating systems for the past six years by the Environmental Research Laboratory, Tucson. While disease has not been a major problem in the cultured stocks, occasional disease problems have caused lowered growth rates and decreased production. Additional disease problems have been noted, but did not appear to adversely affect the growth or survival of cultured fish. Described here are those diseases which have caused decreased production and survival. Others included here were of lesser importance, but were of academic interest for comparison with our past experiences and with those of others as reviewed by Roberts and Sommerville (1982) and Paperna et al. (1983).

Materials and Methods

The tilapias cultured were *Oreochromis mossambicus*, original stocks from Fish Breeders of Idaho, Buhl, Idaho; *O. aureus* from Gila River, Gila Bend, Arizona; a golden hybrid of *O. mossambicus* x *O. urolepis hornorum*, from M. Sipe, Palmetto, Florida; *Tilapia zillii*, from Arizona Fish Growers, Camp Verde, Arizona; and a Taiwanese red strain of *O. mossambicus* from J. Walsh, Tucson, Arizona.

The freshwater culture system consisted of fifty 1,000-l round fiberglass tanks, fifty 250-l round fiberglass tanks, and six 1,000-l rectangular tanks, all housed in a 1,800-m2 fiberglass greenhouse. The water was treated in a 10,000-l settling tank and a 15,000-l biological filter. The biofilter was a fiberglass raceway filled with crushed gravel overlaid with crushed oyster shell as the filter matrix. The flow rate through the biofilter was 150-l/min, with a downflow through the matrix. In an adjacent greenhouse, six 10-m3 raceways and ten 100-m3 raceways were constructed as individual units recycling water through biofilters built into one end of the raceway. These units were used to compare filter systems incorporating rotating biological contacters, gravel beds, water hyacinth *(Eichhornia crassipes)* and a variety of vegetables grown hydroponically using the fish wastes as fertilizers.

The brackishwater system was similar, but consisted of only eight 1,000-l round fiberglass tanks (located in the greenhouse), a 10,000-l settling tank and a 15,000-l raceway-type biological filter filled with a matrix of gravel, sand and crushed oyster shell. The settling tanks and biological filters were located outside the end-wall of the greenhouse. All tanks were equipped with a central (in round tanks) or corner (in rectangular tanks) stand pipe and bottom siphon that maintained water depth and removed bottom detritus. Water collected from the stand pipes was returned by gravity to settling tanks where it was drawn off with a 1-hp pump and passed through the biological filters by gravity flow. After passing the biological filters, the water was pumped by a similar pump into manifolds which supplied the fish tanks. All pipes and fittings were PVC. Supplemental aeration in all tanks was supplied by one or more weighted airstones. Compressed oil-free air was supplied by two 1-hp blowers.

Freshwater was recirculated through the system continuously. Approximately 4% of the system's total volume was discharged daily into the city sewer system. Replacement water and added water to compensate for evaporation loss were continuously added using city water directly. Water for the brackishwater system was city water with added commercial rock salt and synthetic sea salts (Instant Ocean) added to a salinity of approximately 20 to 25 ppt. Salinity was adjusted as needed by addition of freshwater to the system. Water temperature typically averaged 28°C ± 5°C.

The fish were fed a pelleted or a cooker-extruded feed prepared from a variety of experimental formulations. Protein levels were maintained between 25 and 35%. In addition to commonly used

feed commodities, a variety of unusual agricultural by-products were also incorporated into some of the diets. These included halophyte straws, water hyacinth, wheat and sorghum straw, and dried marine green algae concentrates. No apparent adverse effects accompanied the use of experimental feeds that contained these ingredients.

For investigation of bacterial infections, all isolates were obtained from the kidneys or from peritoneal fluid of fish displaying gross signs of infection. Media used were tryptic soy agar (Difco Laboratories, Detroit, Michigan) for fish reared in freshwater and marine agar (Difco), tryptic soy agar plus 2% salt, and TCBS agar (thiosulfate citrate bile sucrose agar; Difco) for fish reared in brackishwater. All cultures were incubated at 28°C. After 24 hours positive cultures were restreaked to obtain pure cultures and incubated for an additional 24 hours. Bacterial isolates were classified using the API 20E multitest system (Analytab Products, Plainview, New York).

Tissue samples for histological study were preserved in 10% neutral buffered formalin or in Davidson's AFA (Humason 1967). After 24 to 72 hours in fixative, preserved tissues were dehydrated through a graded series of ethyl alcohol, cleared in xylene or Hemo De (Fisher Products, Phoenix, Arizona), and embedded in Paraplast-Plus (Monoject Scientific, St. Louis, Missouri). Four-micron-thick sections were prepared using a rotary microtome, mounted on glass slides, and stained with Mayer's hematoxylin and eosin or by McManus' PAS stain (Luna 1968).

Results

Hyperplasia of the gill epithelium and fusion of gill lamellae were commonly observed. Presumably, chronic high levels of ammonia and nitrite (Table 1) were the principal causes of this condition. It was occasionally severe enough to contribute, along with protozoan parasites, to mortalities.

Bacterial infections occasionally caused mortalities in some cultured tilapia populations, and occasional serious epizootics in others. Known fish pathogenic bacteria isolated from affected fish reared in freshwater were *Aeromonas hydrophila* and *Edwardsiella tarda*. *Vibrio* sp., *A. hydrophila*, and an *Aeromonas* sp. were isolated from fish reared in brackishwater (Table 2).

In addition to *E. tarda* isolated from the kidney and/or peritoneal fluid of moribund fish, several other species of bacteria were isolated. These included *Pleisomonas (Aeromonas) shigelloides*, *Pseudomonas putrefaciens*, another *Pseudomonas* sp., and species of Enterobacteriaceae. Many of these latter organisms, in addition to *A. hydrophila*, have been reported to be common components of the microflora of tilapia intestines (Sakata and Koreeda 1986).

Only one epizootic due to a bacterial infection was severe enough to warrant treatment with medicated feed. In that instance, several hundred golden hybrid tilapia of 200-g average weight were being reared in several 1,500-l tanks in the brackishwater system. The affected fish developed severe epidermal ulcers, often with tail and fin rot as well. In many fish,

Table 1. Averages and ranges of ammonia (ionized and unionized), nitrite, nitrate, and pH values observed in culture system water over a one-year period, 1986. (Except for pH, values are mg/l).

	Total ammonia	Nitrite	Nitrate	pH
Average	0.88	0.14	21.60	7.60
Range	N.D. - 19.2	N.D. - 10.5	N.D. - 181	6.44 - 8.53

N.D. = Not detected.

Table 2. Bacteria isolated and principal clinical signs associated with bacterial infections of cultured tilapia.

Principal species	Size	System	Primary lesion	Bacteria isolated	Isolated from
O. mossambicus	~200 g	brackish	epidermal ulcers	*Aeromonas hydrophila*	kidney
Taiwan red	~200 g	brackish	septicemias	*Vibrio* sp. *Aeromonas* sp.	kidney
Golden	~650 g	fresh	severe ascites	*E. tarda* *A. hydrophila*	peritoneal fluid and kidney
O. aureus	fry	fresh	off feed small pin-headed	*A. hydrophila*	peritoneal fluid
Taiwan red	adult	fresh	severe ascites	*E. tarda* *Enterobacteria* spp.	kidney
Golden	350 g	fresh	severe ascites	*Vibrio* sp. *Pseudomonas putrefaciens*	kidney
Golden	~2 kg	fresh	dark body color, severe ascites	*E. tarda* *Enterobacteria* spp. *A. shigelloides* *Pseudomonas* sp.	peritoneal fluid

ulceration of epidermis and the underlying muscle and/or necrosis or the caudal peduncle was so severe that vertebrae were exposed. Mortality of affected fish was significant. A salt tolerant, TCBS positive strain of *A. hydrophila* was isolated from the kidneys of the affected fish. The epizootic was successfully stopped and the appearance of affected fish improved within a few days following the initiation of a 14-day treatment with oxytetracycline (Pfizer) medicated feed. The feed was formulated to contain 1.5 g oxytetracycline per kg feed. This ulcerative syndrome has not reappeared in our culture system.

The most severe mortalities (nearly 100%) from disease that occurred in our culture system were due to infection by the fungus *Saprolegnia* sp. In all cases such infections were associated with low water temperatures (< 15°C). In winter, populations reared in outdoor tanks or in unheated greenhouses developed *Saprolegnia* infections, usually starting on the head or caudal peduncle, as soon as water temperatures fell to 15°C. If water temperatures remained below 15°C, infection typically spread and mortalities occurred within 48 hours. Mortalities usually continued until reaching 100%, or subsided when water temperatures warmed above 15°C. Golden hybrids were most susceptible to *Saprolegnia* followed by *O. mossambicus*, *O. mossambicus* (Taiwan red strain), with *O. aureus* being the most cold tolerant. Formalin treatments (at 25 ppm administered as a single dose and not followed by flushing of the treated tanks) lowered mortality rates temporarily, but were generally ineffective if water temperatures remained low.

A single case of a different fungal infection was found in an adult (~850 g), golden hybrid tilapia. That fish, at harvest, was found to possess an enlarged granulomatosis kidney and prominent cottony patches of aerial hyphae on the surface of the peritoneum covering the kidney and on the peritoneum slightly

Table 3. List of protozoan parasites observed in stocks of cultured tilapia.

Protozoan	Life stage affected	Usual site
Trichodina sp.	all	Skin, gills
Epistylis sp.	subadults, adults	Skin
Ichthyobodo sp.	all	Skin, gill, fins
Apiosoma sp. and *Ambiphrya* sp.	juveniles, subadults and adults	Gills
Icthyophthirius multifiliis	fingerlings	Skin, fins, gills

ventrolateral to the kidney. Cultures of the cottony patch from the peritoneum produced a single colony type, which was considered to be a species of the genus *Paecilomyces*. Members of the genus are saprophytes and are occasional pathogens in man and animals (Barnett and Hunter 1972). The genus is also closely related to the genera *Penicillium* and *Aspergillus*, the latter which contains species known to cause mycoses in cultured tilapia (Olufemi et al. 1983; Olufemi and Roberts 1983). Histological sections of the granulomatous kidney showed it to contain many cysts with hyphae with long chains of oval conidia that were characteristic of the genus *Paecilomyces*. The morphology of the hyphae and conidia in tissue sections was nearly identical to that displayed by the fungus in culture, although it was larger in tissue.

A second fish from the same population also possessed tumor-like granulomatosis lesions of the kidney. However, histological study of the lesions did not reveal fungal hyphae or other microbial agents in the otherwise identical granulomatosis lesions. This finding suggests that the *Paecilomyces* infection was opportunistic, and that these granulomatosis lesions of the kidney were due to some other undetermined cause.

Protozoan epicommensals and parasites were commonly observed on the skin, fins and gills of fry through adult tilapia reared in the freshwater system. Parasitic protozoa were only rarely observed on tilapia reared in the brackishwater system. Occasionally the numbers of these organisms became excessive on the tilapia in the freshwater culture system, causing (or associated with) mortalities. The most commonly observed protozoa are listed in Table 3. *Trichodina* and *Ichthyobodo* were often observed together on fish with eroded fins and shallow epidermal ulcers. Fish with *Epistylis* had patches of the colonial protozoan on the epidermis, which often apparently caused the loss of scales at the attachment site. *Epistylis* colonies were commonly in turn colonized by filamentous green algae. Fry and fingerlings infested with *Icthyophthirius multifiliis* displayed grossly visible "white spots" on the fins and epidermis that easily distinguish "Ich" disease. The peritrichs *Apiosoma* and *Ambiphrya* were commonly observed on the gills of juvenile and adult tilapia. Occasionally their numbers were sufficiently abundant to have contributed to poor growth performance and/or mortalities.

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A Note on Infestation of *Oreochromis niloticus* with *Trichodina* sp. and *Dactylogyrus* sp.

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Abstract

Oreochromis niloticus kept in 3,000-l tanks at the Fisheries Research Station, Limbe, Cameroun, were found to be infested with the ectoparasites *Trichodina* sp. and *Dactylogyrus* sp. Treatments with formalin (250 ppm for 35-40 minutes) or potassium permanganate (5 ppm for 10-15 minutes) completely eliminated the parasites.

Introduction

Until recently, fish cultured in Africa were raised in extensive systems at low stocking densities. This reduced problems from parasites and pathogens (Paperna 1982). The tilapias have been remarkably little affected by parasites and diseases and most literature concerning parasites of tilapias deals with their taxonomy and has little coverage of pathological aspects. However, Roberts and Sommerville (1982) consider this because these species are reared in countries with lack of adequate diagnostic facilities.

The control of ectoparasites of fish is an integral part of intensive fish culture (Meyer 1968).

The objectives of this study were to identify parasites infesting *Oreochromis niloticus* kept in tanks at Limbe, Cameroun, and to test some chemicals for treatment. As descriptions of parasites infestations of tilapias are few, it is hoped that this may encourage other workers to inspect their fish and, if necessary, to treat them accordingly.

Materials and Methods

Fish and tanks/aquaria

O. niloticus fingerlings with an average weight of 44.2 g were taken from

the Bambui Fish Culture Station in the northwest of Cameroun to 3,000-l metal tanks at the Fisheries Research Station, Limbe. Each tank was stocked with 16 fish. Fingerlings were fed with rice bran and groundnut cake at 4% of the body weight/day. Fish were sampled at 2-week intervals for adjustment of feed allowance.

Investigations on prophylaxis and chemical treatments were conducted in six 40-l plastic aquaria. Each aquarium was stocked with four fish collected from the metal tanks after a two-month growing period. Their average weight was 44.2 g (± S.D. = 4.6 g). Stream water was used to fill the tanks and to replace losses due to evaporation. The water in the aquaria was changed frequently. Each aquarium had two small aerators. Water temperatures ranged from 24 to 33 C.

Examination of fish

Fish with ectoparasites showed abnormal behavior: loss of escape reaction; tendency to scrape against the tank walls; rapid opercular movements and a tendency to jump out of the water. Such fish were captured carefully with a hand net. A scalpel was scraped along the skin from head to tail and the resulting cutaneous smear was examined under the microscope. To examine gills for ectoparasites a small pipette was inserted inside the operculum. A little water was projected into the cavity and immediately sucked up; the contents of the pipette was placed in a drop of water on a slide, mixed and covered with a coverslip for microscopic examination. Ectoparasites were identified to genera following Meyer (1966).

Chemical treatments

Two treatment chemicals were used in static water conditions: formalin at 250 ppm and potassium permanganate at 5 ppm. Common salt (sodium chloride) was used in tanks for preventive measures at a rate of 25 g per liter of water.

The length of treatments depended on the behavior of fish in the water. When fish began to show signs of distress and turned on their backs or sides, a water flow was started. The fish quickly recovered. The duration for the formalin treatment based on this method of observation was 35-40 minutes. That for potassium permanganate was 10-15 minutes. Regular inspections of fish were made in an effort to detect the presence of parasites after treatments.

Results and Discussion

Two common ectoparasites were found: *Trichodina* sp. and *Dactylogyrus* sp.

Trichodina sp.

Trichodina parasitized the skin and gills. Internal organs were not examined in this study but this parasite has been reported from the urinary bladder of some European fish (Amlacher 1970). *Trichodina* reproduces by binary fission (Roberts and Sommerville 1982). Fish are infected by direct transmission of parasite. Fryer and Iles (1972) cited by Roberts and Sommerville (1982) reported that in mouth brooders *Trichodina* can invade the mouth and transmit the infection to fry.

No serious pathological effects were seen because the infestations were not heavy. According to Amlacher (1970), serious infestations can be fatal because they reduce the respiratory surface of the gills and destroy the skin.

Dactylogyrus sp.

Dactylogyrus was found only on the gills. Amlacher (1970) reported that it occurs chiefly on the gill filaments but, when present in large numbers can be distributed all over the body. *Dactylogyrus* has a direct life cycle typical of monogenean flukes without any intermediate host and transmission is probably

by means of free-swimming oncomiracidia through the water.

Pathological effects

Infested fish showed rapid opercular movements but serious pathological effects were observed because the infestation was not severe. According to Amlacher (1970) the edges of the gills become thickened and the opercula are held somewhat opened when the infestation is severe. Moreover, there can be destruction of the branchial epithelium and rupture of blood vessels leaching to death of severely infested fish.

Chemical treatments

FORMALIN

Both *Trichodina* and *Dactylogyrus* were completely eliminated by a single treatment with 250 ppm formalin for 35-40 minutes. No adverse effects on the fish were noted. The 35-40 minute treatment was briefer than the one hour treatment recommended in much of the literature for temperatures of 30-32 C. Formalin treatment is widely used for protozoan ectoparasites of fish, but it must be used with caution as heavily parasitized fish may be unable to tolerate this level

POTASSIUM PERMANGANATE

Trichodina and *Dactylogyrus* were most difficult to eradicate with a single application of 5 ppm potassium permanganate. Two repeated applications with a 2-day interval were necessary. Four days after this, the fish ceased to scratch against the tank walls. The chemical was washed out by increasing the flow of water through the tank.

After eradication of *Trichodina* and *Dactylogyrus* with formalin or potassium permanganate, bimonthly applications of sodium chloride at a rate of 25 g per liter of water for 15 minutes successfully prevented reinfestation.

Acknowledgements

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SESSION III: GENETICS AND REPRODUCTION

Gonadal Sex Differentiation in *Oreochromis niloticus*

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Abstract

The histological events in normal gonadal development of *Oreochromis niloticus* observed in this study involve the presence of primordial germ cells (PGCs) in the dorsal root of the developing mesentery, in the mesoderm ventral to the gut and in the gut endoderm right after hatching. PGCs shifted bilaterally from the dorsal root of the developing mesentery to localize in the presumptive gonadal regions in fry 1 to 3 days post-hatching. Paired gonadal anlagen appeared in fry at 9 to 10 days posthatching. Ovarian and testicular differentiation indicated by a flat cavity lying along the lateral side of the gonad and slit-like lumen which appear as splits in the stroma tissue packing the centro-lateral region facing the mesogonium, respectively, were observed in fry at 30 to 33 days posthatching. Oogenesis and spermatogenesis occurred in fry of age 56 days and 3 months posthatching, respectively.

Introduction

Oreochromis niloticus exhibits sexual dimorphism and breeds throughout the year. It is one of the largest species of tilapia, reaching 40 to 50 cm in length; has a wide natural distribution in Africa and has been spread throughout the tropics and subtropics for aquaculture; for example over 50,000 t/year are cultured in the Philippines (Guerrero 1987). This study provides information on gonadal development and sex differentiation of *O. niloticus*. A former study on the develop-

ment of *O. niloticus* from fertilization to 10-days posthatching (Galman 1980) contains illustrations and descr tions of organogenesis but no detailed in. rmation on gonadal development.

Materials and Methods

O. niloticus breeders were secured from the Freshwater Aquaculture Center, Central Luzon State University and allowed to breed in tanks. Weekly inspection of the mouths of female breeders was done to see if incubating eggs were present. The eggs were washed out from the breeder's mouth and the day of hatching noted. The culture of eggs and embryos was carried out in 5-l plastic basins three-quarters filled with tap water and aerated continuously. Spoiled eggs were removed daily. Water was changed everyday or more often as necessary. Water temperature ranged from 25 to 26°C.

After hatching, the larvae were kept in the same container until they reached the active feeding and free swimming stage, when they were transferred to 60-l glass aquaria. Stocking density was kept at 10-15 fry/l for the first 30 days after which a stocking density of 5-6 fry/l was maintained until the end of the observation period. Commercial feed (Tetramin Baby Fish Food) was given at 4% body weight thrice daily; the quantity of feed given adjusted weekly.

From the time of hatching, 10 fry were fixed in Bouin's fluid every day for the first two weeks and every three days for the succeeding weeks until sex differentiation was histologically evident. The region between the pronephros and the anus (approximate location of the gonads) was embedded in paraffin wax; sectioned at 8 µm; stained with Delafield's hematoxylin and eosin; and mounted in Permount (Fisher Chemical Co.).

Results and Discussion

At hatching, when fry were 5 to 6 mm, primordial germ cells (PGCs) were found to concentrate along the dorsomedian region of the peritoneal wall at the root of the developing dorsal mesentery. PGCs are distinguishable from the ordinary mesodermal cells by their definite roundish contour, light-staining cytoplasm and larger nuclei (9-12 µm) and cell sizes (16-18 µm). They are similar to the PGCs in *O. mossambicus* described by Nakamura and Takahashi (1973) and in *T. zillii* by Yoshikawa and Oguri (1978). Some PGCs however, were found in places far from the presumptive gonadal region. Some were found lying in the mesoderm ventral to the gut and in the gut endoderm.

The same observations were made in 6-7 mm fry at 1 to 3 days posthatching. However, there was a gradual shifting of PGCs later from the dorsal mesentery root and its localization in the paired presumptive gonadal regions. Most of the PGCs were found at the site where the paired presumptive gonads would arise. The localization of PGCs at the dorsal root of the developing dorsal mesentery and in the mesoderm ventral to the gut at hatching were similar to the findings of Nakamura and Takahashi (1973) and Boco (1977) in *O. mossambicus* and by Yoshikawa and Oguri (1978) in *T. zillii*. Eckstein and Spira (1965) reported that gonia in *O. aureus* are seen at the somatopleure-splanchnopleure junction at 16 days posthatching. The presence of PGCs in the gut endoderm as reported for *O. niloticus* noted in this study was not observed by Satoh and Egami (1972) in *Oryzias latipes* three days after fertilization.

With PGCs at the dorsal root of the developing mesentery, in the mesoderm ventral to the gut and in the endoderm cells of the gut in newly hatched fry of *O. niloticus*, it may be hypothesized that PGCs of *O. niloticus* originate from the endoderm and pass through the mesoderm on their migration to the gonadal region. This is based on the observation that

PGCs at the gut endoderm were found only in newly hatched fry. At all older stages examined, the PGCs were either at the root of the developing dorsal mesentery or in the mesoderm ventral to the gut. With these observations, the proposed path of migration of PGCs to the gonadal anlagen is from the gut endoderm to the splanchnic mesoderm, then dorsally to the dorsal mesentery, and finally laterally to the gonadal primordia. The proposed PGCs migratory pattern in *O. niloticus* is thus similar to that of *Oryzias latipes* (Satoh and Egami 1972) and *Cyprinus carpio* (Remojo 1979).

The ability of PGCs to migrate from the gut endoderm to the presumptive gonadal sites, as observed in the study, is discussed in some works on PGCs migratory activity (Wolf 1931; Johnston 1951; Baker 1972; Spiegelman and Bennett 1973; Clark and Eddy 1975). PGCs are capable of migration by amoeboid movements (Wolf 1931; Johnston 1951); cytolytic properties (Johnston 1951; Baker 1972); possession of micro-filaments in their cytoplasm (Spiegelman and Bennett 1973) and their ability to change shape (Clark and Eddy 1975).

Paired gonadal anlagen were observed at 9 to 10 days posthatching when fry were about 9 mm. This observation coincides with the findings of Nakamura and Takahashi (1973) in *O. mossambicus*. Eckstein and Spira (1965) and Boco (1977) observed it at an earlier stage, 8 days post-hatching and 5 to 7 days post-hatching, respectively. The gonadal anlagen are bounded by peritoneal cells and contain the PGCs and stromal cells, somatic cells scattered in among the PGCs. The paired gonadal anlagen are suspended from the dorsal peritoneal wall of the coelomic cavity by a thin sheet of somatic cells, the mesogonium. In *T. zillii* (Yoshikawa and Oguri 1978), germ cells intrude into the peritoneal cavity enclosed with few somatic cells at 6 days post-hatching.

After 10 days posthatching, there was an increase in number of germ cells which led to slight enlargement of the gonads. The somatic cells of the gonad also increased slightly in number. No distinction as to sex could be observed in the histology of all examined gonads and they were classified as sexually indifferent gonads at this stage.

Using the criteria of Nakamura and Takahashi (1973) on sex differentiation, namely the appearance of ovocoel or testocoel to indicate femaleness or maleness, sex differentiation of *O. niloticus* in this study took place at 30 to 33 days posthatching when the body length was 9-12 mm. Appearance of ovocoel in females and testocoel or efferent duct in males occurred at the same age. This is similar to the findings in *O. mossambicus* by Nakamura and Takahashi (1973) and Boco (1977). In *O. mossambicus*, however, sex differentiation takes place at 16 to 20 days posthatching when the body length is at an average of 8 to 11 mm (Nakamura and Takahashi 1973; Boco 1977). At 48 to 49 days post-hatching, the ovarian cavity or ovocoel in females and testicular cavity or testocoel in males are more distinct.

In fry 56 days posthatching, oogenic cells in the ovary had enlarged to 30 to 70 µm or approximately 2 to 3 times their former size. This indicates that oocyte growth has occurred. Oocytes of the peri-nucleolus stage and oocytes of younger stages which have started growth were observed. The testis at this point remained quiescent, although the stromal tissues with developing efferent ducts increased distinctly in amount. Spermatogenesis occurred in fry at three months posthatching. Spermatogonia, spermatocytes, and spermatozoa were observed. The ovary in females of this age showed large oocytes of 45 to 80 µm in diameter.

As in other tilapia species such as *O. aureus* (Eckstein and Spira 1965) and *O. mossambicus* (Nakamura and Takahashi 1973), oogenesis starts ahead of spermatogenesis. This is also similar to the findings of Goodrich et al. (1934) in *Lebistes reticulatus*, Johnston (1951) in *Micropterus salmoides salmoides*, Onitake (1972) and Satoh and Egami (1972) in

Oryzias latipes, and Takahashi (1975) in *Poecilia reticulata*. It takes a longer time for oocytes to grow as they have to amass large volumes of cytoplasm including yolk; thus, an earlier start in oogenesis will assure synchrony in the development of the gametes.

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Bidirectional-Backcross Selection for Body Weight in a Red Tilapia

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Abstract

Red tilapia fry (*Oreochromis aureus*-based) were cultured for 105 days in 1984 and subsequently selected by sex and color (red- and normal-colored phenotypes) for high (up) and low (down) body weight. A randomly selected population was also developed to serve as a control. Selection differentials in the up and down lines averaged +24 and -28 g, respectively. In 1985 up, down and randomly selected red males (heterozygous for the red-color trait), were backcrossed to female *O. aureus*. Both red- and normal-colored progeny from test matings were costocked into replicate earthen ponds (four replicates per selection mode), at a combined density of 1 m^{-2} and cultured for 105 days. Feeding regime during both years was essentially ad-libitum feeding of a high-protein pelleted diet.

At harvest, mean body weights (g) of up, down and control lines averaged 133, 128 and 133, respectively. The red phenotype (sexes and selection modes pooled), outperformed the normal-colored phenotype (137 g *vs.* 127 g, $P < 0.05$), and males (phenotypes and selection modes pooled), outperformed females (141 g *vs.* 117 g, $P < 0.05$). ANOVA revealed that all first-order interactions involving sex, phenotype or selection mode were significant ($P < 0.01$). Response to selection for body weight varied among phenotypes and sexes as a result of interactions. Realized heritability estimates ranged from -0.75 for up-selected red females to +1.0 for up-selected normal-colored females. Selection for body weight also apparently influenced sex ratios, indicating a correlated response to selection. Offspring from up, down and control lines were composed of 71, 52 and 61% males, respectively.

Introduction

Tilapia culture in temperate climates includes young-of-year culture (Behrends et al. 1985), and overwintering of large fingerlings followed by a second season of growth (Wohlfarth et al. 1983). Either system could benefit from development of fast-growing strains adapted to 5-7 month growing seasons. Mass selection has been

used to improve production traits in several fish (Gjedrem 1983). This relatively simple technique entails selecting individuals on their own phenotypic merit (Falconer 1981). Thus, to improve growth rate in subsequent generations, only the fastest growing individuals are selected and retained as broodstock. Unfortunately, mass selection for increased body weight in the genus *Oreochromis* has generally resulted in little or no response (Chan May Tchien 1971; Hulata et al. 1986; Teichert-Coddington 1983).

Selective breeding research was initiated in 1982 by the Tennessee Valley Authority to develop fast growing, cold tolerant strains of tilapia suitable for pond culture. This paper describes a mass selection experiment in which red hybrid male tilapia (*O. aureus*-based), both up- and down-selected for body weight, were backcrossed to random-bred *O. aureus* females (US strain).

Materials and Methods

A base population of red tilapia was created to evaluate a backcross male selection breeding program for enhancing young-of-year growth rate. The red gene-complex was transferred into a cold-tolerant population of *O. aureus* via hybridization and recurrent backcrossing (Behrends and Smitherman 1984, in press; Fig. 1). F-1 hybrids and subsequent backcross populations were composed of approximately 50% red- and 50% normal-colored phenotypes confirming that red coloration is a heterozygous trait (Behrends and Smitherman, in press).

Breeding protocol used during 1984 (base population) and 1985 (select populations) was similar. Breeding was conducted at 27-30°C in 700-l tanks. Twenty tanks were each stocked with three randomly selected *O. aureus* females and a single red male. Seven to ten days after stocking, females were captured and eggs and/or fry removed from their bucchal cavities. Individual spawns were placed in separate hatching jars, incubated to the free swimming stage and

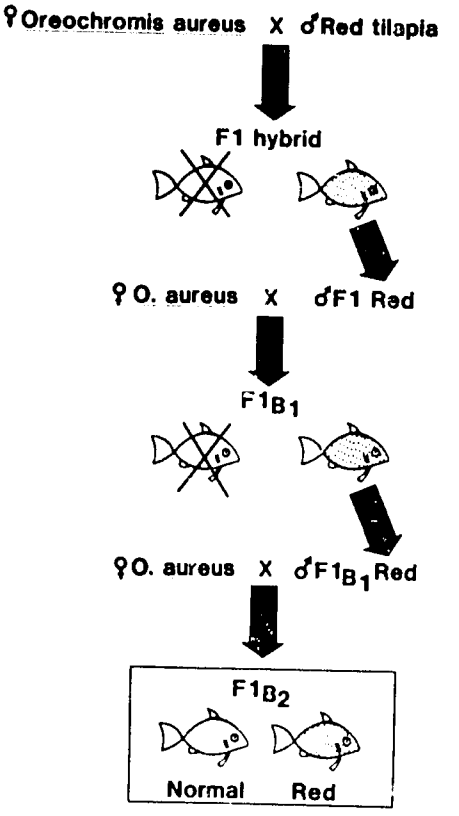

Fig. 1. A base population of red tilapia was developed by repeatedly backcrossing red males (heterozygous for the red-color trait) to random-bred *Oreochromis aureus*.

subsequently nursed for 20-30 days, at which time individual fry ranged from 0.3 g to 0.4 g.

The base population upon which selection was practiced consisted of 949 fish from sixteen families; 12 full-sib and 4 half-sib families. Each family was age-classed according to criteria presented by Hallerman et al. (1983), to account for age-related differences in initial weight. Small differences in initial weight due to environment (age) can result in magnified differences in later growth (Hulata et al. 1986). Thus, four age classes (I, II, III, IV), each differing in age by approximately one day, and each represented by 3-4 spawns, were stocked into replicate 100-m^2 earthen ponds. Equal numbers of both red- and normal-colored fingerlings were

costocked at a combined density equivalent to 8,000/ha. Each age class was replicated four times.

Throughout the pond culture period, fish were fed twice daily, seven days/week, with a 36% crude protein diet (Ralston Purina Company, St. Louis, Missouri). All treatments received the same daily ration, which was progressively increased from 10 kg/ha/day to 40 kg/ha/day. These rates were considered to be in excess of nutritional needs on a per cent body weight basis, but low enough to maintain good water quality. After 75 days of pond culture (July-October), fish were harvested by age-class, separated by color and sex, and individually weighed to the nearest 0.1 g.

Due to highly skewed sex ratios in the red phenotype (84% males) only red males were subjected to selection. Six red males from each pond were retained for broodstock; these consisted of two selected at random, followed by selection of the two largest and two smallest. Thus, 32 each of up-select, down-select and randomly selected males were retained from a total population of 340 red males. Each male was tagged with a color-coded and numbered tag for later identification (Floy Tag Co., Seattle, Washington). Up-selected red males averaged 24 g heavier than the mean for the red male population, while down-selected males were 28 g less than the mean. Standardized selection differentials (selection reach / 1 standard deviation), were 1.2 and -1.4 for up- and down-selected lines, respectively.

In the following year red male broodstock (up, down and random) were backcrossed to random bred female *O. aureus*, (Fig. 2). Thirteen full-sib spawns including 4 up-select, 5 down-select, and 4 random spawns were collected six days after stocking. Hatchery protocol was similar to that described previously with the exception that red and normal-colored sibs within a selection group were separated at hatching and nursed for 20 days prior to stocking into earthen ponds. Equal numbers of red- and normal-colored fingerlings were costocked by selection mode into replicate ponds (four ponds per

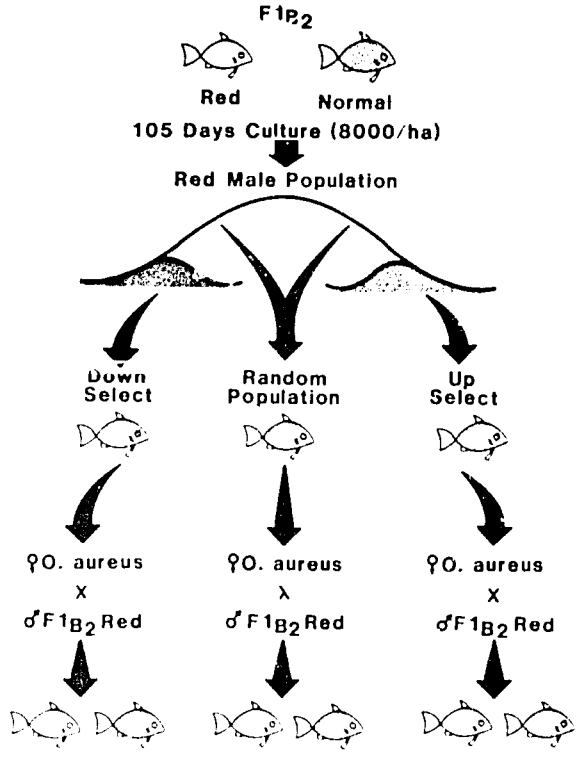

Fig. 2. Red males were bidirectionally selected for body weight. Selection reach was +24 and -28 g in the up- and down-selected populations, respectively. Up-, down- and randomly-selected males were subsequently backcrossed to random-bred *O. aureus* females to produce F1 B3 populations.

selection mode), at a combined density of 10,000/ha. After 105 days of culture, fish were harvested by selection mode, separated by color and sex, and weighed individually to the nearest 0.1 g.

Split plot designs were used during both years. Age and selection mode were designated as whole units during 1984 and 1985, respectively, while phenotype and sex were designated as subunits in both years. The statistical model used during 1984 included effects due to age, pond nested within age, color, sex, and all first and second order interactions. The statistical model used during 1985 included effects due to selection mode, pond nested within selection mode, color, sex, and all first and second order inter-

actions. Body weight data were analyzed by analysis of variance (GLM procedure). Student-Newman-Kuels' Multiple Comparisons Test was used to separate means at a significance level of $P < 0.05$ (Barr et al. 1979). Sex ratio and mortality data, expressed as percentages, were arcsine transformed prior to analysis (Sokal and Rohlf 1981).

Results

Base population

Individual weights at harvest ranged from 15 to 148 g with a mean of 88 ± 16.88 g. Coefficient of variation was 19%. Mean weights at harvest (g) for the four age classes were: 85, 90, 93 and 86 for age classes I, II, III and IV, respectively (Table 1). While age-class means differed by as much as 8 g, differences were not significant ($P > 0.05$). Normal-colored females in age class 4 were significantly heavier than their male counterparts (Table 1).

Color phenotype, sex and the interactions age x sex, sex x phenotype and age x sex x phenotype were all significant factors ($P < 0.01$) affecting growth. Sexually dimorphic growth was influenced by both age and phenotype. Red and normal-colored males (age classes pooled), were 13 g and 6 g heavier than their respective female counterparts. Similar phenotypic differences in sexually dimorphic growth were evident in the parental stocks prior to hybridization (Behrends et al. 1982). Normal-colored tilapia (sexes and age classes pooled) were approximately 7% heavier than their red counterparts (Table 1).

Both mortality and sex ratios differed among phenotypes ($P < 0.05$), but were not influenced by age ($P > 0.05$, Table 2). Mortality of red tilapia averaged 37% compared to 15% for their normal-colored counterparts (Table 2). Historically, mortality in red strains of tilapia has been consistently higher in the red phenotype, both in the hatchery and during pond culture (Behrends et al. 1982; Behrends and Smitherman, in press).

Table 1. Mean weights ($\bar{x}$ ± 1 S.D.) of four age classes of tilapia (base population) as a function of phenotype and sex after 75 days of pond culture. Numbers inside parentheses denote number of individuals represented in mean.[1]

Age class	Weight (g)					
	Red phenotype			Normal phenotype		
	Female	Male	x	Female	Male	x
1	72 ± 15.5 (13)	82 ± 19.0 (84)	80 (97)	86 ± 13.0 (64)	89 ± 16.2 (75)	87 (139)	85 (236)
2	70 ± 10.6 (17)	87 ± 22.4 (78)	84 (95)	85 ± 19.5 (68)	103 ± 21.5 (72)	94 (140)	90 (235)
3	82 ± 18.1 (14)	90 ± 19.0 (88)	89 (102)	93 ± 16.2 (59)	99 ± 18.4 (80)	96 (139)	93 (241)
4	74 ± 16.5 (19)	87 ± 19.5 (90)	85 (109)	91 ± 14.4 (60)	83 ± 16.3 (68)	87 (128)	86 (237)
$\bar{x}$	74 (63)	87 (340)	86 (403)	88 (251)	94 (295)	92 (546)	88.5 ± 16.9 (949)

[1] Means based on pooled weights from four replicate ponds per age class.

Table 2. Mean mortality values and sex ratio data for four age classes of red tilapia. Values represent averages (mortality) or totals (sex ratio data) for four replicate ponds.

Age class	Mortality (%) Red phenotype	Mortality (%) Normal phenotype	$\bar{x}$	Sex ratio data (total count) Red phenotype (# females)	Red phenotype (# males)	Red phenotype (% males)	Normal phenotype (# females)	Normal phenotype (# males)	Normal phenotype (% males)
1	39	13	26	13	84	87	64	75	54
2	41	12	27	17	78	82	68	72	51
3	36	13	25	14	88	86	59	80	58
4	32	20	27	19	90	82	60	68	53
$\bar{x}$	37	15	26	16	85	84	63	74	54

Selected vs. random-bred populations

Table 3 summarizes weight data as a function of selection mode, phenotype and sex. Mean weight of the red phenotype (sexes and selection modes pooled) was 7% heavier than the normal-colored phenotype (Table 3). This is contrary to results reported in the base population (Table 1). In the base population, fry were communally reared, while in the following year, select and random-bred fry were segregated at hatching according to color phenotype. In the base population, communal rearing provided an initial advantage to the normally pigmented fry since their early growth and development was faster (pers. obs., senior author). This initial advantage magnified during later stages of growth.

Population parameters (means ± 1 S.D., and coefficients of variation; sexes and phenotypes pooled), for selected and random bred populations were: up-select (133 ± 28.2, 21.1%), down-select (128 ± 27.3, 21.3%), and random-bred (133 ± 29.8, 22.4%).

Table 3. Mean weights ($\bar{x}$ ± 1 S.D.)[1] of up-select, down-select, and random bred populations of red tilapia as a function of phenotype and sex after 105 days of pond culture. Number inside parentheses denotes number of individuals represented in mean.[1]

Selection mode	Red phenotype Female (g)	Red phenotype Male (g)	Red phenotype x	Normal phenotype Female (g)	Normal phenotype Male (g)	Normal phenotype x	x
Up-selection	113 ± 24.5 (29)	139 ± 27.2 (138)	134 (167)	127 ± 32.8 (72)	136 ± 23.7 (112)	132 (184)	133 (351)
Down-selection	110 ± 19.2 (54)	143 ± 25.2 (116)	133 (170)	116 ± 24.3 (119)	139 ± 26.6 (70)	124 (189)	128 (359)
Random bred	122 ± 32.0 (39)	149 ± 29.1 (130)	143 (169)	115 ± 21.9 (103)	135 ± 23.0 (84)	124 (187)	133 (356)
$\bar{x}$	114 (122)	144 (384)	137 (506)	118 (294)	136 (266)	128 (560)	131 ± 25.3 (1,066)

[1] Means based on pooled weights from four replicate ponds per selection mode.

Full model analysis (ANOVA, GLM procedure), revealed that body weight was strongly influenced by sex ($P < 0.01$), and first-order interactions involving selection mode, sex and phenotype ($P < 0.01$). Differences in body weight among sexes (sexual dimorphism), differed among phenotypes; within the red phenotype, males were 26% heavier than females ($P < 0.01$), while normal-colored males were only 15% heavier than normal-colored females ($P < 0.01$, Table 3).

Significant phenotype x selection mode interactions ($F = 8.1$, $P < 0.01$) revealed that response to selection was phenotype dependent. Therefore, subsequent analyses were performed within phenotype to test the effects of selection and the interactions sex x selection. Selection was not a strong factor affecting body weight or yield in either the red ($P = 0.11$) or normal phenotype ($P = 0.19$), due in part to sex x selection mode interactions ($F = 4.48$, $P < 0.02$). Fig. 3 illustrates the magnitude and direction of sex x selection mode interactions. Note that opposing responses to selection in males and females within phenotype (Fig. 3), tended to obscure the effects of selection within phenotype. Furthermore, differential responses to selection between phenotypes (phenotype x selection mode interaction) also obscured the overall selection response.

Realized heritability estimates for body weight for each sex-phenotype combination are presented in Table 5.

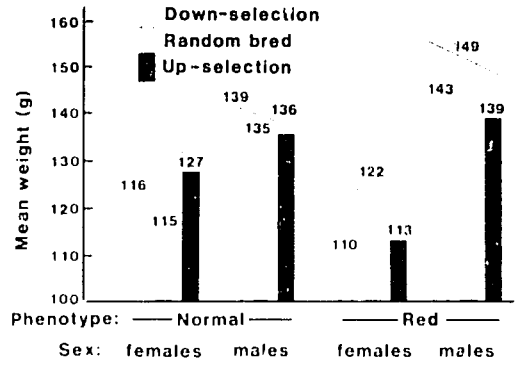

Fig. 3. Mean body weights of red tilapia (*O. aureus*-based) as a function of selection mode, phenotype and sex. Dashed lines illustrate the magnitude and direction of sex x selection mode interactions in each of the phenotypes.

Note that estimates are probably inflated due to intergroup competition resulting from communal rearing practices (Brody et al. 1976). Of the eight heritabilities calculated, three were significantly different from 0 (Table 4).

The strong response to up-selection in the normal-colored phenotype was asymmetrical with respect to sex (female = 1.0 ± 0.12, male = 0.08 ± 0.05). Asymmetrical responses were also reported by Bondari et al. (1983) for an *O. aureus* population subjected to bidirectional selection for body weight.

Mortality was not affected by selection mode ($P > 0.05$), but was influenced by

Table 4. Mean mortality values and sex ratio data for up-select, down-select and random bred populations of red tilapia. Values represent averages (mortality) or totals (sex ratio data) for four replicate ponds.

Selection mode	Mortality (%) Red phenotype	Normal phenotype	x	Sex ratio data (total count) Red phenotype (♂ females)	(♂ males)	(♂ males)	Normal phenotype (♂ females)	(♂ males)	(♂ males)
Up-selection	17	8	12	29	138	83	72	112	61
Down-selection	15	6	10	54	116	68	119	70	37
Random bred	16	7	11	39	130	77	103	84	45
$\bar{x}$	16	7	11	41	128	76	98	89	48

phenotype ($P < 0.05$, Table 4). As in the base population, mortality was twice as high in the red phenotype as in the normal phenotype (Tables 2 and 4).

Sex ratios were influenced by both phenotype, mode of selection, and the interaction phenotype x mode of selection ($P < 0.05$, Table 4). In the random bred population, proportion of males in the red and normal phenotypes averaged 77 and 45%, respectively. These values are somewhat less than values reported in the base population (Table 2). Relative to the random bred population, selection for increased body weight resulted in a correlated 8 and 35% increase in the proportion of males in the red- and normal-colored populations, respectively. Conversely, selection for low body weight resulted in a 12 and 18% decrease in the proportion of males in the red and normal colored populations, respectively (Table 4).

Discussion

Results of this study were complex, sometimes paradoxical, and thus subject to speculation. Response to selection was obscured by interactions involving selection mode, sex and phenotype. Such interactions, if genetic in nature, indicate non-additive sources of genetic variation (Brody et al. 1980), which may be exploited through complex breeding programs, i.e., reciprocal-recurrent-selection (Falconer 1981).

A negative response to up-selection in the red phenotype (Tables 4 and 5), indicates a strong negative epistatic effect of the red gene complex. Paradoxically, while the red phenotype (sexes pooled) did not respond to up-selection, they were significantly heavier than their normal-colored counterparts, irrespective of selection mode (Table 3). We suggest that these differences constitute a measure of heterosis. Chromosome(s) carrying the red-gene complex and associated linked genes were reputedly derived from *O. mossambicus* (Behrends et al. 1982). Homologous chromosome(s) in the normal-colored phenotype have, through introgression, been replaced by *O. aureus* chromosomes. Thus the red phenotype is, and will remain, genetically more heterozygous than its normal-colored counterpart.

Selection for body weight resulted in correlated changes in sex ratios which altered male-female densities. While there is ample documentation showing that individual growth rates in bisexual populations are density dependent, little is known regarding the influence of male-female densities (total density held constant), on growth. Thus selection results and realized heritability estimates may have been influenced by changes in male-female densities. Additional studies should be conducted to determine the magnitude of intra- and inter-sex differences in growth as a function of male-female densities.

Table 5. Realized heritability estimates for red- and normal-colored phenotypes of a red strain of tilapia as a function of selection mode and sex. Standard errors were calculated according to the method of Prout (1962). Asterisks denote estimates that are significantly different from 0.

Selection mode	Sex	Phenotype	
		Red	Normal
Up-selection	Male	−0.83 ± 0.28	0.08 ± 0.05
	Female	−0.75 ± 0.39	1.0 ± 0.12*
	Mean	−0.75 ± 0.47	0.67 ± 0.06*
Down-selection	Male	0.86 ± 0.16*	−0.33 ± 0.08*
	Female	0.43 ± 0.16	−0.07 ± 0.03
	Mean	0.71 ± 0.04*	— 0 —

Proportion of males in the base population averaged 54 and 85% for normal and red colored phenotypes, respectively. Fifty-four per cent is near the expected 1:1 sex ratio common to *O. aureus* (Shelton et al. 1983). The high percentage of red males in the base population indicates linkage, pleiotropy, and/or differential mortality of sexes during early stages of development (see El-Gamal et al., this vol., for data related to early mortality of red tilapias).

Historically, mortality has been consistently higher in the red phenotype, both in the hatchery and during pond culture (Behrends et al. 1982, 1987). Possible reasons for increased mortality also include pleiotropic effects of the red gene complex, linkage and/or differential predation by birds and fish.

To date, no satisfactory sex determining theory has been postulated to explain all the known sex segregations in tilapia hybrids (Wohlfarth and Hulata 1983). In the present study, correlated changes in sex ratios due to selection for body weight indicate that sex determination in *Oreochromis* spp. may be a polygenic threshhold trait (Falconer 1981; Kirpichnikov 1981). Under this hypothesis, genes which influence growth also determine sex, or are closely linked to sex-determining genes. In the context of this study, bidirectional selection for body weight in red male tilapia apparently altered the frequency of growth/sex regulating genes in both red- and normal-colored offspring. Above a specific threshhold, offspring were males, while below the threshold, offspring were females. Hulata et al. (1986), also found that selection for increased body weight in the Ghana strain of *O. niloticus* resulted in male dominated populations.

The polygenic threshhold hypothesis also provides a genetic basis for sexually dimorphic growth in tilapias. The near-normal distribution of sex ratios from pair spawnings of *O. aureus* and *O. niloticus* (Shelton et al. 1983), is consistent with polygenic sex determination. Additional experiments will be required with both pure species and hybrid populations to test and validate the polygenic threshhold theory of sex determination.

Upper limits to selection for body weight in tilapia may be imposed by correlated changes in sex ratios, especially if response to selection is asymmetrical with little or no response in males. Bondari (1983) also reported strong asymmetrical responses to selection for body weight in a population of *O. aureus*. Under these conditions, breeding programs designed to utilize non-additive gene action, i.e., reciprocal recurrent selection, may be productive.

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Development of Biochemical Dichotomous Keys for Identification of American Populations of *Oreochromis aureus, O. mossambicus, O. niloticus, O. urolepis hornorum* and Red Tilapia*

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Abstract

Three populations each of *Oreochromis aureus* and *O. niloticus*, one each of *O. mossambicus* and *O. urolepis hornorum*, and two each of red tilapia derived from hybridization of *O. urolepis hornorum* females and *O. mossambicus* males were compared for the electrophoretic mobilities of their enzymes at 27 enzyme loci using horizontal starch gel electrophoresis. Variation was sufficient to differentiate the species but not all of the populations surveyed. Dichotomous keys based on relative electrophoretic mobilities of isozymes were developed for the identification of the species. The primary diagnostic loci were AAT, EST and SOD in the liver and EST and GPI in the eye. Red tilapia had a high frequency of alleles from *O. mossambicus*, followed by *O. urolepis hornorum*, with low frequencies from *O. aureus* and *O. niloticus*.

Introduction

Electrophoresis of tissue-specific enzymes can be used to determine the purity of fish genomes (McAndrew and Majumdar 1983). Tilapia resemble each other, and some species readily hybridize (Taniguchi et al. 1985). Introgressed populations can be difficult to recognize. Genetic identity of tilapia, including many red tilapias (Behrends et al. 1982; Brummett 1982), has been poorly studied.

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The objectives of the present study were to identify population-specific allozymes, to construct biochemical dichotomous keys to separate species of tilapia and to identify hybrids and introgressed populations.

Methods

Populations

The Auburn University population of *Oreochromis aureus* was obtained from Israel in 1957 as *Tilapia nilotica* and was later re-identified by Trewavas (1966) as *T. aurea*. This population was initiated with three males and one female. The population maintained at Auburn was also distributed to Florida and to the Alabama State Hatchery at Marion in 1960. In this study, Florida population were fish captured from lakes near Leesburg (Crittenden 1962), and Marion population were fish brought back to Auburn to diversify the Auburn line.

The population of *O. mossambicus* was obtained from a fish farmer in Hooper, Colorado, in 1980 (Khater 1985).

The Ivory Coast population of *O. niloticus* has been maintained at Auburn since 1974 when a group of some 100 fry of unknown genetic diversity was received from Fortaleza, Brazil. These fish were offspring of fish from a small group of fish shipped from Bouaké, Côte d'Ivoire in 1971 (Lovshin and da Silva 1974; Smitherman 1988). The Ghana population was randomly selected from fish maintained at the Dor Fish Station in Israel in 1982 (Smitherman 1988). The Egypt population was captured from the Nile delta north of Cairo in 1982 (Khater 1985; Smitherman 1988).

Auburn University obtained its population of *O. urolepis hornorum* from Fortaleza, Brazil, in 1974. These fish originated from the Côte d'Ivoire in 1971 (Smitherman 1988).

Stocks of red tilapia were obtained from the Tennessee Valley Authority (TVA) in Muscle Shoals, Alabama, in 1982 and from a commercial fingerling producer in southern Florida in 1983. *O. urolepis hornorum* ♀ X *O. mossambicus* ♂ hybrids were the original parents of the F_3 (TVA) and F_{11} (Florida) fish used in this study (Behrends et al. 1982; Halstrom 1984). Both F_3 and F_{11} generations originated from the same group of parents in 1980 but were subsequently reared in different areas (Halstrom 1984). Both generations of red tilapias could have been contaminated by *O. aureus* or *O. niloticus* as these fish are cultured in the same areas (Shafland and Pestrak 1982).

Fish from each of these populations were randomly sampled from holding tanks at the Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University, Alabama, USA.

Electrophoresis

The enzyme systems, tissues and gel buffers used in this study are shown in Table 1. Muscle, liver and eye tissue samples from at least 20 individuals of each population of tilapia and at least 10 each of red tilapia populations were compared for the relative electrophoretic mobilities of 22 enzyme loci using standard procedures of horizontal starch gel electrophoresis (Utter et al. 1974), as modified by Brummett (1982). Five additional loci for the enzymes calcium binding protein, carbonic anhydrase, and peptidase were examined for the *O. niloticus* populations (Khater 1985). Gel slices were stained according to the procedures of Shaw and Prasad (1970), as modified by Philipp et al. (1979).

Locus nomenclature was standardized as follows: muscle locus = A; liver locus = B; eye locus = C; mitochondrial locus = D; duplicate loci (Dunham et al. 1980) are designated by Roman numerals, the fastest being I. Allozymes are indicated by Arabic numerals, the fastest being 1. Unless otherwise specified, all bands migrated anodally.

Table 1. Enzyme systems, tissues (loci) and the associated gel buffers, used in this study.

Enzyme**	Enzyme Committee Number	Tissue (Locus)*	Gel Buffer***
Alcohol Dehydrogenase (ADH)	1.1.1.1	L	Tris-Citrate, pH 6.8
Aspartate Amino Transferase (AAT)	2.6.1.1	M,L,Mi	Tris-Citrate, pH 6.8
Calcium Binding Protein (CBP)		M	Tris-Citrate, pH 6.8
Carbonic Anhydrase (CA)	4.2.1.1	L	Tris-Citrate, pH 6.8
Creatine Kinase (CK)	2.7.3.2	M,E	EBT, pH 8.6
Esterase (EST)	3.1.1.1	M,L,E	EBT, pH 8.6
Glucose Phosphate Isomerase (GPI)	5.3.1.9	M,E	EBT, pH 8.6
α-Glycerophosphate Dehydrogenase (α-GPDH)	1.1.1.8	M	Tris-Citrate, pH 6.8
Isocitrate Dehydrogenase (IDH)	1.1.1.42	M	Tris-Citrate, pH 6.8
Lactate Dehydrogenase (LDH)	1.1.1.27	M,L,E	Tris-Citrate, pH 6.8
Malate Dehydrogenase (MDH)	1.1.1.37	M,L,Mi	Tris-Citrate, pH 6.8
Peptidase (PEP)****	3.4.13	L	Tris-Citrate, pH 6.8
Phosphoglucomutase (PGM)	2.7.5.1	M	EBT, pH 8.6
6-Phosphogluconate Dehydrogenase (6-PGDH)	1.1.1.43	L	Tris-Citrate, pH 6.8
Superoxide Dismutase (SOD)	1.15.1.1	L	EBT, pH 8.6

*Tissue abbreviations: M = muscle, L = liver, E = eye, Mi = mitochondria.
**From the procedures of Shaw and Prasad (1970) as modified by Philipp et al. (1979).
***EDTA-Borate-Tris (EBT).
****Three loci were observed in liver.

Results and Discussion

Allele frequencies for polymorphic enzyme loci exhibiting intra- or interspecific variation are in Table 1. Of the 27 loci investigated, nine exhibited interspecific variation: liver ADH; liver and muscle AAT; muscle CK; liver and eye EST; eye GPI; muscle α-GPDH; and liver SOD. Seven loci--liver ADH, liver and muscle AAT, muscle CK, liver EST, liver PEP and liver SOD--showed intraspecific variation.

Liver ADH was polymorphic within the Auburn population of *O. aureus*. Allele B1 was found at a frequency of 0.75, while allele B2 had a frequency of 0.25. Florida and Marion populations were monomorphic for allele B2.

Table 2. Allele frequencies for polymorphic loci of *Oreochromis* spp.

Locus	Allele	*O. aureus* Auburn	*O. aureus* Florida	*O. aureus* Marion	*O. urolepis hornorum*	*O. mossambicus*	*O. niloticus* Ivory Coast	*O. niloticus* Ghana	*O. niloticus* Egypt	Red F3	Red F11
ADH	B1	0.75	0.00	0.00	1.00	0.40	1.00	1.00	1.00	0.00	0.00
	B2	0.25	1.00	1.00	0.00	0.60	0.00	0.00	0.00	1.00	1.00
AAT	B1	1.00	1.00	1.00	1.00	0.00	1.00	0.90	1.00	0.78	0.54
	B2	0.00	0.00	0.00	0.00	1.00	0.00	0.10	0.00	0.22	0.46
	A1	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	-	-
	A2	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	-
	A3	0.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	-	-
CK	A1	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
	A2	0.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.77	-
	A3	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.23	-
EST	BI1	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.41	0.49
	BI2	1.00	1.00	1.00	0.00	1.00	0.00	0.00	0.00	0.57	0.51
	BI3	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.02	0.03
	BII1	1.00	1.00	1.00	0.00	0.00	0.00	0.05	0.25	0.03	0.00
	BII2	0.00	0.00	0.00	0.00	1.00	1.00	0.95	0.75	0.89	1.00
	BII3	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.08	0.00
	BIII1	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.49	0.66
	BIII2	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.51	0.34
	BIII3	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	BIV1	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.50	0.52
	BIV2	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.43	0.39
	BIV3	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	0.07	0.10
	BV1	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.58	0.67
	BV2	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.42	0.33
	CI1	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	0.43	1.00
	CI2	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.57	0.00
	CI3	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.00	0.00
	CII1	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CII2	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.66	0.71
	CII3	0.00	0.00	0.00	1.00	0.00	1.00	1.00	1.00	0.34	0.29
GPI	C1	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.42	0.53
	C2	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	0.58	0.47
PEP	B1	-	-	-	-	-	0.00	1.00	0.64	-	-
	B2	-	-	-	-	-	1.00	0.00	0.36	-	-
SOD	B1	0.72	1.00	1.00	0.00	0.00	1.00	1.00	1.00	0.00	0.00
	B2	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.37
	B3	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	0.63	0.63

Liver AAT was polymorphic among populations of *O. nilcticus*. The Ghana population had a frequency of 0.10 for the B2 allele while Egypt and Ivory Coast populations were monomorphic for the B1 allele.

The muscle AAT locus of Auburn *O. aureus* possessed a faster A2 allele than was found in the Florida or Marion populations which were monomorphic for A3 allele.

Muscle CK varied among populations of *O. aureus*. The Auburn population was monomorphic for the A1 allele, while the Florida and Marion populations were monomorphic for the A2 allele.

Liver EST BI1 was polymorphic for *O. niloticus*. The Ivory Coast population was monomorphic for allele BII2, while Egypt and Ghana populations were polymorphic for alleles BII1 and BII2.

PEP exhibited considerable variation among the populations of *O. niloticus*. The Ivory Coast and Ghana populations were monomorphic for B1 and B2 alleles, respectively. Egypt had both alleles.

The Auburn population of *O. aureus* was polymorphic for B1 and B2 alleles at the liver SOD locus, while both the Florida and Marion populations were monomorphic for allele B1.

Sufficient isozymic variation was found to permit construction of biochemical dichotomous keys for the separation of the four species (Fig. 1). Two dichotomous keys were developed: one employing liver and eye loci resolvable only for the EDTA-borate-tris buffer system; the other employing liver loci resolvable on EDTA-borate-tris and tris-citrate buffer systems.

Inadequate variation among populations of *O. aureus* sampled in this study prevented assignment of all individuals to one of the three populations. However, the Auburn population had faster alleles than the other two populations at both muscle AAT and muscle CK loci. These differentiate the Auburn population from Florida and Marion populations, which were identical at all loci examined. Since these populations were derived from the Auburn population, the differences at these loci may be caused by random genetic drift due to the founder effect. The polymorphisms at the liver SOD and liver ADH loci might be usable to identify populations, if sample sizes are large enough to accurately calculate allele frequencies. These genes might also be usable as genetic markers if they were incorporated into a line in a controlled manner.

Variation among *O. niloticus* samples was insufficient to distinguish the three populations. The Ghana and Egypt populations were somewhat more variable than the Ivory Coast population. However, the polymorphisms that do exist could possibly be used to separate populations if sufficiently large samples were taken.

There were no definitive data indicating the presence of *O. aureus* alleles for ADH B, CK A, EST CII, GPI C or SOD B in either the F_3 (TVA) or F_{11} (Florida) red tilapia. Except for SOD B2 (f = 0.37 in both F_3 and F_{11}), *O. aureus* and *O. niloticus* alleles occur at very low frequencies in the red tilapia. EST BII and CI alleles from *O. aureus* found in low and high frequencies, respectively, in F_3 fish were not found in the F_{11} fish. EST BIV alleles from *O. aureus* remained unchanged at low frequencies. AAT B2 from *O. niloticus* was also lower in the F_{11} than in the F_3 generation. The red tilapia show high frequencies of *O. mossambicus* and *O. urolepis hornorum* alleles, especially at the highly polymorphic esterase loci.

Based on these results, the red tilapia analyzed was a hybrid between *O. mossambicus* and *O. urolepis hornorum* with a small amount of introgression from *O. aureus* and *O. niloticus*. The *O. mossambicus* lineage exhibits the strongest influence, as its alleles were always present at high frequencies. *O. urolepis hornorum* alleles were not always so frequent and ADH B and EST BIII alleles from *O. urolepis hornorum* were apparently eliminated through selection for red color. In the two cases where there is a significant change in allele frequency from F_3 to F_{11} (AAT B and EST BIII), the increase in frequency was toward the *O. mossambicus* allele. This may be due to

A. Enzyme Systems Resolvable with EDTA-Borate-Tris Buffer System.

1.	SOD-Liver	faster than *O. urolepis hornorum*, *O. mossambicus*; equal to *O. aureus* *O. niloticus*; occasionally with polymorphisms	2
1'.	SOD-Liver	much slower than *O. aureus*, *O. niloticus*; equal to *O. urolepis hornorum*, *O. mossambicus*	3
2.	EST-Eye (CI)	slower than *O. urolepis hornorum*, *O. mossambicus*; faster than *O. niloticus*	*O. aureus*
2'.	EST-Eye (CI)	slower than *O. aureus*, *O. urolepis hornorum*, *O. mossambicus*	*O. niloticus*
3.	GPI-Eye	equal to *O. aureus*, *O. niloticus*; slower than *O. mossambicus*	*O. urolepis hornorum*
3'.	GPI-Eye	faster than *O. aureus*, *O. urolepis hornorum*, *O. niloticus*	*O. mossambicus*

B. Enzyme Systems Resolvable in Liver Tissues Only:

1.	SOD-Liver	faster than *O. urolepis hornorum*, *O. mossambicus*; equal to *O. aureus*, *O. niloticus*; occasionally with polymorphism	2
1'.	SOD-Liver	much slower than *O. aureus*, *O. niloticus*; equal to *O. urolepis hornorum*, *O. mossambicus*	3
2.	EST-Liver (BIII)	Locus present	*O. niloticus*
2'.	EST-Liver (BIII)	Locus absent	*O. aureus*
3.	AAT-Liver	equal to *O. aureus*, *O. niloticus*; faster than *O. mossambicus*	*O. urolepis hornorum*
3'.	AAT-Liver	slower than *O. aureus*, *O. urolepis hornorum*, *O. niloticus*	*O. mossambicus*

Fig. 1. Keys for the identification of four *Oreochromis* species based on relative electrophoretic mobilities of enzymes.

repeated selection for the improvement of the red coloration, a mutation originally observed in *O. mossambicus*.

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Cytogenetical Characterization of *Oreochromis niloticus, O. mossambicus* and Their Hybrid

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Abstract

Cytogenetical analyses were carried out with Ag-staining, C- and G-banding on *Oreochromis niloticus, O. mossambicus* and their F1 hybrid *O. niloticus* x *O. mossambicus*. Karyotypes were similar, with 44 chromosomes of which 4 were readily identifiable by size; the other 40 were difficult to classify into submetacentric (sm) or subtelocentric (st) because of the small size of their short arms, where present. Distribution of biarmed chromosomes differentiated *O. niloticus* with a mode of 14 biarmed, from *O. mossambicus* with a mode of 6; the hybrid had an intermediate mode of 10. Ag-staining techniques, evidencing those nucleolus organizer regions (NORs) active in the preceding interphase, discriminated the parental species (maximum 5-6 Ag-stained NORs) from the hybrid (maximum 4 Ag-stained NORs). C-banding techniques identified several homolog pairs and constitutive heterochromatin associated with ribosomal genes in some NOR-bearing chromosomes of both species.

Introduction

About 70 species of Tilapiinae have been subdivided into the genera *Oreochromis, Sarotherodon* and *Tilapia* according to their different spawning behavior and parental care (Trewavas 1982). Twenty of these have been analysed karyologically, and a high homogeneity appears: 2n = 44, with 2 pairs of marker chromosomes much larger than the others, and minor differences in the number of biarmed chromosomes.

The evolution of the Tilapiinae has therefore not been characterized by major important karyological changes. This is further substantiated by the ease with which many tilapias hybridize (Wohlfarth and Hulata 1983).

Karyotype studies were made on *Oreochromis niloticus* and *O. mossambicus*, and their hybrid *O. niloticus* x *O. mossambicus*. The karyotypes of the parental species are already known (Table 1). The aim of this work was to investigate fine differences

Table 1. Chromosomal studies on *Oreochromis niloticus*, *O. mossambicus* and hybrids.

Species	2n	Karyotype	NF	Number of biarmed	Reference
O. niloticus	44				Chervinski (1964)
	44				Jalabert et al. (1971)
	40				Badr and El-Dib (1976)
	44	1m+8sm+13st, a	62	18	Arai and Koike (1980)
	44	ZW ♀, ZZ ♂			Nijjhar et al. (1983)
	44	1m+9sm+6sm—st+7st	64	20	Majumdar and McAndrew (1986)
	44			14 (9-17)	present work
O. mossambicus	44	44m	88		Natarajan and Subrahmanyam (1974)
	44	9st+26a	62		Fukuoka and Muramoto (1975)
	44	3st+19t	44		Prasad and Manna (1976)
	44	3st+19t	44		Krishnaja and Rege (1980)
	44	3sm+19st	44-50	6	Thompson (1981)
	44	3sm+4sm—st+15st	50	6	Majumdar and McAndrew (1986)
	44			6 (2-10)	present work
Hybrid (*O. niloticus*, *O. mossambicus*)	44	3m+2st+17a		10	Sanchez et al. (1983)
F_1 *O. niloticus* x *O. mossambicus*	44			10 (5-14)	present work

a : acrocentric
m : metacentric
NF : nombre fondamental
sm : submetacentric
st : subtelocentric
t : telocentric

using C-, G-banding and Ag-staining, which detect active nucleolus organizer regions (NORs). Few references to C-banding in tilapias exist (Kornfield et al. 1979; Majumdar and McAndrew 1986) and NOR-bearing chromosomes are reported only in *Sarotherodon galileus* (Kornfield et al. 1979). No comparison of plates treated with different techniques was made. Banding techniques may also give information on sex chromosomes, the presence of which was reported in *O. niloticus* by Nijjhar et al. (1983), but not confirmed in later work (Majumdar and McAndrew 1986).

Karyotype analysis of the hybrid may be useful for the direct comparison on the same plate of parental haploid chromosome complements, and for detection of F1 hybrids.

Cytogenetic markers are useful in aquaculture for stock identification, and for controls in breeding and selection programs, particularly as tilapias have been widely hybridized and distributed all over the world.

Materials and Methods

Oreochromis niloticus, *O. mossambicus* and their F_1 hybrid, *O. niloticus* x *O. mossambicus*, were studied (Table 2). The fish were bred and reared in the laboratories of the Department of Animal and Human Biology, University of Rome. The *O. niloticus* strain came from

Table 2. Summary of cytogenetic observations made on *Oreochromis niloticus*, *O. mossambicus* and their hybrid.

Species	Number of animals (m, f, juv)	Number of metaphases examined	Number of metaphases karyotyped	Cells with 2n < 44	Cells with 2n = 44	Number of C-banded metaphases	Number of G-banded metaphases	Number of Ag-stained metaphases	Number of associations*
O. niloticus	5 (2, 1, 2)	148	18	20	128	48	30	104	17
O. mossambicus	4 (2, 2, 0)	147	26	22	125	37	48	77	0
F_1 *O. niloticus* x *O. mossambicus*	4 (1, 2, 1)	112	15	10	102	34	26	93	3

*Association : arm to arm pairing of NOR bearing chromosomes

Lake Manzala (Egypt); *O. mossambicus* obtained from Station Piscicole de Godomey (Bénin) was originally from Mozambique.

Three hours before being killed, the fish received a Velbe injection (Lilly, 1 mg/ml, 0.2 ml/20 g weight). Metaphase plates from homogenized head kidney were prepared according to the air-drying method (Hitotsumachi et al. 1969). Plates were Ag stained by the method of Howell and Black (1980), treated with potassium and hexacyanoferrate to remove Ag nitrate, Giemsa stained, then either (a) C-banded by the method of Sumner (1972) as modified by Bickham (1979), or (b) G-banded according to Seabright (1972). After each staining technique, the same metaphase plate was photographed.

Tilapia karyotypes are characterized by 44 chromosomes, four are large and readily classified; among the other 40, some have short arms, small and gradually decreasing in size. It is difficult to distinguish submetacentric (sm), submeta-subtelocentric (sm-st) borderline chromosomes, and subtelocentric (st) chromosomes. Even in the same individual, different plates with different condensation levels show different numbers of biarmed chromosomes. The experimental error in measuring the centromeric index (Levan et al. 1964) is greater than the variability in the length of the short arms. In defining here the number of biarmed chromosomes we give the range of variation of the biarmed chromosomes numbers and the mode because of artifacts and difficulties of categorization. Chromosomes were recorded as biarmed when short arms were recognized for size and condensation levels comparable to the long arms.

Results

Table 2 summarizes the cytogenetic observations.

The 62 *O. niloticus* metaphase plates revealed 9-17 biarmed chromosomes with a mode of 14 (Fig. 1a). The representative karyotype is illustrated in Fig. 2a (upper rows); 14 biarmed chromosomes were evident. The 65 *O. mossambicus* cells revealed a lower number of biarmed chromosomes (Fig. 1b) around a mode of 6, as evident in the karyotype in Fig. 2b (upper rows).

In the hybrid 2n was 44; cells with lower diploid number due to chromosomal losses during preparation (Table 2) had similar frequencies as in the parental species. The 37 metaphases studied for biarmed composition gave a mode corresponding to the expected value, 10, i.e., the sum of the parental haploid sets (Fig. 1c). The karyotype is shown in Fig. 2c (upper rows).

Particular emphasis was given to the morphology of the first chromosome pair, which is reported to be heteromorphic in *O. niloticus* females (Nijjhar et al. 1983). In both species, this first pair is frequently variable morphologically, but no sex-dependent variability was observed (Figs. 3a, b). In the hybrid (Fig. 3c), the two parental chromosomes 1 were recognizable, as *O. mossambicus* 1 usually have more evident short arms than *O. niloticus* 1. By direct comparison, *O. mossambicus* chromosome 1 therefore appears smaller than that of *O. niloticus* 1.

C-banding (Fig. 2, lower rows) showed centromeric constitutive heterochromatin in most chromosomes of the two species and the hybrid. Although it did not permit

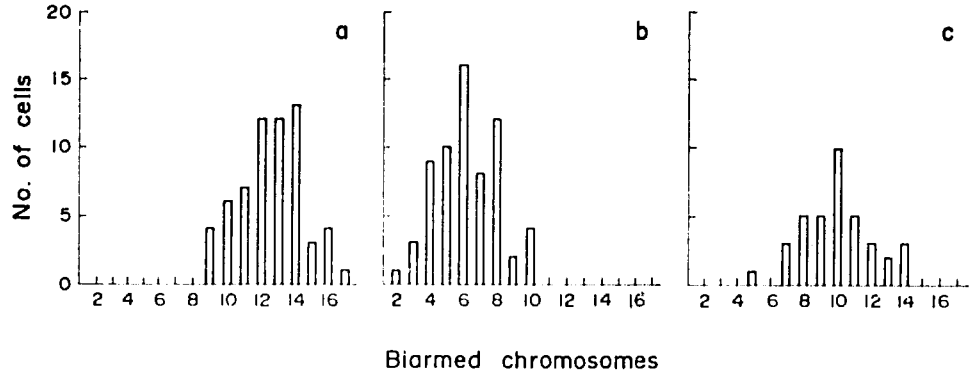

Fig. 1. Distribution of biarmed chromosomes in *Oreochromis niloticus* (a), *O. mossambicus* (b) and *O. niloticus* x *O. mossambicus* (c) identified with Ag-NOR staining.

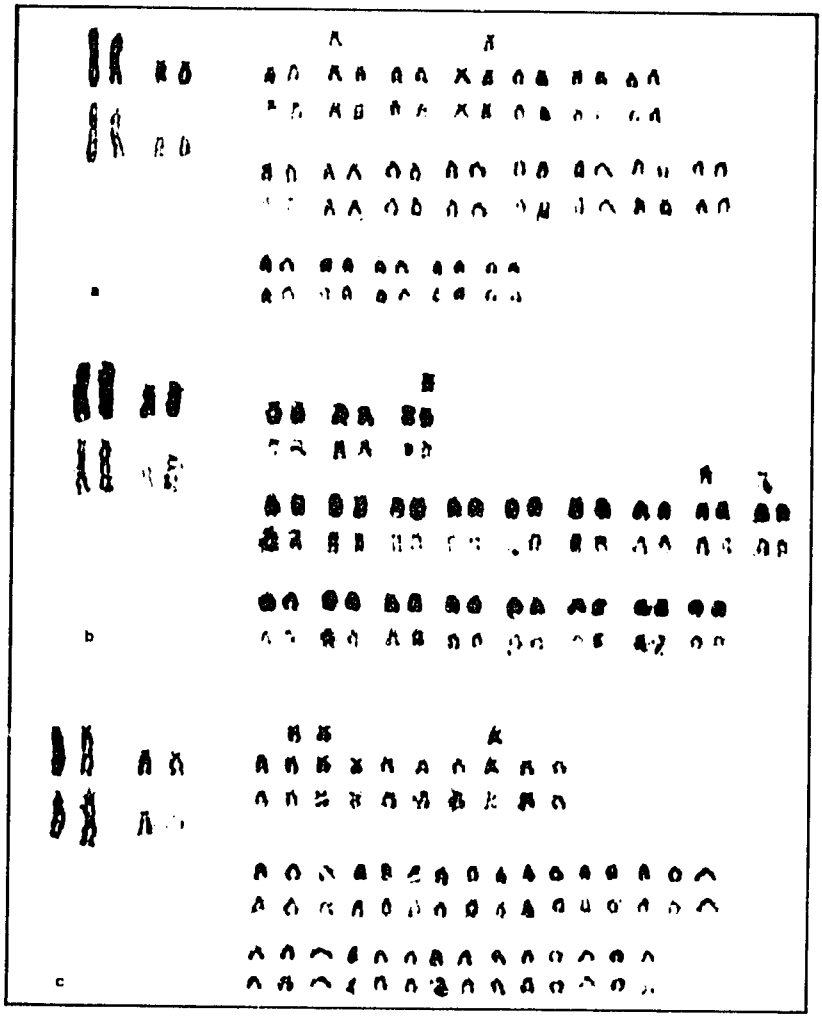

Fig. 2. Giemsa-stained (upper rows) and C-banded (lower rows) karyotypes of *Oreochromis niloticus* (a), *O. mossambicus* (b), *O. niloticus* x *O. mossambicus* (c). Ag-stained NOR-bearing chromosomes are also shown.

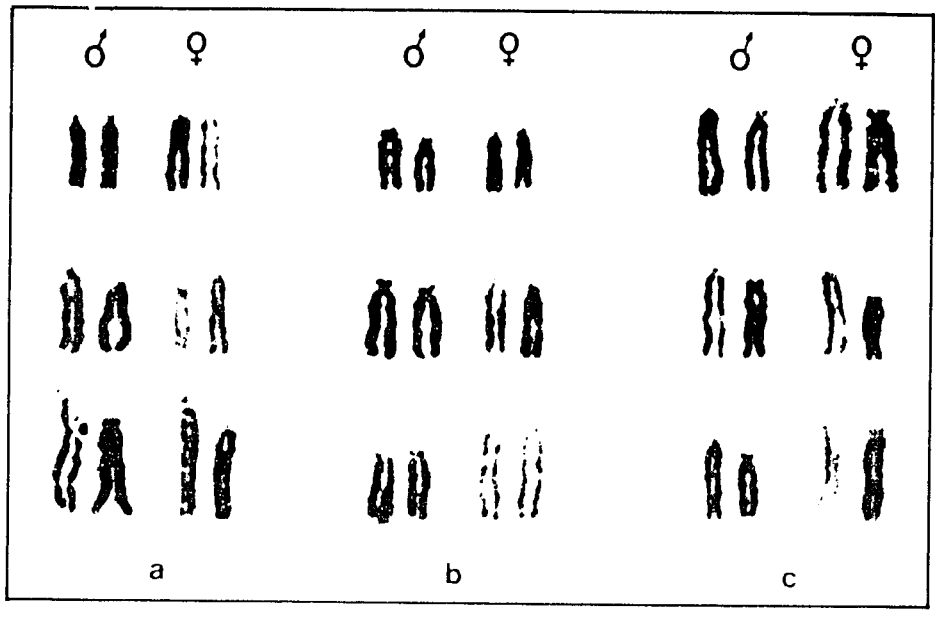

Fig. 3. Three sets of Giemsa-stained pairs of chromosomes (No. 1) from one male and one female in *Oreochromis niloticus* (a), *O. mossambicus* (b), *O. niloticus* x *O. mossambicus* (c).

identification of marker chromosomes, it enabled the matching of several homolog pairs. *O. niloticus* chromosomes 6, slightly heteromorhic in Giemsa, were matched after C-banding for their heterochromatic short arms. The short arms of many biarmed chromosomes in parental species and hybrids were entirely C-banded. C-banding did not identify any sex-specific patterns.

G-banding was performed on over 100 metaphase plates from the two species and the hybrid. Only in *O. niloticus* was a repetitive pattern obtained (Fig. 4).

Ag-staining revealed the presence of NORs on short arms of some biarmed and subtelocentric chromosomes in both species and the hybrid (Fig. 5). Cells commonly had 2 or 3 Ag-stained NORs. The parental species had a maximum of 6

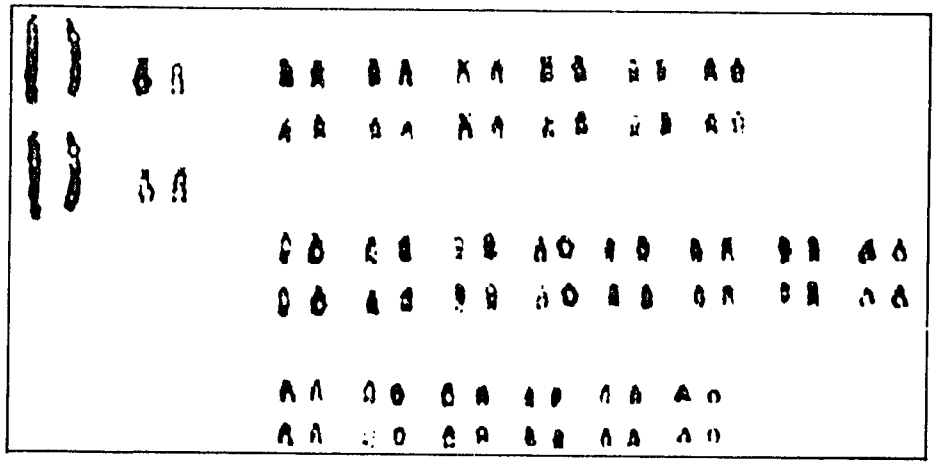

Fig. 4. Giemsa-stained (upper rows) and G-banded (lower rows) karyotypes of *Oreochromis niloticus*.

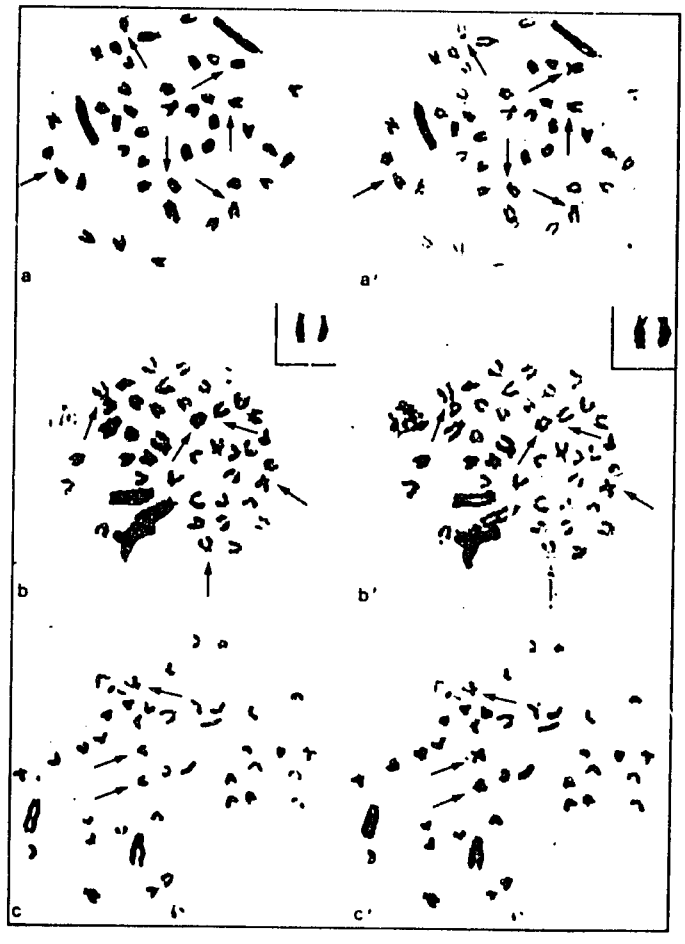

Fig. 5. Giemsa-stained (left) and Ag-stained metaphase plates (right) in *Oreochromis niloticus* (a, a'), *O. mossambicus* (b, b'), *O. niloticus* x *O. mossambicus* (c, c'). Arrows indicate NOR-bearing chromosomes.

Ag-stained NOR-bearing chromosomes per cell; the hybrid had a maximum of 4 (Fig. 6). The parental species had at least 3 pairs of NOR-bearing chromosomes. This number could be low, as the small size of the NORs made detection difficult. In *O. mossambicus* there was evidence of a 4th NOR-bearing pair: in addition to cells bearing 5 or 6 NORs on small chromosomes, several other cells (8/77) showed marks on one homolog from the 2nd pair (Fig. 5b', insert). It was difficult to recognize NOR-bearing pairs and to compare the species to determine homologies, also because after C-banding some NORs were C-positive in both species (Fig. 2).

Discussion

Previous data on *O. niloticus* and *O. mossambicus* karyotypes (Table 1) differ from these data in defining the number of biarmed chromosomes and consequently the "nombre fondamental" (NF, Matthey 1949). The present data indicate fewer biarmed chromosomes in *O. niloticus* than other authors. Majumdar and McAndrew (1986), in their work on seven species of Tilapiinae, inferred that most differences in data were the result of inaccuracy in classification, in other words technical artifacts. Kornfield (1984), in a review on cichlid genetics, pointed out that in spite of the use of standard definitions for

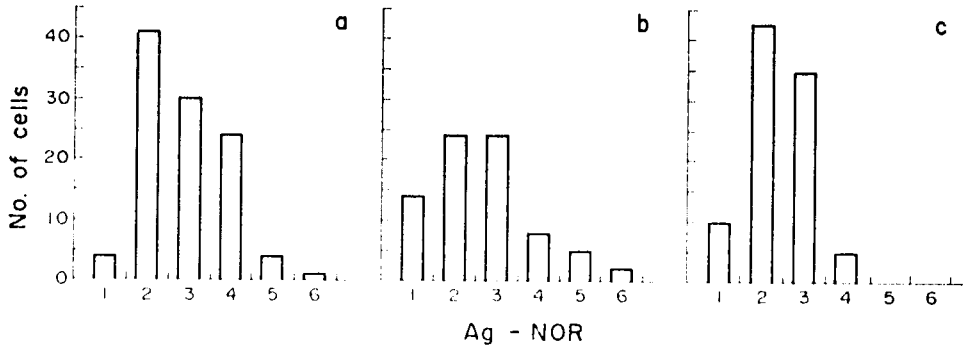

Fig. 6. Distribution of Ag-stained NOR-bearing chromosomes. *Oreochromis niloticus* (a), *O. mossambicus* (b), *O. niloticus* x *O. mossambicus* (c).

chromosome morphology (Levan et al. 1964) discrepancies were typical for studies of the same species.

These differences in data are probably because calculations of centromeric index cannot be performed correctly due to the minute differences among chromosomes and the size of short arms.

Results from all studies on both species reveal that *O. niloticus* has more biarmed chromosomes than *O. mossambicus*, and that their karyotypes have differentiated little.

After C-banding, there was no evidence of great changes in constitutive heterochromatin. The association between constitutive heterochromatin and ribosomal genes could be one cause of morphological variability of biarmed chromosomes and thus discrepancies among different studies. The more studies are performed on teleosts with Ag-staining techniques, the more morphological variability of NORs is reported (Gold 1984; Moreira-Filho et al. 1984; Sola et al. 1984).

The discovery of Ag-stained NORs on pair No. 2 in *O. mossambicus* is an important difference from *O. niloticus*, even though the marker was found only on a few plates in a single specimen. Further studies with chromomicin A_3, which stains structural NORs (Amemiya and Gold 1986; Mayr et al. 1986a, 1986b) and not functional NORs as does Ag (Miller et al. 1976a, 1976b; Croce et al. 1977), are being carried out to confirm this. In neither species or in the hybrid were there the high number of associations (Table 2) reported by Kornfield et al. (1979) for *Tilapia zillii*.

The number of Ag-stained NORs in the hybrid was seldom 4, never 5 or 6 (Fig. 6). Even though in the parental species the frequency of cells with 5 or 6 Ag-stained NORs is low, the number of active NORs in the hybrid may indicate a regulation process which suppresses NOR activity of the chromosomes from one of the parents. This has been observed in other vertebrate interspecific hybrids (Howell 1977).

The data on biarmed number for *O. niloticus* x *O. mossambicus* are similar to those of supposed hybrid populations from Mexico studied by Sanchez et al. (1983). Those populations were the result of the introduction of *O. mossambicus* and *O. niloticus*. The authors pointed out the limited value of conventional karyotypic analysis to clarify the biological identity of the introduced species. However, neither C-banding nor Ag-staining techniques provided chromosomal markers.

G-banding techniques can give more information but were not successful, except with *O. niloticus*. It is, however, impossible to identify marker chromosomes without direct comparison with other species. Some biarmed chromosomes showed short arms faintly stained after G-banding. Presumably

these were C-positive, as reported for *Anguilla anguilla* and *A. rostrata* (Sola et al. 1984). The difficulties in performing G-banding make this technique unsuitable for rapid identification.

Another difference between the two species is the morphology of chromosome 1. This is probably due to a major extension of constitutive heterochromatin in *O. niloticus*, but the C-banding pattern is not informative enough. Use of this pair as marker chromosomes to detect the F1 hybrid requires many observations due to the high morphological variability in the parental species, sometimes overlapping those of the hybrid.

This variability could explain the heteromorphism found in *O. niloticus* females by Nijjhar et al. (1983). These data indicate that morphologically differentiated sex-chromosomes are not identifiable, even through C-banding. These results are similar to those of Majumdar and McAndrew (1986) for the same species, and consistent with their and Kornfield's (1984) postulation that a strong chromosomal sex determination is unlikely in such fish as tilapias, where such "evolutionary malleability of sexuality exists".

Acknowledgement

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Viability of Red and Normal-Colored *Oreochromis aureus* and *O. niloticus* Hybrids*

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Abstract

Viability of red and normal-colored hybrids of *Oreochromis aureus* and *O. niloticus* was compared. Full-sib groups were produced by making reciprocal crosses between a red *O. aureus* hybrid and *O. aureus* and between a red *O. niloticus* hybrid and *O. niloticus*. The ratio of red:normal-colored embryos in each group was essentially 1:1. Hatchability of red embryos was significantly lower than that of normal-colored embryos. Fry were grown in a hatchery for 3 weeks; viability of the two color phenotypes was not different. Viability of red and normal-colored fry was the same during sex reversal. However, when the groups were grown for an additional 6 weeks in 20-m^2 concrete tanks, survival of red sibs was significantly less ($P = 0.05$). Fish were grown to market size in 20-m^2 concrete tanks and 0.04-ha earthen ponds. Survival of red sibs was significantly less than that for normal-colored sibs both in open tanks and ponds. These data indicate that subviability is a negative pleiotropic effect of the red genotype.

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Introduction

Red tilapia are more marketable than normal-colored fish in certain locales (Fitzgerald 1979; Galbreath and Barnes 1981). However, before commercial culture of red tilapia can be recommended, the pleiotropic effects of this phenotype on important production traits must be identified and quantified. Many abnormal body colors have negative pleiotropic effects (Tave 1986). For example, Wohlfarth and Moav (1970) found that both blue and gold common carp had lower growth rates than normal-colored common carp. Albino channel catfish produced eggs that had lower hatchability and produced progeny that were less viable than normal-colored channel catfish (Bondari 1984).

The pleiotropic effects of the alleles that produce red pigmentation in tilapia on important production characteristics such as viability and growth are not known. Previous research with red tilapia at Auburn University suggested that red tilapia were less viable than their normal-colored counterparts. The objective of this study was to determine viability of red *Oreochromis aureus* and *O. niloticus* hybrids.

Materials and Methods

Fry production

Broodstocks used in this study were: 1. *O. aureus* (Auburn University strain); 2. *O. niloticus* (Auburn University-Egypt strain); 3. red *O. aureus* hybrids (commercial strain of red tilapia backcrossed to *O. aureus* for three generations); and 4. red *O. niloticus* hybrids (commercial strain of red tilapia backcrossed to *O. niloticus* for three generations). The single species broodstocks were produced at the Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University, Alabama. The red hybrids were produced at the Tennessee Valley Authority (TVA) Agricultural Research Farm, Muscle Shoals, Alabama. These broodstocks were used to make the following matings:

O. aureus x *O. aureus* (A x A)

O. niloticus x *O. niloticus* (N x N)

O. aureus x *O. aureus* red hybrid (A x R)

O. niloticus red hybrid x *O. niloticus* (R x N)

Matings were made in nets (hapas) suspended in 360-l tanks; confining broodfish in hapas facilitated monitoring of spawning activity.

One week after stocking broodfish, eggs and sac fry were removed from females and incubated in McDonald jars. At the eyed stage (4 days old), a sample of each family was examined microscopically to enumerate the color phenotypes. Melanistic pigmentation existed only in the normal-colored embryos. As soon as swim-up fry were obtained, color phenotypes for each family were enumerated.

Sex reversal

One week after hatching, each of the four genetic groups of fry was divided into two subgroups: one subgroup was given feed containing 60 ppm 17α-ethynyltestosterone *ad libitum;* the other subgroup received feed with no hormone. This study was conducted at an indoor hatchery at the TVA Agricultural Research Farm.

Two hundred and seventy fry of each group were stocked in three replicate hapas suspended in 360-l tanks. For those groups that had both color phenotypes (A x R and R x N), 45 normal-colored fry and 45 red fry were stocked in each hapa. After 22 days, fish were enumerated and survival was determined.

Fingerling production

Fry from each hormone-treated or control group were pooled, transported to the Auburn University, and randomly

reallocated to three outdoor concrete tanks (20 m^2; 0.75 m deep). Eighty-eight fry from each group (average weights ranging from 0.38 to 0.62 g) were stocked in each tank. For those groups that had both phenotypes (A x R and R x N), 44 normal-colored and 44 red fry were stocked in each tank. Fry were fed *ad libitum* once daily with 32% protein crumbles and/or 36% protein floating catfish feed. After 42 days, fish were enumerated and survival was determined.

Growout

Concrete tanks. Fingerlings from each hormone-treated or control group were pooled and randomly reallocated to three replicate outdoor concrete tanks (20 m^2; 0.75 m deep) at Auburn University. Thirty fingerlings from each group (average weights ranging from 16 to 24 g) were stocked in each tank. For those groups that had both color phenotypes (A x R and R x N), 15 normal-colored and 15 red fingerlings were stocked in each tank. Fingerlings were fed 32% protein floating catfish feed *ad libitum*. After 95 days, fish were enumerated and survival was determined.

Earthen ponds. Sex-reversed, 4- to 7-g fingerlings were stocked in 0.04-ha earthen ponds. Each genetic group was randomly allocated to two ponds; each pond was stocked with 150 fingerlings. For those groups that had both color phenotypes (A x R and R x N), 75 normal-colored and 75 red fingerlings were stocked in each pond. Fish were fed *ad libitum* with 32% protein floating catfish feed. After 98 days, fish were harvested and survival was determined.

Statistical analysis

ANOVA was used to compare viability of the two color phenotypes within each genetic group. Duncan's Multiple Range Test (Steel and Torrie 1980) was used to assess differences among the means.

Results

Hatchability

Data for hatchability are presented in Table 1. Samples of eggs examined microscopically indicated that the ratio of red:normal-colored embryos was 1:1 for A x R and R x N. However, percentage abnormalities and percentage mortality for red embryos was large in some families. Red embryos from A x R and R x N had the lowest hatchability (P = 0.01). Hatchability of normal-colored embryos in the A x R cross was greater (P = 0.01) than that for their red siblings and did not differ from the hatchability of *O. aureus*. In the R x N cross, there was no difference (P = 0.05) in hatchability for the sibling subgroups. However, their hatchability was lower (P = 0.01) than that for *O. niloticus*. Hatchability of the two red groups combined was lower (P = 0.01) than that for the normal-colored embryos combined.

Table 1. Hatchability of *Oreochromis aureus* (A), *O. niloticus* (N) and their red hybrids. Means followed by the same letter are not significantly different (P = 0.05), Duncan's Multiple Range Test.

Group	Color	Hatchability (%)		
		Mean	Range	CV
A x A	Normal	90.7^{ab}	87-94	4.1
A x R	Normal	85.8^{ab}	74-100	9.8
A x R	Red	43.0^{c}	07-89	60.6
N x N	Normal	94.6^{a}	89-98	4.0
R x N	Normal	69.7^{abc}	46-96	33.0
R x N	Red	63.8^{bc}	45-84	34.4

Sex reversal

During sex reversal, there was no difference in viability among the groups (P = 0.05). Survival rates ranged from 96 to 100%.

Fingerling production

Data on viability during the fingerling phase are presented in Table 2. During fingerling production, viability ranged from 84.7 to 99.6. The four red subgroups were significantly less viable (P = 0.05).

While the viability of the red fish from the R x N cross was not different (P = 0.05) than that of their normal-colored full-sibs or *O. niloticus*, the red fish in A x R cross were less viable (P = 0.05) than their normal-colored counterparts, or *O. aureus*.

Growout

Concrete tanks. During growout in concrete tanks, where fish reached average weights ranging from 130 to 276 g, viability of red tilapia was significantly lower (P = 0.05) than that of their normal-colored counterparts in all groups (Table 2).

Earthen ponds. Data on viability during growout are presented in Table 3. In earthen ponds, where fish reached average weights ranging from 186 to 289 g, the observed mean viability of red tilapia was slightly greater than that in concrete tanks, but was still significantly lower (P = 0.05) than that of their normal-colored full-sibs.

Discussion

For all cultured stages, cumulative mortalities for red and normal-colored A x R siblings were 72% and 19%, respectively; cumulative mortalities for red and normal-colored R x N siblings were 57% and 34%, respectively.

The reduced viability of the red sibs in both the A x R and R x N groups indicates that the red genotype has a negative pleiotropic effect on survival. The substitution of a mutant allele for a normal allele often has secondary effects because biochemical pathways are altered to produce the mutant phenotype (Tave 1986). Viability of red tilapia was evaluated within two groups. The major genetic difference of the fish within both the A x R and R x N groups was body color. Consequently, differences in viability of the color phenotypes within each group was due to the pleiotropic effects of the red genotype.

Table 2. Viability of *Oreochromis aureus* (A), *O. niloticus* (N) and their red hybrids (R) during fingerling production and growout in concrete tanks. Means followed by the same letter are not significantly different (P = 0.05), Duncan's Multiple Range Test.

Group	Color	Sex reversed	Mean survival (%)			
			Fingerling	CV	Growout	CV
A x A	Normal	-	98.5[a]	1.3	97.8[a]	3.9
A x A	Normal	+	99.6[a]	0.7	98.9[a]	1.9
A x R	Normal	-	98.5[a]	2.7	100[a]	0.0
A x R	Normal	+	97.0[a]	3.6	100[a]	0.0
A x R	Red	-	86.4[bc]	7.9	82.2[b]	4.7
A x R	Red	+	85.6[c]	7.7	71.1[c]	14.3
N x N	Normal	-	97.2[a]	4.1	98.9[a]	1.9
N x N	Normal	+	99.2[a]	1.3	98.9[a]	1.9
R x N	Normal	-	97.0[a]	2.7	97.8[a]	3.9
R x N	Normal	+	98.9[a]	1.6	96.7[a]	4.9
R x N	Red	-	93.2[ab]	7.3	77.8[b]	4.9
R x N	Red	+	95.4[a]	3.4	70.0[c]	6.7

Table 3. Viability of *Oreochromis aureus* (A), *O. niloticus* (N) and their red hybrids (R) during growout in earthen ponds. All groups were sex reversed. Means followed by the same letter are not significantly different (P = 0.05), Duncan's Multiple Range Test.

Group	Color	Survival (%)		
		Mean	Range	CV
A x A	Normal	94.0[a]	93-95	2.0
A x R	Normal	94.0[a]	91-97	5.0
A x R	Red	81.3[b]	80-83	2.3
N x N	Normal	94.3[a]	94-95	0.5
R x N	Normal	94.0[a]	92-96	3.0
R x N	Red	85.3[b]	81-89	6.6

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Behavior and Gonadal Structure of Intergeneric *(Oreochromis-Sarotherodon)* Tilapia Hybrids

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Abstract

Comparative behavioral and cytological (light and electron microscope) studies were conducted on F1 and F2 hybrids of *Oreochromis* spp., *Oreochromis* sp./*Sarotherodon galilaeus* hybrids and Taiwanese red tilapias *(O. mossambicus/O. niloticus)*. Crosses were made by artificial fertilization or natural spawning. For the cross *O. niloticus* x *O. aureus*, F1 hybrid progeny treated with 17α-methyltestosterone were studied. Additional studies were made on red *O. mossambicus*. The intergeneric F1 and F2 hybrids demonstrated a variety of behavioral patterns both in fry-parent interactions as well as in the behavior of adults during reproduction. These action patterns include fragments of the maternal - or paternal - repertoire, inherited from their parents. All intergeneric *Oreochromis* x *Sarotherodon* and reciprocal crosses gave highly biased sex ratios: 90-98% male.

The ovaries of females from the intergeneric crosses showed two types of abnormality: cessation of secondary yolk development (which prevented completion of oogenesis) and ambisexuality (occurrence of spermatogonial cysts on the ovigerous lamellae with misdevelopment of chorionic structures and follicles). The testes of hybrid males showed extensive development of interstitial tissue, separating and surrounding the sperm-producing tubule. These results are discussed in relation to the use of tilapia hybrids in aquaculture.

Introduction

Tilapias are constantly gaining importance in aquaculture, especially in the tropics and subtropics. In Israel, during the last few years, intensive studies have been carried out to improve the quality of the tilapias for domestic consumption and export. Using various techniques and culture systems, the main research goals have been to achieve high yields at low cost, and to produce more attractive fish for the consumer. To increase yields, various species of *Oreochromis* have been hybridized, frequently yielding all-male populations for fish growers (Schwartz 1983).

There is little difficulty in producing hybrids between various *Oreochromis* spp. (Wohlfarth and Hulata 1983). *O. niloticus* x *O. aureus* F1 hybrids (Fishelson 1962) are widely cultured from Africa to East Asia. The maternal mouth-brooding behavior of all *Oreochromis* spp. is similar and has been well described (Peters 1963; Fishelson 1966, 1983). However, in some *Sarotherodon* spp., such as *S. galilaeus* studied here, both parents mouth brood the young (Fishelson and Heinrich 1963). Thus, these two genera exhibit very different reproductive behavior and do not hybridize voluntarily. An additional difference between *Oreochromis* and *Sarotherodon* lies in the response of the postlarvae to their parents: in *Oreochromis*, they respond positively to retrieving motions of the mother, whereas in *Sarotherodon* neither parents nor young interact positively and the released young form schools and avoid their parents (Fishelson 1966, 1983). There are no *Oreochromis-Sarotherodon* hybrids in current commercial use.

Modern tilapia culture often involves manipulation of natural genotypes and production of abnormal phenotypes, hybrids or sex-reversed fish. This paper describes some behavioral and gonadal structural studies on some intergeneric (*Oreochromis-Sarotherodon*) hybrids as well as some hybrids of *O. niloticus* x *O. aureus* that underwent sex reversal of fry (Rothbard et al. 1983).

Methods

Behavioral studies on parents and fry

Table 1 lists the sources of the fish used in this study, the crosses performed, the techniques employed and the numbers of parents studied.

Parental fish were kept for easy observation in 200- to 400-l aquaria at 26°C and 12L:12D photoperiod and fed daily with fish pellets of 25% protein. Fry were transferred to 60-l aquaria under similar conditions fed with dry Tetra fish food and Artemia nauplii. Parent-fry relationships were studied both with live parental fish and using a 'surrogate' mother model: a black wax ball of 50-mm diameter was presented to the newly swimming larvae twice a day for 5 minutes starting on the 14th day of incubation. Brooding females were kept isolated in 200-l aquaria and not fed.

Comparison of the gonadal structure of F1 and F2 Oreochromis-Sarotherodon *hybrids*

Table 2 lists the number of hybrids and red tilapia (obtained from Taiwan; Pruginin et al., this vol.) for which the gross structure of the gonads and cytology were studied.

For histological studies, gonadal tissue was fixed in Bouins fluid, embedded in wax, sectioned histologically at 10 µm and stained in Delafield's hematoxylin-eosin or iron-hematoxylin. For electron microscopy (EM), parts of organs were fixed in 3.5% glutaraldehyde at pH 7.2; postwashed with buffered cacodylate; stained with 1.0% osmium tetroxide and embedded in Epon. The blocks were sectioned and studied with a Jelco 9 EM.

Results and Discussion

Behavioral studies

Table 3 summarizes the results of successful intergeneric hybrid crosses.

The percentage fertilization rate in all these crosses was 80-90% and the survival rate of fry in artificial incubators and up to exogenous feeding was 60-80%. Very high percentages of male progeny were observed.

The growth performance of the F1 progeny was not lower than that of their *Oreochromis* parent in the brood and

Table 1. Sources of fish used, crosses produced and number of parents studied. The female parent is given first for all crosses. The red Taiwanese stock is a hybrid line.

Type of fish	Origin	Generation F1	Generation F2	Fertilization Artificial	Fertilization Natural	No. of parents studied
Oreochromis mossambicus x *Sarotherodon galilaeus*	E. Africa / Israel	+	+	+	+	12
S. galilaeus x *O. mossambicus*	Israel / E. Africa	+	—	+	—	24
O. niloticus x *S. galilaeus*	W. Africa / Israel	+	+	+	+	20
S. galilaeus x *O. aureus*	Israel / Israel	+	+	+	+	28
S. galilacus x *O. niloticus*	Israel / W. Africa	+	+	+	+	18
Taiwanese red (an *O. mossambicus*/ *O. niloticus* hybrid)	Taiwan	+	+	—	+	28
Red tilapia (*O. mossambicus*)	Singapore	+	+	—	+	34

Table 2. Types of tilapia hybrids for which the morphology and cytology of gonads were studied. The female parent is given first for all crosses. The red Taiwanese stock is a hybrid line.

Type of fish	Generation	No. of gonads studied LM	No. of gonads studied EM
Oreochromis mossambicus x *Sarotherodon galilaeus*	F1	10	4
O. mossambicus x *S. galilaeus*	F2	2	1
S. galilaeus x *O. aureus*	F1	6	2
S. galilaeus x *O. aureus*	F2	3	1
O. niloticus x *S. galilaeus*	F1	6	2
O. niloticus x *S. galilaeus*	F2	2	2
O. niloticus x *O. aureus**	F1	8	2
Taiwanese red (an *O. mossambicus* /*O. niloticus* hybrid)	?	4	1

LM = Light microscopy.
EM = Electron microscopy.
*F1 hybrid fry that received 17α-methyltestosterone to sex-reverse females; obtained from the Kibbutz Gan-Shmuel hatchery.

Table 3. Percentage of males and females in F1 of various crosses of *Oreochromis* spp. and *Sarotherodon galilaeus*, produced by artificial fertilization. The female parent is given first.

Type of cross	No. of successful crosses	No. adults*	Sex ratio of progeny (%) Male	Female
S. galilaeus x *O. niloticus*	4	180	95	5
O. niloticus x *S. galilaeus*	2	80	96	4
S. galilaeus x *O. aureus*	3	80	90	10
O. aureus x *S. galilaeus*	2	60	98	2
S. galilaeus x *O. mossambicus*	2	60	96	4

*Fish grown until sexual differentiation (50-70 mm TL).

higher by 10-20% of that of natural *S. galilaeus*. The growth of the F1 progeny to adults was typical for our aquarium conditions, and at one-and-a-half years of age at 160-200 mm TL, they were mature.

The reproductive behavioral traits of adult male F1 hybrids of *O. aureus* x *S. galilaeus* and *O. niloticus* x *S. galilaeus*, particularly aggressiveness and territoriality, were intermediate between the two types of behavior expected for the parental fish, i.e., *aureus/niloticus* type - streng, aggressive territorial behavior (defense of the nest), deep nest excavation and prolonged courting plus fertilization of broods of several females; *galilaeus* type - weak, transient aggression, shallow nest excavation and courting of selected females (Fishelson 1966).

Here out of 24 males observed, 18 showed the *galilaeus* type behavior and 6 the *aureus/niloticus* type. For the former, as soon as one female positively responded to courtship, the male remained bonded to her, forming a monogamous pair. Such a pair selected the site for spawning and remained together until the fertilized eggs were picked up by the female. In all the 26 spawnings of F1 hybrids observed, no male was observed picking up eggs for brooding. It would thus appear that the paternal mouth-brooding habit of *S. galilaeus* was lost in the F1 hybrids.

In all the F1 hybrids that showed *aureus/niloticus* type, the males established their territories and selected the sites for nest building. The females readily entered to spawn. Following each act of fertilization, the females collected batches of eggs in their mouths and, after collecting all, left the males. The males then continued to court and fertilize other females. The maternal mouth brooding continued for 14-15 days.

The released F2 fry behaved towards the mother fish as typical *Oreochromis* mouth-brooded fry. On each retrieving motion of the mother fish, as well as of the surrogate mother (black ball), the fry reacted quickly, approaching for contact (Peters 1965a, 1965b) and entering the mother's mouth. This response and its duration were as described by Peters (1963), Heinrich (1967) and Fishelson (1983).

Of the adult F1 generation of crosses with *S. galilaeus*, 16 females brooded their eggs successfully producing F2 progeny. Among the *O. mossambicus* x *S. galilaeus* line, only two females succeeded in reproducing and the survival rate of their progeny was very low (5-8%).

The F1 mouth-brooding intergeneric hybrid females were carefully separated from other fish, by inserting partitions in the aquaria or isolating them in groups in separate containers. At this stage, all the brooding hybrid females became darker on the lower parts of head and belly. After 14 days the release of young began. The female F2 fish frequently showed swelling of the abdomen as observed in pre-spawning tilapia females, but did not produce even a single spawn. Left together

with males, their response to courting was minimal. In such fish, the abdomen shrank back to its normal state after about 10-12 days. This process occurred 3-4 times a year in the same female. Squeezing out eggs from such females showed that many of them were in a state of degeneration.

The F2 hybrid fry did not differ morphologically from normal fry of tilapias but displayed an array of behavioral patterns differing not only among families of different crosses, but also among broods from different females of the same cross. Thus, for example, in *S. galilaeus* x *O. niloticus* F1 female and F2 progeny interactions, 12 families demonstrated the following variants:

Variant A: The female releases the juveniles and does not take care of them. If the juvenile attempts to make contact with her and to enter her closed mouth, the female escapes this approach, distancing herself from the young. After 2-3 days of such attempts, the young form a school.

Variant B: The released young fish form at once a dense school. The female attempts to retrieve them, but they swim away whenever she approaches. Only rarely does the mother fish succeed in catching a few young and keeping them for a while in her mouth.

Variant C: The released young fish, following the female, usually below her, form a school closely watched by the mother who swims over them, showing aggressive behavior toward other fish or people approaching the aquarium.

All three variants show a strong dominance of the *galilaeus* pattern, in which the bond within the group of juvenile fish is stronger than that of their attachment to the mother (Fishelson 1983).

Tests of these behavior patterns with ball-models gave the same results, dominated by escape responses in 18 cases of the 20 cases observed. In the two instances of F2 hybrid of *S. galilaeus* x *O. niloticus* and *O. aureus* x *S. galilaeus* F2 hyrids, that responded positively to the models, this response was of a very short duration, only 2-3 days, and not the 14-16 days as observed in both normal and hybrid lines of *Oreochromis* spp. (Fishelson 1966).

Gonadal structure

Two major abnormalities were found in the ovaries of females from the hybrid crosses made in our laboratory and hormone-treated fish received from Kibbutz Gan Shmuel: abnormal yolk development and ambisexuality (presence of spermatogonial cysts; Table 4).

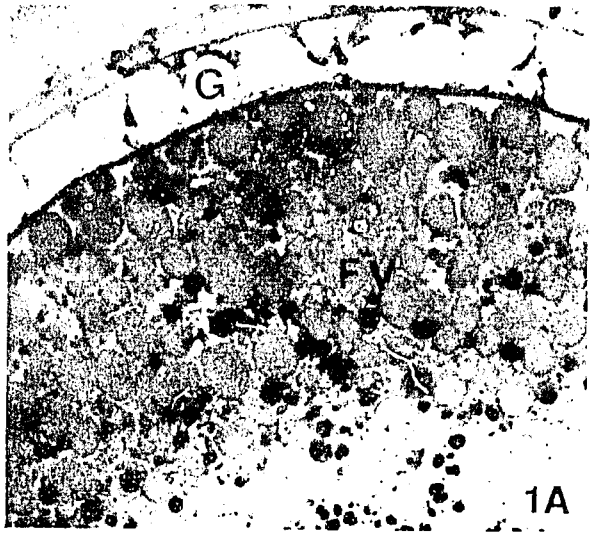
Table 4. Frequency of spermatogonial cysts in ovaries of various hybrids of tilapia. The female parent is given first.

Hybrid	Generation	Dissected ovaries	
		All female	With male cysts
O. niloticus x *S. galilaeus*	F1	8	2
O. aureus x *S. galilaeus*	F1	5	3
S. galilaeus x *O. niloticus*	F2	3	7
O. aureus x *S. galilaeus*	F2	3	4
O. mossambicus x *S. galilaeus*	F1	0	8
Taiwanese red (*O. mossambicus*/*O. niloticus*)	F1	3	5
Testosterone treated hybrids of *O. niloticus* x *O. aureus*	F1	10	15
Red tilapia (*O. mossambicus*)	F1	10	0

ABNORMAL YOLK DEVELOPMENT

In F2 hybrids of *S. galilaeus* x *O. aureus* and *O. niloticus* x *S. galilaeus*, and in F1 hybrids of *O. mossambicus* x *S. galilaeus*, secondary yolk deposition was arrested within ripening oocytes. Ripe preovulatory oocytes, developed peripheral fat vacuoles whereas in normal oocytes yolk granules are found all over the egg (Figs. 1a and 1b). The mass of the fat vacuoles greatly enlarged the abnormal ovary and such eggs contained 75-80% fat, almost twice the normal amount. In a few cases, many of the abnormal eggs were found released into the ovarian lumen. In two *O. niloticus* x *S. galilaeus* F2 females of 320 and 360 g, the ovarian weights were 72 g and 89 g, and the total numbers of ripe eggs counted were 4,000 and 6,000, respectively: much higher than the normal number of eggs/spawning from any mouth-brooding species. Most of these eggs appeared to be degenerating.

A further abnormality was found in ripe eggs in which yolk *was* deposited. In these eggs, the yolk granules, instead of taking the normal oval or rounded shapes, assumed various irregular configurations,



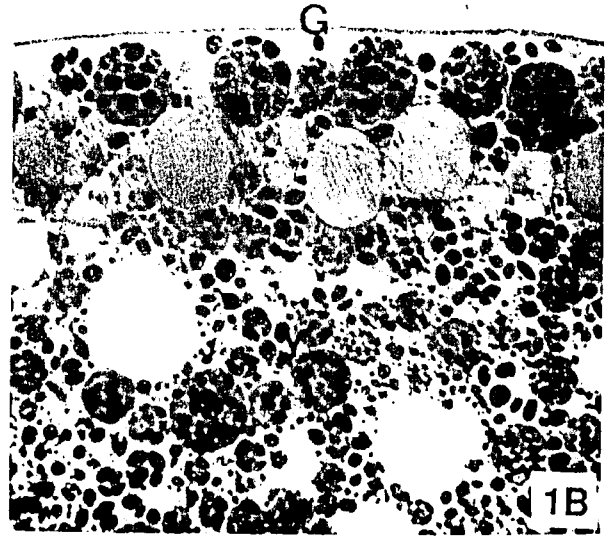


Fig. 1A. Part of a preovulatory egg of an F2 hybrid of *Oreochromis mossambicus* x *Sarotherodon galilaeus*, lacking peripheral yolk granules. B. Part of a normal egg of *O. aureus* with yolk granules within vacuoles of the cortical cytoplasm: FV = fat vacuoles; G = granulosa: (x 400).

either pressed together or dispersed. Electron microscopy studies of such oocytes revealed abnormal development of the chorion and adjacent follicular layers (Figs. 2a and 2b). In normal tilapia oocytes, the chorion is formed by 10-12 microlayers of fibrillar material. These are porous and oocyte cytoplasmic strands cross them to join cytoplasmic extensions of the granulosa (Fig. 2a). In the abnormal oocytes, the chorion is underdeveloped and the follicular cells are deformed and vacuolated (Fig. 2b). This may block the transport of yolk into the fat vacuoles, preformed in the oocyte during vitellogenesis. This condition was sometimes accompanied by a strong proliferation of interstitial cells and connective tissue around the oocytes, strongly resembling changes observed in ovaries of protogynous sex-changing fishes.

AMBISEXUALITY

Ambisexuality of female gonads was found in all hybrids studied. This was observed as cysts of spermatogonia dispersed on the ovigerous lamellae between the oocytes (Fig. 3). In juvenile fish of 18-22 mm TL, the gonads already contain male and female germ cells (Fig. 4). In female pre-adult hybrids of 60 mm TL and over, the oocytes develop and multiply, whereas the male anlage remain as dispersed cysts. This type of ambisexuality was particularly prominent in the progeny of *O. mossambicus* x *S. galilaeus* F1 and to a lesser degree in other types of hybrids (Table 4).

Ambisexuality in natural and hormone-treated populations of cichlids has been previously reported by Peters (1975), Shelton et al. (1978) and Rothbard

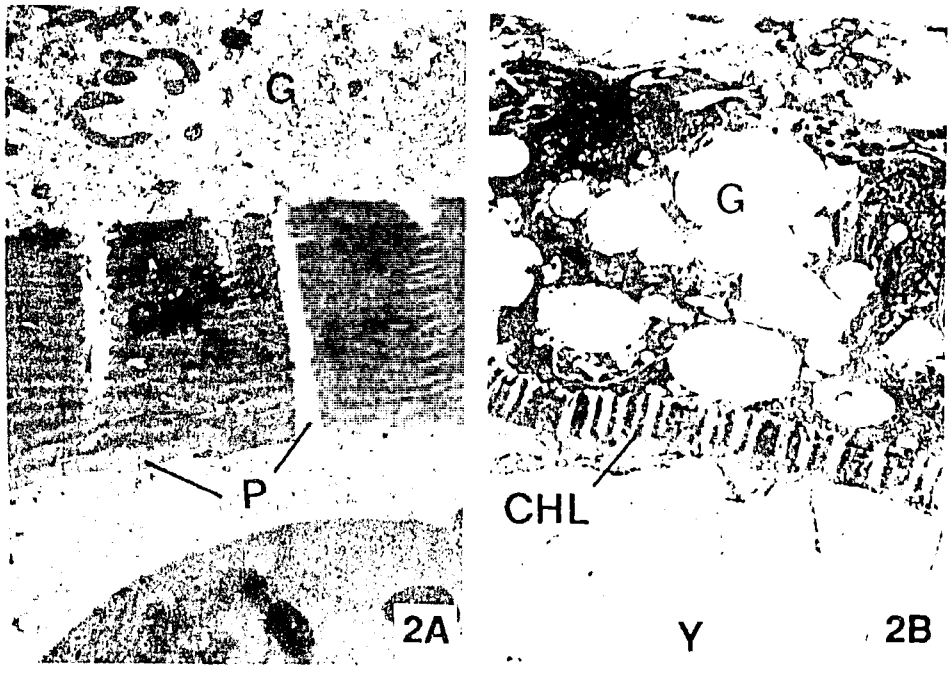


Fig. 2A. Electron micrograph of the chorion and adjacent structures of a normal egg of *Oreochromis aureus* (x 3,000). B. The same of an abnormal chorion of an F1 hybrid of *O. niloticus* x *Sarotherodon galilaeus* (x 2,000): CHL = chorionic layers; P = pore with extensions of ooplasm; G = granulosa cells of the follicle; Y = yolk vacuole.

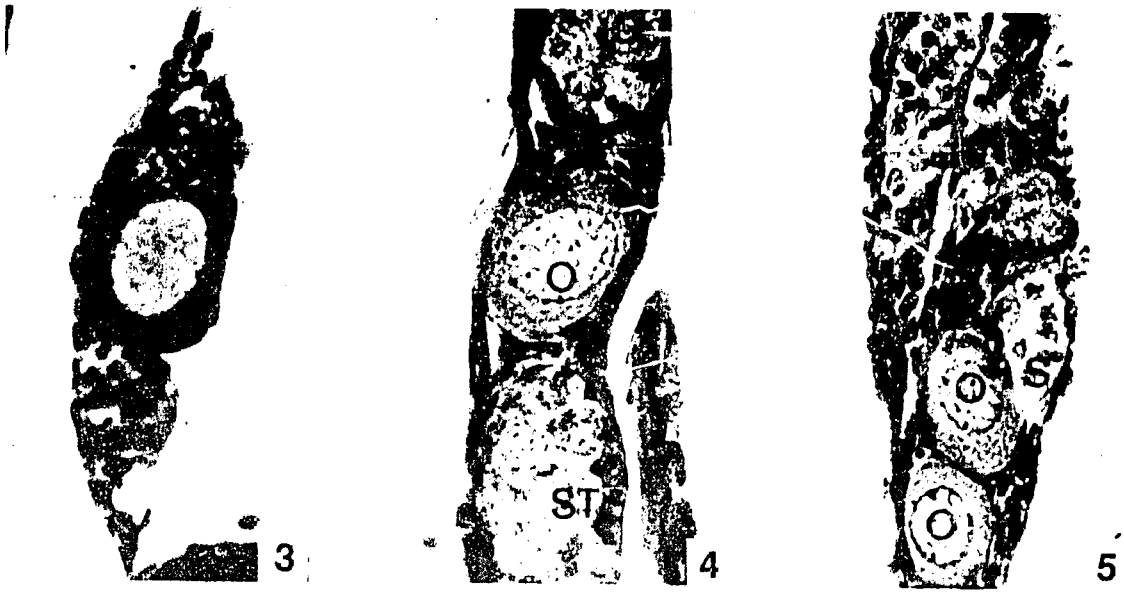


Fig. 3. A cyst of spermatogonia and an oocyte on an ovigerous lamella of an adult F1 hybrid of *Oreochromis niloticus* x *Sarotherodon galilaeus* (x 400).
Fig. 4. The gonad of a 20-mm TL F2 hybrid of *Oreochromis niloticus* x *Sarotherodon galilaeus* with male (ST) and female (O) cells (x 400).
Fig. 5. The bisexual gonad of 17α-methyltestosterone treated juvenile F1 hybrids of *Oreochromis niloticus* x *O. aureus* with oocytes (O) and spermatogonia (S) (x 400).

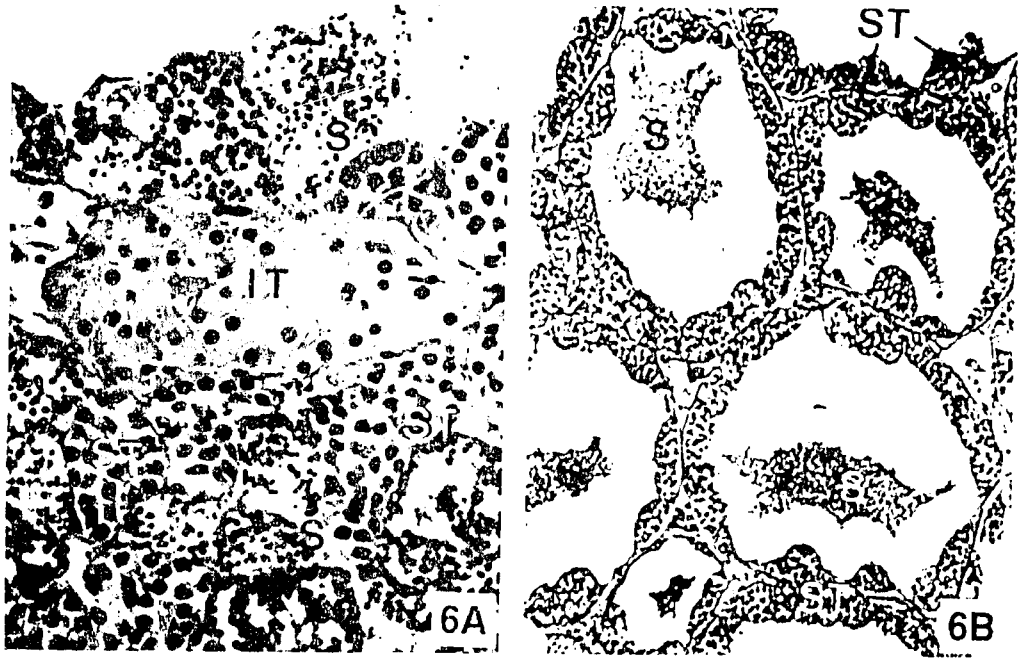


Fig. 6A. The gonad of a sex-reversed adult hybrid F1 male of *Oreochromis niloticus* x *O. aureus* with interstitial tissue (IT); spermatocytes (ST) and spermatozoa (S). B. The same of an *O. aureus* male with sperm (x 350).

et al. (1983). (See also Pruginin et al., this vol., for observations on red tilapia.)

The addition of testosterone to the food of postlarval F1 and F2 hybrids of the various intrageneric crosses induced ambisexuality at a very early postlarval stage, in general resembling results described by Fishelson (1975) for *Anthias squamipinnis*. The adult gonad of treated fish was masculine, with a few egg-cells dispersed between the cysts (Fig. 5). These intersexes later grew into phenotypic males with testes at various stages of spermatogenesis, frequently with a very few remnants of luteinized oocytes, but with extensive interstitial tissue, as compared to normal (Figs. 6a and 6b). Whether they can be functional males is not known.

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Breeding Characteristics and Growth Performance of Philippine Red Tilapia

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Abstract

The breeding characteristics and growth performance of Philippine red tilapias were investigated. Progeny of six different color phenotypes were obtained from red-gold parents: I, Red-gold orange; II, Red-gold with black spots; III, Uniform pink; IV, Pink with black spots; V, Albinos with black eyes and VI, Uniform gray. Crossing females of I, II, IV and III with III males yielded, respectively, 75%, 77%, 80% and 100% red progeny. A direct relationship between fecundity and female weight was demonstrated ($r = 0.87$). Fecundity ranged from 300 to 500 eggs/spawn. Semi-intensive culture trials in 250- to 300-m^2 cages of three genetic groups (F1, F3 backcross and F3 inbred generation) yielded 15 to 33 t/ha (0.9-2.6 kg/m^3). The effect of inbreeding was shown; a 38% decrease in growth performance was observed in the F3 inbred generation. The growth performance index ($\phi' = 3.2$) was similar to that of Taiwanese red tilapia and other *Oreochromis* species. The consequences of these characteristics for the potential of red tilapia culture are discussed.

Introduction

Red tilapia (a collective name for a large number of red, orange, gold and pink phenotypes) have become objects of interest for fish culturists and researchers throughout the world (Wohlfarth and Hulata 1983). Red-orange or golden coloration is favored in some consumer markets in comparison to the ordinary black or grayish color (Radan 1979; Fitzgerald 1979; Behrends et al. 1982).

The Philippine red tilapia was introduced from Singapore in 1978 and subsequently crossed with *O. niloticus* from Taiwan, Japan and Singapore (W. Ang, pers. comm.). Then its culture performance was tested in freshwater (Galman 1987) and in brackishwater (V. Corre and M. Sanchez, pers. comm.; Behrends et al. 1982). Several recent research projects have improved our knowledge of its genetic structure (Behrends and Smitherman 1984; Kuo and Tsay 1984, 1985). The present work continues our earlier study on Philippine red tilapia (Galman and Avtalion 1983) and deals with its capacity to breed true all year-round as well as its culture performance under commercial conditions.

Materials and Methods

First generation progenies of founder stocks (red-orange gold with black spots on the anterodorsal region (Radan 1979; Briggs 1981) kept by the Philippines Development Alternatives Foundation (PDAF) were kept in concrete tanks, aquaria and net enclosures in earthen ponds in Gintong Biyaya Hatchery, PDAF, Metro Manila, Philippines.

The different color phenotypes of the Philippine red tilapia as well as their respective dominant pigmentations are summarized in Table 1.

Broodstock (50-100 g) were kept in 80 concrete tanks (one side made of glass) (120 x 180 x 60 cm) at densities of 9 females to 3 males/tank. From the founder stock, three successive generations were obtained using the mating scheme summarized in Fig. 1 in which Roman figures (I to VI) refer to phenotypes described in Table 1. As mentioned, one backcross was performed using females (I) of the second generation and males of the first generation.

Water in the tank was changed once or twice weekly depending on quality. Accumulated wastes were siphoned out when necessary. Mouth-brooding females were gently isolated and their eggs were removed, counted and placed in incubators (Rothbard and Hulata 1980). Females that had spawned were replaced with reserve ripe females. From December to February when the photoperiod was shorter, 2 hours of artificial lighting (40-watt fluorescent lamps) were carried out. Fry were grown until 4 g in suspended net enclosures (3 x 1.5 x 0.5 m) in aerated earthen ponds (6 x 3 x 1 m) at a stocking density of 220/m^2. Fish were fed as recommended by Jauncey and Ross (1982) (Table 3).

Fry (3.2 ± 0.9 g) of three genetic groups have been used for cage culture: 1st generation of phenotypes I to V, 3rd

Table 1. Different color phenotypes and pigmentation of the Philippine red tilapia.

No.	Phenotype description	Gold-orange red (OR)	Pink (P)	Gray (Gr)	Black spots (BS)	Albino (Al)
I.	Red gold-orange	+	-	-	-	-
II.	As I, with black spots	+	-	-	+	-
III.	Uniform pink	-	+	-	-	-
IV.	Pink with black spots	-	+	-	+	-
V.	Albino with black eyes	-	-	-	-	+
VI.	Uniform gray	-	-	+	-	-

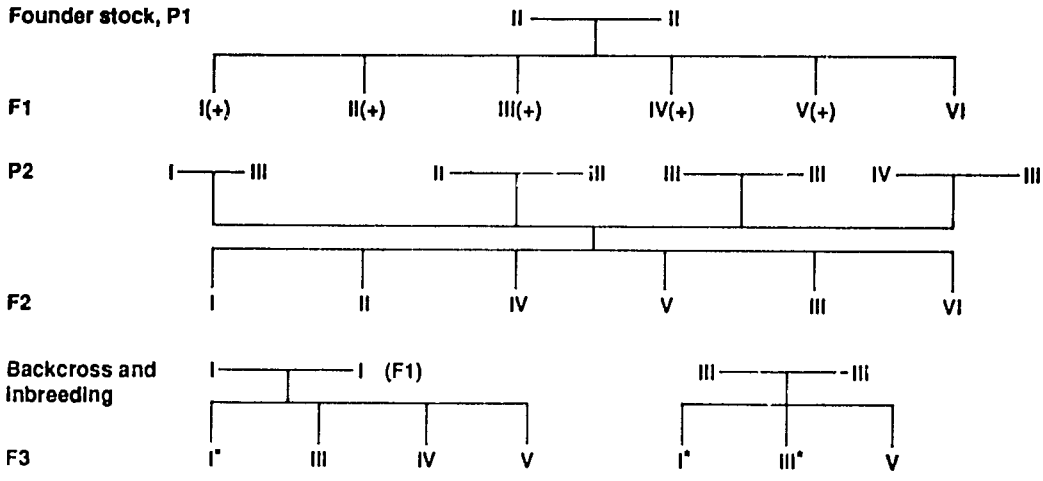

Fig. 1. Mating scheme of the various Philippine red tilapias.

+: these phenotypes in the F1 generation were used for culture trials.

*: these phenotypes of the F3 generation obtained from backcross (BC) and inbreeding were used for culture trials in cages (average depth = 1.5 m) in a southern coastal site of Laguna de Bay, a very eutrophic lake located close to Manila. The experimental conditions of cage culture are summarized in Table 2.

Table 2. Experimental conditions and results of cage culture trials in Laguna de Bay, Philippines, with supplemental feeding. Survival was 90 ± 10%. Cage trials 1-10 used F1 phenotypes I to VI. Cage trials 11-16 used F3 phenotype I obtained by backcrossing. Cage trials 17 to 18 used F3 phenotypes I and III, obtained by inbreeding. For details of phenotypes, see Table 1.

Vol. (m^3)	Cage (No.)	Area (m^2)	Mo	CP (days)	D (g)	Bi (g)	Bf (g)	FW (g)	P/m^3 (kg)
450	1	300	1	283	13.93	18.6	1,094	191.9	2.43
450	2	300	9	146	11.11	15.5	666	144.4	1.52
450	3	300	10	125	13.3	18.6	782	137.2	1.73
450	4	300	11	85	8.89	12.4	693	182.4	1.54
375	5	250	1	134	5.33	6.2	347	182.6	0.93
375	6	250	5	137	5.33	6.2	403	212.1	1.07
375	7	250	5	143	16.00	18.6	719	126.1	1.92
375	8	250	5	162	12.00	13.9	810	189.5	2.16
375	9	250	5	164	16.00	18.6	807	189.5	2.16
375	10	250	5	162	10.67	12.4	794	208.9	2.12
375	11	250	5	168	5.33	6.2	351	184.7	0.94
375	12	250	10	143	5.33	6.2	348	183.2	0.93
375	13	250	9	152	5.33	6.2	450	236.8	1.20
375	14	250	8	177	10.66	12.4	963	253.4	2.57
375	15	250	10	183	6.93	8.1	624	252.6	1.66
375	16	250	4	201	8.00	9.3	715	250.9	1.91
375	17	250	8	189	6.67	7.75	382	148.2	1.02
375	18	250	11	242	6.67	7.75	488	205.2	1.30

Mo, month at which stocking was made; CP, duration of culture; D, stocking density (number of fingerlings/m^3; Bi, initial biomass; Bf, final biomass; FW, individual final weight; P, production/m^3.

generation of phenotype I obtained by backcross and 3rd generation of phenotypes I and III obtained by inbreeding. They were randomly selected and stocked at different densities.

Results and Discussion

The crosses between the females of different phenotypes I, II, III and IV with males of phenotype III resulted in 75% colored progeny of different phenotypes as detailed in Table 4. The backcross of OR offspring (from I x III) with the male parent yielded 75-100% colored of different phenotypes in various combinations. Inbreeding after three generations of phenotype III yielded 100% red, pink and albinos which indicates that this phenotype is homozygous.

All red progenies could therefore be produced by selective breeding. In fact, individuals of pink phenotypes which have been repeatedly used throughout three generations, yielded exclusively red fish mostly of the pink phenotype, but also of gold-red and black-eye albino (lethal trait) phenotypes.

Fecundity and fry production

There was a strong correlation between the weight of females and the number of the larvae produced (Fig. 2). Older females weighing over 500 g (3-5 years old) produced many eggs/spawn

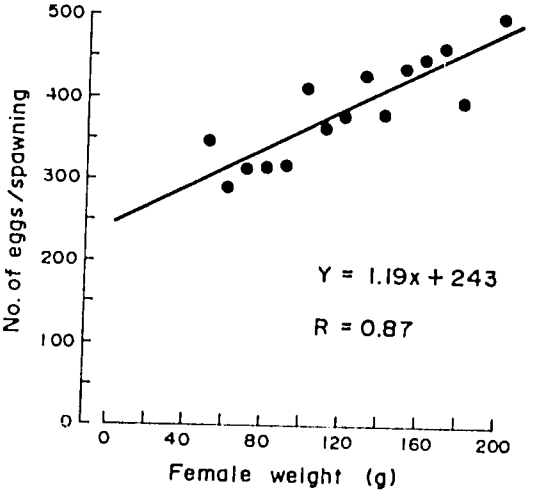

Fig. 2. Relationship between fecundity (number of eggs per spawning) and the weight of female Philippine red tilapia. Each point represents a mean of 30 females.

Table 3. Feeds and feeding methods for Philippine red tilapia fry (1 mg-1 g), fingerlings (5-50 g), broodstock and growout in cages: B = moist balls; Pe = pellets; Po = powder; % protein values in brackets.

Component	Fry B(35)	Fingerlings Pe(30)	Broodstock Pe(20)	Growout Po(15)
Rice bran (14)	52.5	65.0	87.0	88.5
Fish meal (63)	37.5	25.0	3.0	1.5
Leucaena leucocephala leaf meal (25)	9.0	9.5	9.5	9.5
Vitamin-mineral mix (Squibb)	1.0	0.5	0.5	0.5
Daily ration as % body weight	10-20	10-20	5-10	3-5

Table 4. Results of crosses of different phenotypes of Philippine red tilapia. F: Female; M: Males; S.D. = Standard deviation; +, present; ++, abundant; +++, dominant.

Cross F x M	No. of spawnings	% of colored progeny ± S.D.	% of gray ± S.D.	Frequency of progenies Phenotype I	II	III	IV	V
I III	100	75 ± 7.7	25 ± 7.7	+++	+	++	-	+
II III	101	77 ± 6.9	23 ± 6.9	+++	+++	++	+	+
IV III	13	80 ± 13.4	20 ± 13.4	+	++	+	++	+
III III	24	100	0	++	-	+++	-	+

(1,200-3,000) but their spawning frequency was between 4-6 weeks compared to 3 weeks in younger females 4-24 months old weighing 40-200 g and giving an annual average of 300-500 fry/spawn.

A continuous fry production was obtained throughout the year (Table 5). A 50% decrease (only 80-100 spawns compared with an average of 180 for summer months) during months of lower temperature was observed (Table 5). The low fry production for the hot months of March and April was also expected after more than five spawning cycles. At relatively high densities used in this study, the average number of spawning females in each tank in most cases was 2-4/month and rarely more than 4/month (Fig. 3). Despite artificially lengthening the photoperiod, we had decreased production which might possibly be due to spawning fatigue.

Growth performance

Growth performance in cage culture was remarkably good (Table 2). The maximum yield obtained was 2.57 kg/m^3 at a stocking density of 10.66/m^3 and the highest mean individual weight was 253.4 g. The interrelationships between different parameters (volume, surface area, culture period, season, stocking density, initial biomass, final biomass, final weight and production) were analyzed by Principal Coordinate Analysis (Daget 1976). A correlation matrix (Table 6) was constructed for the cage culture data and production/m^3 was highly correlated with stocking density, D (r = 0.78) and initial biomass, Bi (r = 0.74). The final individual weight (FW) has an inverse relationship with initial biomass (Bi) (r = -0.53) and stocking density (D) (r = 0.50). A final weight higher than 250 g could probably

Table 5. Average monthly fry production ± S.D. in 80 concrete tanks (120 x 180 x 60 cm) stocked with 3 males and 9 females.

Month	Average number of of fry/spawning ± S.D.	Number of spawnings	Total production in 80 tanks
May, 1984	297 ± 97	185	47,817
June	225 ± 67	234	52,650
July	280 ± 73	210	58,800
August	316 ± 111	153	48,348
September	349 ± 84	175	61,075
October	369 ± 75	152	56,520
November	360 ± 54	157	29,280
December	300 ± 54	83	29,880
January, 1985	298 ± 72	103	26,522
February	300 ± 76	82	24,600
March	261 ± 100	103	26,883
April	291 ± 92	90	26,190

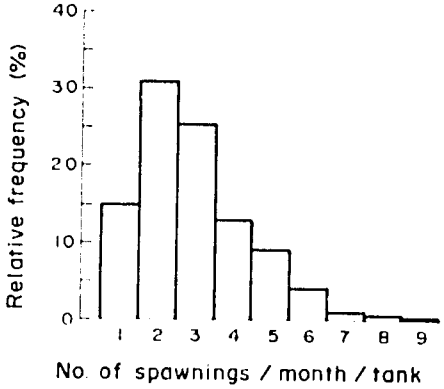

Fig. 3. Frequency of spawnings per month per tank of Philippine red tilapia stocked in 80 1.2 x 1.8 x 0.6 m tanks with 9 females and 3 males each; data are from 1,857 spawnings over a period of 12 months.

Table 6. Correlation matrix between the different parameters and the performance of Philippine red tilapia in cage culture. For explanation of symbols, see Table 2.

	Volume	Area	CP	Mo	D	Bi	Bf	P	FW
Volume	1.0	0.79	-0.08	0.17	0.35	0.56	0.43	0.19	-0.36
Area		1.0	-0.3	0.21	0.36	0.51	0.60	0.44	-0.07
CP			1.0	-0.29	0.06	0.06	0.31	0.32	0.28
Mo				1.0	-0.20	-0.15	-0.20	0.23	0.03
D					1.0	0.98	0.78	0.78	-0.50
Bi						1.0	0.82	0.74	-0.53
Bf							1.0	0.97	0.03
P								1.0	0.13
FW									1.0

be obtained if D were decreased or supplemental feeding increased.

The growth performance of these Philippine red tilapia was compared with that of other *Oreochromis* species using the growth performance index ϕ' (Moreau et al. 1986). It was similar to that of Taiwanese red tilapia as well as to those of *O. niloticus*, *O. mossambicus* and *O. aureus* (Table 7).

Table 8 shows that the two genetic groups tested: F1 and F3 displayed similar daily growth increment (1.3 ± 0.15 g and 1.4 ± 0.2 g, respectively). In contrast, a marked decrease was observed in the growth rate of F3 inbred generation (0.8 ± 0.04). Introgressive hybridization experiments with purebred *O. niloticus* are now in progress with the aim of increasing their growth rate (Galman 1987). Similarly, such a kind of introgressive hybridization was carried out in the USA between the red tilapia (Florida strain) and *O. aureus* to increase tolerance to low temperatures and improve growth rate (Behrends and Smitherman 1984).

The lower growth rate at the F3 inbred generation (Table 8) is probably due to loss of heterozygosity and to genetic recombination of negative traits. The segregation of a red gene in red tilapia hybrids to produce homozygous population was reported by Behrends et al. (pers. comm.). This homozygosity of the red

Table 7. Growth potential of red tilapias and some pure *Oreochromis* species, expressed by using ϕ' (Pauly et al., this volume): PRT, Philippine red tilapia; TRT, Taiwan red tilapia.

Species of hybrid	Mean ϕ' ± S.D.	Reference
PRT	3.31	Galman (1987)
PRT	3.18 ± 0.12	Galman (1987)
TRT	3.15 ± 0.08	Liao and Chang (1983)
O. niloticus	3.20 ± 0.48	Moreau and Pauly, this volume
O. mossambicus	3.19 ± 0.20	
O. aureus	3.15 ± 0.14	

Table 8. Comparative individual growth rates G (g/day) and production P (kg/m^3) of three different generations of red tilapia in cage culture (surface area: 300 m^2 under semi-intensive conditions, 90 ± 10% survival; initial weight 3.2 ± 0.9. For further explanations of abbreviations, see Table 2.

	F1 (I to V)				F3 (I) from backcross				F3 (I and III) from inbreeding	
Trial number	5	6	8	10	12	13	15	16	17	18
CP	134	143	162	162	143	152	183	201	189	242
D	5.3	5.3	12	11	5.3	5.3	7.0	8.0	6.7	6.7
Bi	6.7	6.2	14	12.4	6.2	6.2	8.1	9.3	7.8	7.8
Bf	347	403	810	794	348	450	624	715	382	488
FW	183	212	237	209	183	237	252	251	148	205
P	0.93	1.07	2.16	2.12	0.93	1.20	1.70	1.91	1.0	1.3
G	1.34	1.53	1.15	1.27	1.26	1.54	2.41	1.37	0.77	0.84

genes, which was obtained by inbreeding had a negative impact on the growth rate of the fish. Therefore, this kind of inbreeding selection especially in small and confined hatcheries should be carried out with careful broodstock management to prevent a high level of homozygosity and subsequent deterioration of its genetic qualities. This is now occurring in the Philippines as a result of introduction of small founder stocks of *O. niloticus*, introgressive hybridization by *O. mossambicus* and inbreeding (Macaranas et al. 1986; Pullin and Capili, this vol.).

Although the red tilapia, hybrid or pure (Mires, this vol.; Ferreira 1986), show a bright potential for aquaculture, the inheritance of the red pigmentation needs further studies.

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The Use of Electrophoresis as a Technique for the Identification and Control of Tilapia Breeding Stocks in Israel

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Abstract

Of the methods used for commercial production of all-male tilapia populations in Israel, crossing *Oreochromis niloticus* females and *O. aureus* males produces most of the tilapia fingerlings cultured commercially. This technique needs continuous control of the parental breeding stocks. The appropriate methodology and electrophoretic genetic markers used for this control are described and discussed.

Introduction

Different methods have been used to produce all-male population for commercial tilapia culture, e.g., manual sexing, sex inversion and interspecific crosses (Wohlfarth and Hulata 1983). Although the sex inversion technique (Rothbard et al. 1983) has been shown to be commercially feasible in Israel, most of the fingerlings produced recently (9-10 million/year) were offsprings of interspecific crosses between *O. niloticus* females and *O. aureus* males (Mires 1985). However, this latter technique needs careful control of the breeding stocks.

The findings of Hickling (1960) and Fishelson (1962) and the work of Chen (1969) and Jalabert et al. (1971) on the unusual sex ratios obtained by crossing different species of tilapias (reviewed by Hammerman and Avtalion 1979; Wohlfarth and Hulata 1983) had a strong impact on tilapia culture by providing one of the most effective tools for monosex culture. It is now well admitted that consistent production of all-male broods in tilapias by interspecific hybridization depends on the genetic purity of the parental stocks.

Several electrophoretic systems have been developed and proposed to identify tilapia species and hybrids (Chen and Tsusuki 1970; Avtalion et al. 1975; Herzberg 1978; Kornfield et al. 1979; Cruz et al. 1982; MacAndrew and Majumdar 1983; Wu and Wu 1983; Basiao and Taniguchi 1984). The electrophoretic system developed by Avtalion and Wodjani (1971) and Avtalion et al. (1975, 1976) has proved to be commercially applicable in Israel by performing periodic checks of the sera of broodstocks used in the production of all-male broods. These sera were tested for specific markers, such as serum esterase, transferrin and male specific proteins (Avtalion et al. 1984) using polyacrylamide gel electrophoresis. These markers allow easy differentiation between *O. niloticus* and *O. aureus* and the identification of their F_1 hybrids. Using this system, all individuals presenting xenogeneic markers were eliminated from parental stocks, thus maintaining stocks producing nearly 100% male hybrids. The recent significance and implications of such systems to tilapia culture are given in this present paper.

Materials and Methods

Fish stocks. Purebred stocks of *Oreochromis* species were obtained from Bar-Ilan University, the Fish and Aquaculture Research Station (Dor and Dagan) and Kibbutz Ein Hamifratz. The origins of these species were as follows: *O. niloticus* from Ghana and *O. aureus* originally from Lake Hula, in north Israel.

Preparation of serum samples and electrophoretic analyses. Sera of purebred stocks were prepared and tested using polyacrylamide gel electrophoresis and then stained for esterase and transferrins as previously described (Avtalion and Wodjani 1971; Galman and Avtalion 1983).

Results and Discussion

Purebred stocks of *O. niloticus* and *O. aureus* present electrophoretic patterns consisting of a total of five distinct transferrin bands (Fig. 1), occurring in various combinations in both species, and of two different esterase bands one each occurring in each species (Fig. 2) as shown by Avtalion (1982).

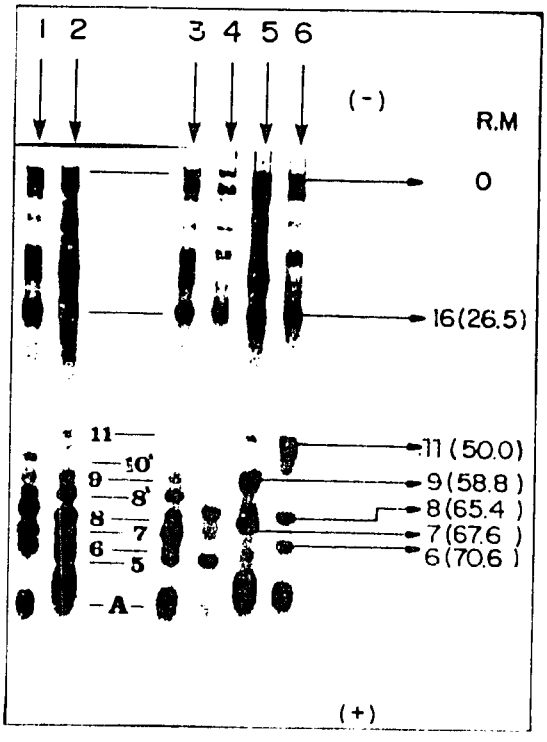

Fig. 1. 5.5% polyacrylamide gel electropherogram of sera from *Oreochromis niloticus* (5), *O. aureus* (6) and their hybrids (-14). *O. aureus* possesses bands 6 and 8, *O. niloticus* possesses bands 7 and 9. The hybrids show polymorphic patterns consisting of different transferrin band combinations (5-16). Relative mobilities (%), shown in brackets extreme right, were computed using band 11 as reference point (R.M. = 50%), which is consistently present in both females and males of all *Oreochromis* and *Sarotherodon* species. A, albumin.

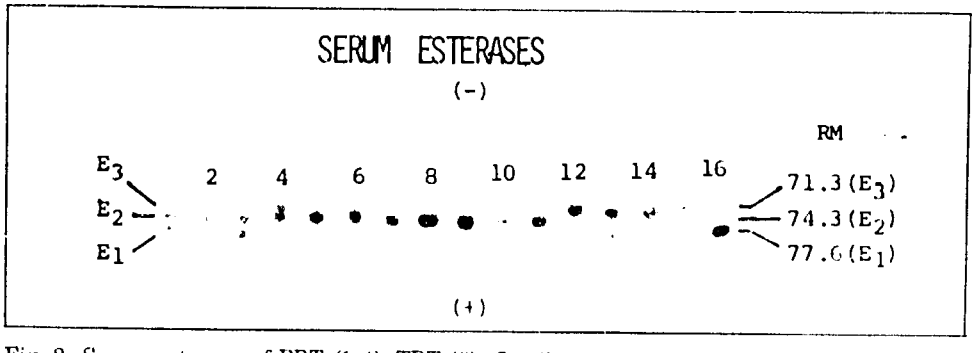

Fig. 2. Serum esterase of PRT (1-4), TRT (5), *O. niloticus* (6-9); *O. mossambicus* (10-12); *O. hornorum* (13-15) and *O. aureus* (16). Note that E1 is present in most of PRT tested.

The identification of pure *Oreochromis* species by the use of transferrin markers is based on the constant presence of bands 7 and 9 in *O. niloticus* and their total absence in *O. aureus*. However, in *O. aureus*, band 8 is always present, whereas band 6 is absent in few individuals. Although some individuals of *O. niloticus* were found to posses a weak band 8, band 6 was never found in purebred stocks of the local species. The esterase markers were proved to be highly specific showing no intraspecific polymorphism with *O. niloticus* possessing E2 and *O. aureus* E1 bands. Hybrids can easily be detected since both E1 and E2 are present in their electropherograms (Avtalion 1982; Galman and Avtalion 1983). These markers are listed in Table 1.

Individuals showing transferrin patterns with the non-appropriate xenogeneic markers were discarded when broodstocks were selected for commercial production of all-male hybrids. In the hatchery of Kibbutz Ein Hamifratz, 4.5 million hybrid fingerlings consisting of over 90% males

Table 1. Transferrin and esterase markers in purebred Israeli stocks of *Oreochromis aureus, O. niloticus* and their F1 hybrids.

Species	Transferrin						Esterase		
	9	8	7	6	5	5'	1	2	3
O. niloticus (N)	+	P	+	-	+	-	-	+	-
O. aureus (A)	-	+	-	+	-	+	+	-	-
N x A hybrids	P	P	P	P	P	P	+	+	-

(+) = always present; (P) = polymorphic

are produced annually using stocks of *O. niloticus* and *O. aureus* possessing the above specific transferrin and esterase band patterns (Mires 1983). Over 10 million hybrid fingerlings consisting of 95-100% males of the same species combinations were also produced in Kibbutz Nir-David in 1985 (S. Sarig, pers. comm.).

Contamination is avoided by periodic electrophoretic control of broodstocks (e.g., Kibbutz Ein Hamifratz) and/or by isolating each species in well-separated hatchery ponds (e.g., in Kibbutz Nir-David). In both hatcheries, parental stocks are marked and tagged before electrophoretic identification and progeny to be used to replace breeders are investigated with the use of electrophoresis (Mires 1983).

The application of interspecific hybridization, as commercially practised in Israel, is only feasible if the purity of stocks can be guaranteed by a periodic control of the breeding stocks. Otherwise, sex reversal (Rothbard et al. 1983; Guerrero and Guerrero, this vol.) is an alternative solution. The electrophoretic checking of stocks needs qualified technicians and laboratory facilities and is time consuming. A promising method based on cell surface immunological markers is now under investigation (Timan and Avtalion, in press). This would permit easy identification of breeding stocks by farmers.

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Feasibility of Commercial Production of Sex-Reversed Nile Tilapia Fingerlings in the Philippines

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Abstract

Broodfish of Nile tilapia *(Oreochromis niloticus)*, weighing 80 to 270 g. were stocked at 3-week intervals in concrete tanks at a biomass density of 400 g/m^2 with a female to male ratio of 2:1 by weight for fry production. Over six million fry were produced in the facility from March 1985 to March 1986 utilizing a breeding area of 1,800-2,000 m^2.

More than one million fry were treated for sex reversal in outdoor nursery tanks from May to November 1985. The fry were stocked at densities of 500-1,000/m^2 and fed with a commercial diet (SRT-95) containing 30 ppm of 17α-methyltestosterone for 21 days. Mean survival of the fry was 78.1% after the treatment period.

Four thousand treated fish were reared to maturation in lake cages for 73 days with feeding. Three thousand of these fish were hand-sexed to determine effectiveness of the sex-reversal treatment. The percentage of males obtained was 99%.

Commercial application of the sex-reversal technique for the production of all-male Nile tilapia in the Philippines is highly feasible and economical.

Introduction

Nile tilapia *(Oreochromis niloticus)* is the most important tilapia species cultured in the Philippines. An annual yield of more than 50,000 t of tilapia, over 90% of which were *O. niloticus*, was reported by the Philippine Bureau of Fisheries and Aquatic Resources for 1985. Commercial farming of Nile tilapia is done in freshwater ponds and cages (Guerrero 1986).

As in other countries, the problem of overcrowding in ponds of tilapias in the Philippines caused by prolific breeding has been a major constraint to development (Sevilleja 1985). Pond-reared tilapias mature as early as two months of age and are capable of breeding once every month

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thereafter. Overcrowding in ponds results in low yields of harvest-size fish.

One of the techniques tested in the Philippines for population control of tilapia is artificial sex reversal (Guerrero and Abella 1976; Guerrero 1979). The technique involves using feed containing synthetic male hormones (17α-methyltestosterone and 17α-ethynyltestosterone) to sexually undifferentiated tilapia fry at an effective dosage and duration. Commercial production of sex-reversed tilapia fingerlings has been done in Israel (Rothbard et al. 1983) and Taiwan (Liao and Chen 1983).

Rothbard et al. (1983) obtained 98-100% male tilapia with sex-reversal treatment of fry in outdoor concrete tanks using 60 ppm of 17α-ethynyltestosterone in the diet for 28-29 days. The outdoor method of treating tilapia fry in ponds using net enclosures for sex reversal was first demonstrated by Buddle (1984). Guerrero and Guerrero (1985a) reported the effective sex-reversal treatment of *O. niloticus* fry in outdoor tanks and net enclosures in earthen ponds using a diet with 30 ppm of 17α-methyltestosterone for 21 days.

This study was conducted at the Manila Electric Company (Meralco) Foundation's Agro-Aquatic Development Center (MF-AADC) in Jala-jala, Rizal, Philippines, to determine the feasibility of commercially producing sex-reversed Nile tilapia fingerlings.

Materials and Methods

Nile tilapia broodfish of 80 to 270 g were stocked in 18-20 concrete breeding tanks, each measuring 20 x 5 x 1.2 m, at a density of 400 g/m^2 with a ratio of 267 g females to 133 g males (Guerrero and Guerrero 1985b). Water depth in the tanks was maintained at 0.8 m with water from a deep well having a total hardness of 70 mg/l and pH of 8.1.

The broodfish were fed a powdered diet (24% crude protein) at 3% of biomass per day in two feedings (0800 and 1600 hours) in the first week of the breeding cycle of 21 days (Guerrero and Guerrero 1985c). The feeding rate was reduced to 2.5% for the second week and to 2% for the last week. Spent broodfish were conditioned for 1 to 2 weeks prior to restocking in the breeding tanks.

Swim-up fry were collected with fine-mesh dip nets from the tanks 10-11 days after stocking of broodfish and counted. The collected fry were conditioned in fine-mesh net enclosures (2 x 2 x 1 m) for 1 day before their transfer to the sex-reversal treatment units. About 31% of the fry produced in the facility from May to October 1985 were treated.

The procedures of Guerrero and Guerrero (1985a) were adopted for outdoor treatment of tilapia fry for artificial sex reversal. A commercial sex-reversal feed (SRT-95) containing 30 ppm of 17α-methyltestosterone was used. In the first week of treatment, 9-11 mm Nile tilapia fry were stocked at 100/m^2 in 2 x 2 x 1 m fine-mesh net enclosures installed in concrete tanks. The fry were then transferred to 5 x 2 x 1 m concrete tanks at densities of 500-750/m^2 for the last two weeks of treatment. The feeding rates were 20% of biomass per day (given in four feedings) for the first week, 15% for the second week and 12% for the last week. Mean body weights of the fingerlings after treatment were measured.

To maintain optimum water quality in the treatment tanks, a daily 5% water exchange using a flow-through system was applied. Temperature of the water in the tanks was monitored daily at 0600 and 1400 hours.

Four thousand of the treated fingerlings were reared in 4-mm mesh, 3 x 3 x 3 m net cages installed in Laguna Lake at a density of 100/m^2 with feeding for a nursery period of 3-4 weeks. The fish were then transferred to similar cages with 20-mm mesh at a density of 50/m^2 and grown to maturation for 73 days with feeding. Three thousand of the mature fish were hand-sexed by examination of the urogenital papilla to determine the percentage of males.

Results and Discussion

A total of 6,035,327 *O. niloticus* fry or 8 fry/m2/day were produced in the MF-AADC facility from March 1985 to March 1986 (Table 1).

Of the 1,081,300 fry treated for sex reversal in outdoor concrete tanks, 78.1% survived (Table 2). Mortality of fry during treatment was mainly due to stress. One kilogram of the sex reversal feed (SRT-95) was sufficient for treatment of 6,600 fry for the 3-week period. Cost of producing one thousand treated fish with the hormone feed was estimated to be US$0.58.

Water temperature in the rearing tanks ranged from 22 to 34°C. The fingerlings had mean weights of 0.25-0.3 g after the 3-week treatment.

The 3,000 fish reared in lake cages that were hand-sexed comprised 99% males.

To apply tilapia sex reversal successfully on a commercial scale, several requirements must be satisfied. First, a massive fry production system must be in place; second, the treatment should be done in existing nursery facilities; and third, the cost of effective treatment must be economical.

The results of this study clearly indicate the commercial feasibility of producing sex-reversed Nile tilapia fingerlings in the Philippines.

Our methods differed from those of Rothbard et al. (1983) in Israel in that we used outdoor concrete tanks rather than earthen ponds for producing the fry and

Table 1. Fry production of *Oreochromis niloticus* in concrete tanks of the MF-AADC from March 1985 to March 1986.

Month	No. tanks	No. fry
March	20	325,479
April	20	434,410
May	20	456,232
June	20	350,642
July	20	703,894
August	18	533,723
September	18	558,144
October	18	397,015
November	20	468,399
December	20	388,653
January	20	400,743
February	18	506,250
March	18	511,743
Total		6,035,327

Table 2. Survival rates of *Oreochromis niloticus* fry treated for sex reversal in outdoor tanks of MF-AADC from May to November 1985.

Month	No. fry treated	% survival
May	55,000	81.5
June	154,000	77.4
July	126,000	72.7
August	126,000	81.4
September	119,000	84.4
October	260,000	77.9
November	240,500	71.2
	Total 1,081,300	Mean 78.1

treated them with a diet containing 30 ppm of 17α-methyltestosterone instead of a diet with 60 ppm of 17α-ethynyltestosterone. Moreover, Rothbard et al. (1983) utilized shaded outdoor tanks with algae control for their sex-reversal treatment which lasted for 28-29 days in contrast to our treatment which was done in unshaded outdoor tanks for 21 days. The lower survival rate of treated fish (50.3%) reported in Israel was attributed to very high densities of fry and ectoparasite infestation.

Acknowledgements

We are grateful for the assistance and support of Messrs. Benjamin Raneses and Nestor Jacaban of the Meralco Foundation's Agro-Aquatic Development Center.

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Practical Methods for Chilled and Frozen Storage of Tilapia Spermatozoa

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Abstract

Practical methods for storing milt from the tilapia *Oreochromis mossambicus* are described. Spermatozoa, protected from freezing damage by methanol and skim milk powder, were cooled to -196°C and retained an average post-thaw motility of 39%. Spermatozoa suspended in an egg-yolk-citrate diluent containing sodium pyruvate were stored for 20 days at 4-5°C and produced 90% fertility.

Introduction

Storage of spermatozoa is an important technique in the control of reproduction of many domesticated animals, and its application to aquaculture is becoming widespread. Short-term chilled storage, for a period of days or weeks, is a relatively simple way of solving hatchery-related problems of non-coincident maturation of broodstock and requires little in the way of sophisticated equipment. Indefinite frozen storage in liquid nitrogen, while more costly in terms of equipment requirements, allows the culturist and researcher more flexibility in performing genetic crosses and manipulations such as induced gynogenesis, and opens up the possibility of banking of genetic material from valuable characterized stocks.

Preliminary methods for chilled and frozen storage of tilapia spermatozoa have been published (Harvey 1983; Harvey and

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Kelley 1984). Subsequent studies in our laboratory have been aimed at extending the period of chilled storage through addition of energy sources to the diluent and removal of unnecessary technical complications from freezing methods. The procedures described here will permit routine storage of milt in a domestic refrigerator (5°C) for 3-4 weeks, and in liquid nitrogen indefinitely.

Fish Stocks and Collection of Milt

Milt was collected from *Oreochromis mossambicus* males after sedation in 900 ppm 2-phenoxyethanol and clearing of the bladder with gentle abdominal pressure. Milt was drawn up in plastic freezing straws or glass capillaries and kept for up to two hours at room temperature (22°C) before dilution and storage. Pre-dilution motility was checked in a hanging drop preparation at 400X magnification, and samples having more than 5-10% progressively motile spermatozoa were discarded.

Freezing of Spermatozoa

The diluent for cryopreservation was simplified from Harvey (1983) by substitution of tap water for Ginsburg saline:

1) Prepare a 5% v/v solution of reagent grade absolute methanol in tap water as intracellular cryoprotectant
2) Add 15% w/v skim milk powder as extracellular cryoprotectant; dissolve. Post-thaw motility can be improved by adjusting the pH of the diluent to 7.4 by dropwise addition of 1N NaOH.

Milt and diluent (1 part milt plus 9 parts diluent) were gently mixed at room temperature and drawn up into 0.5 ml plastic straws used for freezing of bull and human spermatozoa. No equilibration time was necessary between dilution and freezing, as the methanol cryoprotectant penetrates cells rapidly.

Cooling rate is determined by the container holding the sperm (in this case the plastic straws) and the way in which these containers are cooled. Cooling rates between 10 and 80°C/minute (measured between -10 and -40°C) were obtained by placing the straws vertically in a cooling basket made from a tin can suspended over a covered dewar flask of liquid nitrogen so that the bottom of the can just contacted the liquid. The bottom of the can was perforated and fitted with an inner wire mesh spacer so that the straws did not contact the liquid nitrogen directly. Cooling was slower with more straws in the basket. After freezing was complete (about 5-10 minutes), the straws were quickly transferred to liquid nitrogen for storage.

Sperm was thawed when eggs had been squeezed from a female and were waiting in a clean, dry container. Thawing was by agitation of the frozen straws in a water bath and was done by holding the plugged end clear of the liquid and proceeding until the milt had thawed to a slurry that could just be expelled onto the eggs. For a 55°C water bath approximately 6 seconds of immersion were required to reach this point (1,000°C/minute); a 90°C water bath thawed the straws in approximately 3 seconds (1,700°C/minute). Thawed sperm was mixed with approximately 4x its volume in tap or aquarium water and immediately added to the eggs.

Results

Post-thaw motility was measured for cooling rates between 10 and 80°C/minute and averaged 39.2 ± 14% (n = 60). Post-thaw motility greater than 14% correlates strongly with fertility (Harvey 1983). Results using the method presented here were dependent on both cooling rate and thawing rate. At slow cooling rates (10-16°C/minute obtained by placing several straws within a plastic goblet in the cooling basket), varying the thawing rate

between 250 and 1,700°C/minute had no significant effect on post-thaw motility (n = 12). At faster cooling rates (600°C/minute obtained with 6 straws loose in the cooling basket), thawing at 250°C/minute produced significantly lower post-thaw motility (18 ± 4.3; n = 5) than did thawing at 1,700°C/minute (42.4 ± 9.9; n = 5).

Short-Term Chilled Storage of Tilapia Milt

The diluent for short-term storage of milt was improved from Harvey and Kelley (1984) by addition of sodium pyruvate as an energy source. It was prepared as follows, and could be stored for several hours in the refrigerator before use:

> Mix equal volumes of fresh chicken egg yolk and 3% (w/v) sodium citrate in distilled water;
> Add the antibiotic gentamycin sulfate to a final concentration of 400 µg/ml; or use a combination of 1,000 IU penicillin + 800 µg streptomycin sulfate/ml;
> Add 5.5 mg/ml sodium pyruvate and mix well.

Diluent and milt were gently mixed in a ratio of 1:1 and stored in capped polypropylene vials at 4-5°C. The vials were filled no more than 1/3 full to ensure adequate gas exchange.

Sperm cells settled to the bottom of the storage container and were gently resuspended upon removal of the container before fertilization. Allowing the mixed suspension to stand at room temperature for one hour resulted in a significant enhancement of motility, presumably by permitting a degree of repair of membrane structures altered by prolonged cold storage. Fertilization was done by adding 20 ml of stored, mixed milt to 580 ml tap or aquarium water; this amount of activated sperm was sufficient for 100 eggs.

In a typical storage experiment, milt from three *Oreochromis mossambicus* was diluted and stored by the above procedure for 20 days. Post-thaw motility was 14 ± 6.2% with a maximum of 23%; fertility was 90.8 ± 1.9% expressed as percentage of fry alive and apparently normal to the time of absorption of the yolk sac.

Acknowledgement

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Comparative Growth Tests of *Oreochromis niloticus* x *O. aureus* Hybrids Derived from Different Farms in Israel, in Polyculture

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Abstract

Growth rates of overwintered males of several tilapia hybrid populations from Israeli commercial and experimental farms were compared in polyculture ponds when stocked together with common silver and grass carps and freshwater prawns. Tested tilapia hybrids included *Oreochromis niloticus* x *O. aureus* F_1 hybrids from two commercial stocks and one experimental stock; commercially sex-inversed F_2 generation of that hybrid; and experimental *O. niloticus* x *O. urolepis hornorum* and Taiwanese red tilapia x *O. aureus* hybrids. A large variation in growth rate among *O. niloticus* x *O. aureus* hybrids from different farms was demonstrated. Commercially sex-inversed F_2 generation of that hybrid had lower mean weight gain and higher variance of weight than F_1 hybrids. The *O. niloticus* x *O. urolepis hornorum* hybrid had a relatively poor growth. The last two stocks are not recommended for use by farmers.

Introduction

Choice of good stocks adapted to culture is important for increasing tilapia production (Wohlfarth and Hulata 1983). Among various interspecific tilapia hybrids, compared during 1979-1983, the *Oreochromis niloticus* x *O. aureus* hybrid commercially cultured in Israel, was found most suitable for a number of production trials: growth rate, sex ratio, cold tolerance and coloration (Wohlfarth et al. 1983). A number of experimental and commercial *O. niloticus* x *O. aureus* hybrid populations, derived from different parental stocks available in Israel, are compared in this study to evaluate the nature and magnitude of variation among these stocks.

Materials and Methods

Fish stocks

All tested groups consisted of males only, manually sexed according to external sex characteristics, and overwintered from the previous year either on the commercial farms or at Dor.

Samples of *O. niloticus* x *O. aureus* F_1 hybrids produced by the commercial fish farms at Kibbutz Ein Hamifratz (E.H.) and Kibbutz Nir David (N.D.) and the experimental station at Dor (D.) were tested in 1984, together with a sample of *O. niloticus* x *O. urolepis hornorum* (n x h) F_1 all-male hybrid produced at Dor. In 1985, the E.H. hybrid was not available and a sex-inversed F_2 *O. niloticus* x *O. aureus* hybrid population (Rothbard et al. 1983 from the commercial fish farm at Kibbutz Gan Shmuel was used instead, together with wild-type color segregants from an experimental cross of Taiwanese red tilapia females and *O. aureus* males.

All tested tilapia groups were each separately stocked into three replicated 0.1-ha earthen ponds, in polyculture. In 1984 all ponds were also stocked with red tilapias of Taiwanese origin (Galman and Avtalion 1983; Rothbard et al. 1983) and *O. mossambicus* x *O. aureus* hybrids as part of the polyculture complement (see Table 1). In addition to tilapias, ponds in both tests were stocked with common *(Cyprinus carpio),* silver *(Hypophthalmichthys molitrix)* and grass carp *(Ctenopharyngodon idella)* and freshwater prawn *(Macrobrachium rosenbergii)* (see Tables 1 and 2 for further details).

Pond management

Nutrients were applied daily, 6 days/week. These consisted of dry poultry manure and 25% protein feed pellets. In 1984 manure was applied at an initial rate of 50 kg dry matter/ha which was increased every 2 weeks by 25 kg/ha/day to a maximum of 175 kg/ha/day. In 1985, manure was applied at a constant rate of 50 kg dry matter/ha/day.

Table 1. Technical details of the 1984 comparative growth test of different tilapia hybrids [145 culture days (10 May-3 October), except for the E.H. hybrid which was stocked 4 days later].

Species or hybrid	Stocking density (No./ha)	Mean weight (g)			Daily gain (g/fish/day)[2]		Survival
		Initial	Intermediate[1]	Final	1st period	2nd period	(%)
Tested tilapias[3]							
O. niloticus x *O. aureus* (Nir David)	9,000[4]	69	263	442	2.16 x	3.31 x	94
O. niloticus x *O. aureus* (Dor)	9,000[4]	57	247	373	2.11 x	2.35 y	97
O. niloticus x *O. aureus* (Ein Hamifratz)	9,000[4]	140	276	422	1.57 y	2.71 y	93
O. niloticus x *O. urolepis hornorum*	9,000[4]	77	251	381	1.94 x	2.40 y	89
Polyculture complement							
Red tilapia	350	79		253			51
O. mossambicus x *O. aureus*	550	389		565			92
Common carp (*Cyprinus carpio*)	2,500[5]	190		704			97
Silver carp (*Hypophthalmichthys molitrix*)	500	95		1,208			95
Grass carp (*Ctenopharyngodon idella*)	250	131		587			96
Prawns (*Macrobrachium rosenbergii*)	20,000	0.3		40			81

1 Partial harvest at 90 days.
2 Figures in each column sharing same letter are not significantly different (Duncan's Multiple Range Test, P = 0.05).
3 Each of the four hybrids in a separate set of three ponds, together with the other components of the polyculture which were stocked equally to all 12 ponds.
4 Reduced by partial harvest to 5,250/ha at 90 days.
5 Reduced by partial harvest to 2,000/ha at 90 days.

Table 2. Technical details of the 1985 comparative growth test of different tilapia hybrids [140 culture days (5 June-23 October)].

Species or hybrid	Stocking density (No./ha)	Mean weight Initial	Mean weight Final	Daily gain (g/fish/day)[1]	Survival (%)	Coefficient of variation (of weight)
Tested tilapias[2]						
O. niloticus x *O. aureus* F_1 (Nir David)	3,000	199	577	3.54 x	99	n/a
O. niloticus x *O. aureus* F_1 (Dor)	3,000	330	698	2.70 y	95	10.3
O. niloticus x *O. aureus* F_2 (sex-inversed)	3,000	292	606	2.30 y	95	16.8
Red tilapia x *O. aureus*	3,000	359	692	2.45 y	98	n/a
Polyculture complement						
Common carp (*Cyprinus carpio*)	2,000	548	2,153		97	
Silver carp (*Hypophthalmichthys molitrix*)	500	144	1,367		96	
Prawns (*Macrobrachium rosenbergii*)	12,500	2.3	49		88	

[1] Figures sharing same letter are not significantly different (Duncan's Multiple Range Test, P = 0.05).
[2] Each of the four hybrids in a separate set of three ponds, together with the other components of the polyculture which were stocked equally to all 12 ponds.

The supplemental feeding rate was calculated from fish biomass (4% of common and grass carp biomass and 2% of tilapia biomass), adjusted every 2 weeks by sampling the ponds. Mean weights were estimated from samples of at least 30 fish. In 1984 the computation used the mean weights of all tilapia groups, and one-third of the computed ration was applied daily. In 1985 the computation used the mean weight of the heaviest tilapia group, and the full computed ration was applied daily.

In 1984 a partial harvest was performed after 90 days (August 8) to reduce the fish biomass in the ponds. Forty per cent of the hybrid tilapia and 20% of the common carp were removed from each pond.

Analysis

At the termination of the tests all fish were sorted, counted and weighed. In 1985, individual weighing was also carried out on the tilapias of two groups: the Dor F_1 hybrid and the F_2 sex-inversed fish. ANOVA and Duncan's Multiple Range Test were applied to evaluate the significance of differences in growth rates among tested tilapia hybrids.

Results

The results from 1984 are presented in Table 1 and Fig. 1 During the first period (up to the intermediate partial harvest), three groups had similar growth rate (ca. 2.0 g/day), while the E.H. hybrid grew significantly slower (ca. 1.8 g/day). In the second period the N.D. hybrid grew faster than the other three groups, which did not differ among each other. Over the total growth period the N.D. hybrid showed the fastest growth rate.

The results from 1985 are presented in Table 2 and Fig. 2. Growth rate of the N.D. hybrid was faster than that of the other groups. The F_2 sex-inversed tilapias showed a slower growth than the N.D. hybrid, but did not differ significantly from the Dor hybrid. The frequency distribution of individual weights was wider in the F_2 than in the Dor F_1 hybrid (Fig. 3), with coefficients of variation of 16.8 and 10.3, respectively. The hybrid between red tilapia and *O. aureus* had a slightly faster growth than the F_2 sex-inversed hybrid.

Discussion

The results of the two tests indicate that genetic variation exists among *O.*

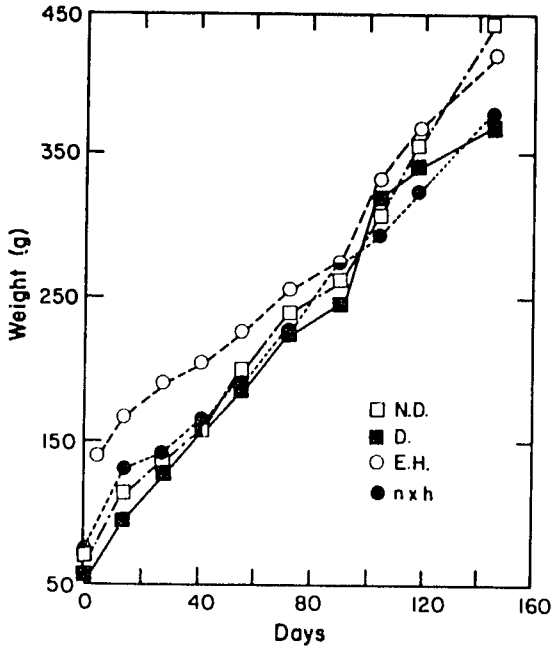

Fig. 1. Growth curves of males of tilapia hybrids stocked in polyculture with carps and freshwater prawns in experimental ponds in Israel. The plotted points are means of over 30 fish. Growth period lasted 145 days, from 10 May to 3 October 1984. E.H. and N.D. are commercial stocks of the *Oreochromis niloticus* x *O. aureus* hybrid; D is an experimental stock of the same hybrid; and n x h is an experimental stock of *O. niloticus* x *O. urolepis hornorum* hybrid. For further details, see Table 1 and text.

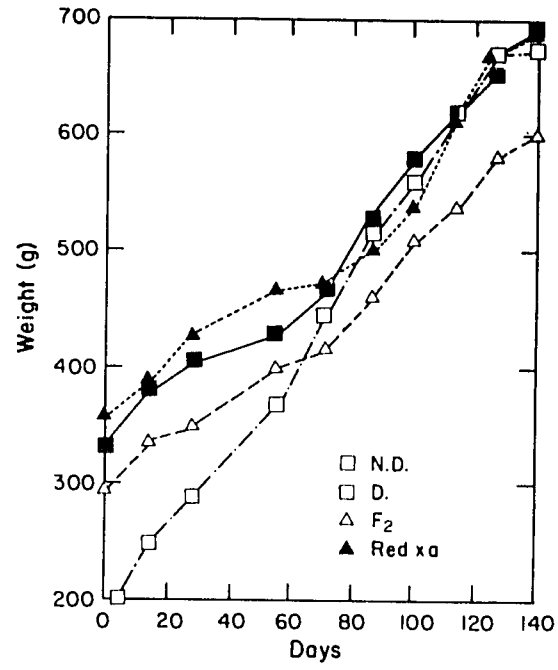

Fig. 2. Growth curves of males of tilapia hybrids stocked in polyculture with carps and freshwater prawns in experimental ponds in Israel. The plotted points are means of over 30 fish. Growth period lasted 140 days, from 5 June to 23 October 1985. N.D. is a commercial stock of the *Oreochromis niloticus* x *O. aureus* hybrid and D is an experimental stock of the same hybrid: F_2 is a commercially sex-inversed population of F_2 generation of *O. niloticus* x *O. aureus;* Red x a is the wild-type color segregant of Taiwanese red tilapia x *O. aureus* hybrid. For further details, see Table 2 and text.

niloticus and *O. aureus* stocks of different commercial and experimental farms in Israel, and that this is expressed in the performance of their hybrids. The N.D. hybrid grew faster than all others tested. The E H. hybrid grew surprisingly slowly in the first part of the 1984 test, despite its much higher initial weight. However, since all ponds were fed the same amount of pellets (calculated from the mean weight of all groups) this group received a smaller amount of feed in relation to its weight than the other three groups. The E.H. hybrid showed a much better relative growth after the partial harvest, when mean weights of all four groups were similar.

The *O. niloticus* x *O. urolepis hornorum* hybrid had a relatively poor performance in the 1984 test, as well as in previous ones (Wohlfarth et al., in press). It is not recommended to the farmers.

Sex inversion of tilapias has become widespread in Israel in recent years, as an alternative to the use of the standard hybrid which often consists of less than 100% male progenies (Rothbard et al. 1983). In the mistaken belief that the genetic constitution of fish is of no major importance, when practically all-male populations are produced by hormonal induction, F_1 hybrid fish have been used as broodstock and the sex of their F_2 progenies inversed. Here, the F_2 fish

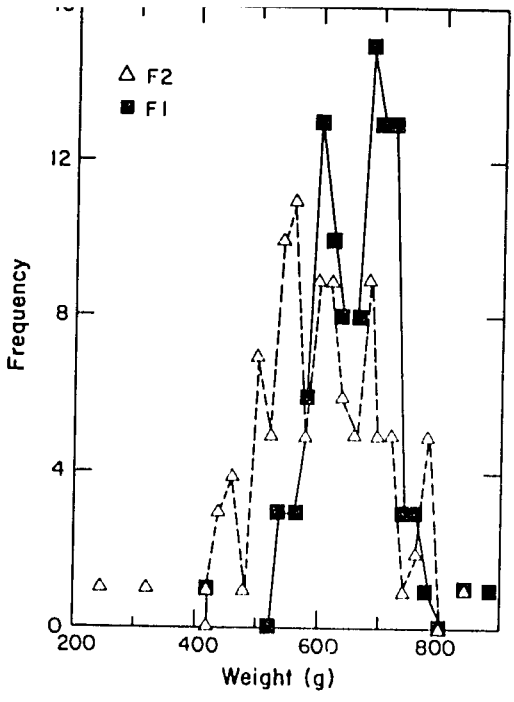

Fig. 3. Size-frequency distributions of samples of tilapia hybrids at harvest of ponds in the 1985 growth test. F_1 is the experimental *Oreochromis niloticus* x *O. aureus* hybrid from Dor (sample size, n = 101). F_2 is a commercially sex-inversed population of F_2 generation of *O. niloticus* x *O. aureus* (n = 102).

tested had a lower mean weight at harvest and a higher variance of weight than F_1 fish, as expected by genetic theory, but more work is needed to investigate this. We believe, however, that the use of good all-male F_1 hybrids is preferred over the use of F_2 (or other) sex-inversed populations, because it helps preserve the pure parental species, and because growth is not lost during the period of the inversion treatment.

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Growth of Two Strains of *Oreochromis niloticus* and Their F_1, F_2 and Backcross Hybrids*

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Abstract

First-year growth of Auburn University-Egypt and Auburn University-Ivory Coast strains of *Oreochromis niloticus* and their F_1, F_2 and backcross hybrids was evaluated in a 60-day yield trial in hapas. At harvest, average group weights ranged from 20.8 to 32.8 g and average group lengths ranged from 102 to 119 mm. Egypt strain was significantly ($P = 0.05$) larger than Ivory Coast strain. All hybrid groups were significantly larger than both parental strains. Heterosis for length and weight in the F_1, F_2 and backcross hybrids was 9.5% and 28.3%, 11.8% and 36.6%, and 11.3% and 32.1%, respectively. The larger F_1 hybrid produced the larger F_2 hybrid. Both F_2 hybrids were larger than their parental F_1 hybrids. Backcross hybrids that were produced by crossing F_1 hybrid females to Egypt males were significantly larger than those that were produced by crossing F_1 hybrid females to Ivory Coast males. F_2 and backcross hybrids were larger because of maternal heterosis; hybrid females produced larger progeny. Results from this study suggest that first-year growth of *O. niloticus* can be improved by using hybrid females

Introduction

Although there have been many crossbreeding studies with tilapia, the majority have been conducted to improve yield through the production of monosex populations by interspecific hybridization. Only two intraspecific crossbreeding studies have been reported for tilapia, and both have been done with *Oreochromis niloticus* (Khater 1985; Uraiwan and Phanitchai 1986). No study has compared growth of F_2 or backcross intraspecific hybrids.

The objective of this study was to evaluate early growth of the Auburn University-Egypt and Auburn University-Ivory Coast strains and their reciprocal F_1 hybrids, F_2 hybrids and backcross hybrids.

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Materials and Methods

The two parental strains of *O. niloticus* used to produce the ten genetic groups that were evaluated in this study originated in Egypt (Khater 1985) and Ivory Coast (Tave and Smitherman 1980). The Auburn University-Egypt strain was collected from the Ismailia Canal, a distributary of the Nile River. This strain was transported to the Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University, Alabama, in May 1982. The Auburn University-Ivory Coast strain originated in the tributaries of the Niger River and Lake Volta, and a foundation stock was shipped to Bouake Fish Station, Côte d'Ivoire (Nugent 1988). From there, descendants were shipped to the Pentecoste Fish Research Station, Ceara, Brazil; from Pentecoste, descendants were shipped to Auburn in 1974. Reciprocal F_1 hybrids that were used as broodstock in this study were produced by Khater (1985).

The parental strains and reciprocal F_1 hybrids were used to produce the following ten genetic groups that were evaluated for growth:

Parental strains

Egypt ♀ X Egypt ♂ (E)
Ivory Coast ♀ X Ivory Coast ♂ (I)

F_1 hybrids

Egypt ♀ X Ivory Coast ♂ (EI)
Ivory Coast ♀ X Egypt ♂ (IE)

F_2 hybrids

EI F_1 hybrid ♀ X EI F_1 hybrid ♂ (EI x EI)
IE F_1 hybrid ♀ X IE F_1 hybrid ♂ (IE x IE)

Backcross hybrids

EI F_1 hybrid ♀ X Egypt ♂ (EI x E)
IE F_1 hybrid ♀ X Egypt ♂ (IE x E)
EI F_1 hybrid ♀ X Ivory Coast ♂ (EI x I)
IE F_1 hybrid ♀ X Ivory Coast ♂ (IE x I)

All broodstock were year class I fish. Average size of females were : E - 215 mm and 164 g; I - 189 mm and 142 g; EI - 195 mm and 133 g; IE - 255 mm and 290 g. All matings were made in 2-m^3 hapas suspended in 20-m^2 concrete tanks on 13 July 1986. There were four replicate spawning hapas for each mating except IE x I, which had two replicates. Four females and two males were assigned to each hapa. All groups spawned by 23 July, except for the IE x I mating, which spawned 10 days later. Group matings produced an average of 7.4 spawns (range 2 to 14).

Swim-up fry from each group were collected and pooled, and a random sample of 500 were stocked in 0.054-m^3 hapas that were suspended in 20-m^2 concrete tanks. A random sample of 100 fry from each group was stocked in each of five replicate hapas on 23 July. Groups were randomly assigned to the hapas. Fry in each hapa were fed an equal amount of feed daily; fry were fed finely ground trout feed (40% protein) at approximately 30% body weight daily, split into three equal feedings. On 7 August fry from the five replicate hapas from each group were pooled, and a random sample of 250 fry per group were stocked for grow-out.

Growth was evaluated over a 60-day growing season. Fry were stocked in 1-m^3 hapas suspended in 20-m^2 concrete tanks. All ten groups were stocked into individual hapas that were suspended in each of five replicate 20-m^2 concrete tanks. Assignment of the groups to the hapas in each tank was random. Fifty fry from each group were randomly assigned to each of the five replicate hapas. Fry in each hapa were fed an equal amount of feed daily; fry were fed finely ground trout feed (40% protein) at 20% total average body weight daily, split into two equal feedings. The amount of feed was adjusted every 15 days.

Fish were measured at stocking and on days 15 and 30. At stocking, a random sample of 15 fry from each group was measured to the nearest millimeter, and on days 15 and 30, a random sample of 30 fish in each hapa was measured to the nearest millimeter.

On day 31, the fish from the five replicates from each group were pooled, and a random sample of 75 fish per group was reallocated to 2-m^3 hapas suspended in a 0.1-ha earthen pond. Twenty-five fish from each group were randomly assigned to each of three replicate hapas; groups were assigned to the hapas in a random manner. Fish were fed with catfish feed (36% protein) *ad libitum* for 30 days.

On day 45, a random sample of 10 fish from each hapa were measured to the nearest millimeter. On day 60, all fish were measured to the nearest millimeter and weighed to the nearest 0.1 g.

Because the IE x I mating spawned 10 days later, stocking and sampling dates for that group were always 10 days later than for that for the other nine groups.

Data were analyzed by analysis of variance. Difference among group means were determined by Duncan's multiple range test. Comparisons among groups were done using the F-test. Heterosis (H) in the hybrid groups was calculated by using the following formula:

$$H = \left(\frac{\text{Mean of hybrids} - \text{Mean of parental strains}}{\text{Mean of parental strains}} \right) 100$$

Results and Discussion

Survival at day 30 ranged from 98 to 100%; at day 60, it ranged from 96 to 100%.

Mean lengths and weights are listed in Table 1. The superiority of some groups could be detected as early as day 15. Final rankings of the groups were similar to those observed during sample dates. The Auburn University-Egypt strain was significantly larger than the Auburn University-Ivory Coast strain at harvest. The significant difference between these two strains was similar to that observed by Khater and Smitherman (this vol.) during first-year growth in both earthen ponds and plastic pools.

At harvest, all hybrid groups were significantly larger than both parental strains (Table 1). Both F_1 hybrids were significantly larger than both parental strains. Heterosis for length and weight was 9.5% and 28.3%, respectively (Table 2). Length and weight of F_1 hybrids were 6.9% and 18.6% greater than E, respectively. The EI hybrid was significantly larger than its reciprocal, and it produced the better F_2 hybrid--EI x EI (Table 1).

Table 1. Mean total lengths in millimeters at stocking (day 0), days 15, 30 and 45 and means, ranges, standard deviations (SD) and coefficients of variation (CV) for length and weight in grams at day 60 (harvest in Egypt (E) and Ivory Coast (I) strains of *Oreochromis niloticus* and their F_1, F_2 and backcross hybrids). Means followed by the same letter are not significantly different (P = 0.05).

	Day 0	Day 15	Day 30	Day 45	Day 60							
					Length				Weight			
	Length	Length	Length	Length	Mean	Range	SD	CV	Mean	Range	SD	CV
Parental groups												
E	15.4	45.4bcd	61.4e	87.4bcd	106.8f	102-115	2.6	2.4	24.5f	22.0-29.0	1.7	7.0
I	15.2	43.2d	58.2f	82.5e	101.7g	97-107	2.1	2.1	20.8g	18.5-25.0	1.6	7.9
F_1 hybrids												
EI	15.3	48.4abc	68.0bc	90.0bc	117.9a	106-123	2.3	1.9	30.4c	28.0-35.0	2.0	6.7
IE	15.2	46.6abcd	65.4cd	85.7de	110.4e	100-120	4.4	4.0	27.7e	24.5-35.0	2.2	7.7
F_2 hybrids												
EI x EI	15.5	46.6abcd	72.6a	97.7a	118.6a	114-126	2.9	2.4	32.8a	27.0-40.6	3.0	9.3
IE x IE	15.6	43.6cd	65.2cd	87.7bcd	114.6c	109-125	3.4	3.0	29.1d	26.8-36.8	1.9	6.4
Backcross hybrids												
EI x E	15.4	51.4a	70.0ab	98.0a	118.1a	112-126	2.1	2.7	31.1b	26.9-38.5	2.6	8.5
IE x E	15.2	50.2ab	67.4bc	96.0a	117.5ab	109-134	3.7	5.2	30.4c	26.0-47.6	4.3	14.3
EI x I	15.2	45.4bcd	66.0c	91.7b	116.2b	108-125	3.9	3.3	30.2c	26.8-38.6	2.2	7.4
IE x I	15.3	42.4d	62.6de	88.0bcd	112.2d	109-120	3.0	2.7	28.0e	24.0-33.0	2.5	8.9

Table 2. Average group total lengths in millimeters, weights in grams, and heterosis for the parental strains, F_1 hybrids, F_2 hybrids, and backcross hybrids in *Oreochromis niloticus*. Means followed by the same letter are not significantly different (F-test; P = 0.05).

	Length	Weight	Heterosis (%)	
			Length	Weight
Parental strains	104.25c	22.65c		
F_1 hybrids	114.15b	29.05b	9.5	28.3
F_2 hybrids	116.60a	30.95a	11.8	36.6
Backcross hybrids	116.00a	29.92a	11.3	32.1

The heterosis for weight in the F_1 hybrids was greater than in the study by Khater (1985); therein, heterosis was 2.3% for E-I F_1 hybrids, 11.6% for Egypt-Ghana F_1 hybrids, and 6.1% for Ghana-Ivory Coast F_1 hybrids. The results of these two studies are in contrast to the results obtained by Uraiwan and Phanitchai (1986) who found heterosis of -28.3% when they hybridized two strains of *O. niloticus* in Thailand.

F_2 hybrids were significantly larger than F_1 hybrids and parental strains, but were not significantly larger than backcross hybrids (Table 2). Heterosis for length and weight for the F_2 hybrids was 11.8% and 36.6%, respectively, and was greater than for the other hybrids (Table 2). Length and weight of the F_2 hybrids were 9.2% and 26.3% greater than E, respectively. The EI x EI hybrid was significantly larger than the IE x IE hybrid (Table 1). Both F_2 hybrids were larger than their parental F_1 hybrids.

Backcross hybrids were significantly larger than both the parental strains and the F_1 hybrids. Heterosis for length and weight of the backcross hybrids was 5.4% and 22.1% greater than E, respectively.

The usual explanation for heterosis is that it is controlled by dominance effects, so heterosis of the F_2 hybrids should be one-half that of the F_1 hybrids and heterosis of the backcross hybrids should be the average of the F_1 hybrids and the parent with which they were backcrossed (Falconer 1981). In this study, heterosis of F_2 and backcross hybrids was greater than that of the F_1 hybrids.

The superiority of the F_2 and backcross hybrids may be due to maternal heterosis. Maternal heterosis is expressed in the progeny of F_1 females (Falconer 1981). Average weights and lengths of progeny (E, I, EI and IE) produced by parental strain females were 109.2 mm and 25.8 g; average lengths and weights of progeny (EI x EI, IE x IE, EI x E, EI x I, IE x E and IE x I) produced by F_1 hybrid females were 116.1 mm and 30.3 g. Mean lengths and weights of progeny produced by F_1 hybrid females were 6.3% and 17.1% greater, respectively, than those produced by E and I females.

The effect of maternal heterosis on length and weight relative to the parental strains can be detected by comparing E with the average of EI x E and IE x E and by comparing I with the average of EI x I and IE x I. In each comparison, the males are the same, but females from a parental strain are compared to hybrid females. Hybrid females produced progeny that were 11.0 mm and 6.25 g larger than E females and 12.5 mm and 8.9 g larger than I females.

The EI x E backcross hybrid was significantly heavier than all other backcross hybrids, but it was not significantly longer than the IE X E hybrid. Backcross hybrids that were produced by backcrossing F_1 hybrid females to Egypt strain males were significantly larger than those that were produced by backcrossing F_1 hybrid females to Ivory Coast strain males (Table 1).

Coefficients of variation (CV's) for length ranged from 1.9 to 4.0%, and CV's

for weight ranged from 6.7 to 14.3% (Table 1). CV's in the parental and hybrid groups were similar. The small CV's found in this study are similar to those found in an earlier study with Auburn University-Ivory Coast strain of *O. niloticus* (Tave and Smitherman 1980).

Size differences of female broodstock and initial size differences among the groups did not influence the results of this study. Siraj et al. (1983) showed that female size influenced sac fry length, but that it had no influence on fry length at day 20. In this study, the largest fish at harvest were produced by females that ranked third (average body weight). Average stocking lengths ranged from 15.2 to 15.6 mm (Table 1). The group with the largest average stocking length was not the largest group at harvest.

The results from this study suggest that early growth in *O. niloticus* can be improved by using crossbred females.

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Effects of Dietary 17α-Methyltestosterone on Sex Reversal and Growth of *Oreochromis aureus**

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Abstract

Oreochromis aureus swim-up fry were fed 0, 1, 10 or 60 ppm 17α-methyltestosterone (MT) for 30 days. Fish that had been fed 0 ppm MT for the first 30 days (phase I) were subsequently fed 0 ppm MT (0-0), 10 ppm MT (0-10), or 60 ppm MT (0-60) for the next 57 days (phase II); fish that had been fed 60 ppm MT during phase I were subsequently fed 0 ppm MT (60-0), 10 ppm MT (60-10), or 60 ppm MT (60-60) during phase II. One group of fish was fed 1 ppm MT (1-1) and another group was fed 10 ppm MT (10-10) during the entire 87-day experiment. The 60-0, 60-10 and 60-60 treatments produced monosex male populations. The 10-10 treatments produced a 99%-male population. The 0-10, 0-60 and 1-1 ppm MT treatments did not alter the sex ratio. During phase I, average weight increased significantly as MT concentration increased. The group fed 60 ppm MT grew 16.4% more than the nontreated control. Final weights for all MT-treated groups were significantly greater than that of the nontreated group. Ten ppm MT-treated feed may produce 100% male populations if the hormone is administered when fry begin to feed and if fish are fed to satiation. In the 0-10 and 0-60 treatment, females had greater anabolic response than males.

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Introduction

Hormonal sex reversal of tilapia to produce monosex populations has been an active area of research for the past 20 years. Research in this area was reviewed by Hunter and Donaldson (1983). Recently, several studies have suggested that hormone-treated, sex-reversed fish grow faster than nontreated fish (Guerrero 1975; Hanson et al. 1983; Muhaya 1985). On the other hand, Anderson and Smitherman (1978) found that growth of normal males was faster than that of sex-reversed males in both *Oreochromis aureus* and *O. niloticus*. They attributed this to competition from progeny of both hormone-treated and nontreated fish, as 0.25% to 2.5% of stocked fish were females.

Although many studies have shown that male tilapia grow faster than females (Tave, this vol.), little is known about the relative growth rates of males and females under the influence of an exogenous androgen. Sex ratios resulting from various levels and durations of exogenous androgens have also not been determined.

The objectives of this study were to evaluate the effects of different dietary 17α-methyltestosterone (MT) treatments on sex reversal and on growth of *O. aureus*.

Materials and Methods

On 29 June 1986, three female and two male Auburn University strain *O. aureus* were stocked into each of twelve 2-m^3 hapas suspended in 3.05-m diameter circular plastic pools at the Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University, Alabama. On 6 July eggs and/or fry were collected and placed in McDonald hatching jars for incubation. Sixteen sib-lots totalling approximately 20,000 fry of the same developmental stage were produced.

On 12 July one hundred and fifty swim-up fry were randomly allocated to each of twenty-four 2-m^3 hapas suspended in eight 20-m^2 concrete tanks (three hapas per tank) for phase I of the experiment. Fry were fed finely ground trout chow with 0 (control), 1, 10 or 60 ppm MT. Groups fed 1 ppm and 10 ppm MT-treated feed had three replications, while groups fed 0 ppm and 60 ppm MT-treated feed had nine replications. Treatments were assigned to the hapas at random. After 30 days (11 August), fry were harvested and group weights from each hapa were determined. The fish from the replications for each treatment were then pooled and held in hapas until stocking in phase II.

On 14 August fry from each treatment were randomly reallocated to forty-eight 2-m^3 hapas that were suspended in a 0.1-ha earthen pond for phase II of the experiment. The group fed 0 ppm MT feed during phase I was divided into three subgroups which were fed either 0, 10 or 60 ppm MT-treated feed for the next 57 days. These groups were designated 0-0, 0-10 and 0-60, respectively. The group fed 60 ppm MT-treated feed in phase I was divided into three subgroups which were fed either 0, 10 or 60 ppm during phase II. These groups were designated 60-0, 60-10 and 60-60, respectively. The groups that had been fed 1 ppm or 10 ppm MT-treated feed received the same feed during phase II. They were designated 1-1 and 10-10, respectively. Each treatment had six replications, and fifteen fish were randomly assigned to each replicate hapa. Fish were fed *ad libitum*. On 10 October each fish was manually sexed and weighed to the nearest 0.1 g.

For phase I, commercial trout chow (40% protein) was ground and screened through a 1-mm sieve. For phase II, commercial floating catfish fingerling feed (36% protein) was used. Hormone-treated feed was prepared as described by Shelton et al. (1978). After appropriate amounts of MT were added to the feed, it was dried, and soybean oil was added at 5% of total weight. Feed was stored in plastic bags and frozen.

Sex ratios were tested for goodness of fit by chi-square. Growth rates were analyzed by analysis of variance. Differences among group means were

analyzed by Duncan's new multiple range test. Regression analysis was used to determine the relationship between dietary MT concentration during phase I and growth. Weight differences between males and females were assessed by Student's t test (Steel and Torrie 1980).

Results and Discussion

Percentages of males and females for each hormone treatment are listed in Table 1. *O. aureus* has the WZ sex-determining system (Guerrero 1975), so males and females are assumed to be produced in equal numbers. Percentages of males in the 0-0 (control) and 0-10 treated groups were significantly ($P = 0.05$) greater than 50% ($\chi^2 = 4.44$, $df = 1$, $P > 0.025$ and $\chi^2 = 4.85$, $df = 1$ $P > 0.025$, respectively). The higher percentages of males in these groups may be due to sampling error at the end of the sex reversal period. The 0-60 and 1-1 treatments did not alter sex ratio from the assumed 1:1 ratio.

Table 1. Percentages of males and females obtained from eight dietary 17α-methyltestosterone hormone treatments of *Oreochromis aureus* fry.

Treatments	Females	Males
0-0	39	61
0-10	37	63
0-60	43	57
1-1	47	53
10-10	1	99
60-0	0	100
60-10	0	100
60-60	0	100

The 60-0, 60-10 and 60-60 treatments produced all-male populations. The 10-10 treatment produced a population which was nearly 100% male; only one female was not sex-reversed. Previous research with tilapia has shown that 25 to 60 ppm androgen in feed produced all-male populations (Clemens and Inslee 1968; Guerrero 1975; Tayamen 1977; Shelton et al. 1978; Rodriguez-Guerrero 1979; Owusu-Frimpong and Nijjhar 1981; Obi 1982), but that 15 ppm was less effective (Guerrero 1975). Data from the present experiment suggest that 10 ppm can produce monosex populations.

Fry were started on hormone-treated feed 1 week after hatching. This early start may have enabled the 10-10 treatment to produce a population which was almost 100% male. Shelton et al. (1978) found that sex reversal of androgen-treated *O. aureus* improved when fry began to eat hormone-treated feed at about 3 weeks posthatching rather than at 4 weeks posthatching.

Fry were fed *ad libitum*, and this may be another factor which contributed to the production of a near 100% male population in the 10-10 treatment. Rodriguez-Guerrero (1979) found that if feeding rate did not satisfy the metabolic demand of fry, hormone-treated feed was not effective in producing monosex populations.

Average weights at the end of phase I are shown in Table 2. Average weight increased as MT concentration increased ($Y = 3.25 + 0.0088X$; $r = 0.81$). Fish fed 60 ppm MT gained 16.4% more than the control group. Survival rates during phase I were not different.

Average body weights of males, females, and males and females combined (overall) and survival rates at the end of the experiment are listed in Table 3. Overall average weights of all hormone-treated groups were significantly greater than that of the control group. Average final weights in the 60-10, 10-10, 0-10, 60-60 and 1-1 treatments were 21.7%, 15.7%, 13.8%, 13.6% and 13.2% greater than that of the control group, respectively. These results demonstrated that MT had a positive anabolic effect on growth, even at levels as low as 1 ppm.

The 60-0 treatment is the treatment that is traditionally used to sex-reverse tilapia. This treatment produced a significant increase in weight; fish in the 60-0 group weighed 13.2% more than fish in the control group.

These results are similar to those found by Hanson et al. (1983). The 60-10

Table 2. The effects of various concentrations of dietary 17α-methyltestosterone (MT) on final average weight and survival of *Oreochromis aureus* during phase I (30 days).

Treatments (ppm MT)	Ave. weight (g)	Average survival (%)
0	3.23	90.4
1	3.27	89.3
10	3.44	93.6
60	3.76	90.7

Table 3. The effects of various concentrations and durations of dietary 17α-methyltestosterone (MT) on final average weight (g) of males, females, and males and females combined (overall) of *Oreochromis aureus*. Mean weights followed by the same letter are not statistically different (P = 0.05).

Treatments	Final average weight (g)		
	Overall	Male	Female
0-0	71.9^{c}	76.0	66.7
0-10	81.8^{b}	82.6	80.4
0-60	79.8^{b}	81.1	78.3
1-1	81.4^{b}	85.4	77.0
10-10	83.2^{ab}	83.2	86.5*
60-0	81.4^{b}	81.4	
60-10	87.5^{a}	87.5	
60-60	81.7^{b}	81.7	

*Only one female.

ppm MT-treatment produced the best growth; the 60-60 treatment produced a significant depression in growth when compared to that of the 60-10 treatment.

Weight differences between males and females decreased as hormone level increased. In the control, males grew 13.9% more than females, and the difference was significant [P (t = 6.769) > 0.001]. In the 1-1 treatment, males grew 10.9% more than females, and the difference was significant [P (t = 6.113) > 0.001]. In the 0-10 and 0-60 treatments, males grew 2.7% and 3.5% more than females, respectively, but the differences were not statistically different. MT enhanced weight gain in both sexes, but it had a greater effect on females.

The addition of an exogenous androgen may elicit a greater response in females, because the percentage increase in hormone level is far greater than that in males. Males have a significantly greater concentration of natural androgens than females. Rothbard et al. (1987) reported that 7-week-old male tilapia have a higher testosterone level than females. Additionally, there may be a threshold response. If the natural concentration of androgens in males is high enough to elicit the response threshold, additional hormone would only increase growth slightly. If the natural concentration in females, on the other hand, is below the threshold, the addition of an exogenous androgen would elicit a large response.

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Genetic Variation of Scale Circulus Spacing (CIRC) in a Tilapia Hybrid *(Oreochromis mossambicus* x *O. urolepis hornorum)**

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Abstract

The heritability of circulus spacing on fish scales is of interest because circulus spacing (CIRC) can be used to compare the size-specific growth rate of individuals, strains and species under natural or aquaculture conditions. A sib correlation method was used to estimate heritabilities of CIRC in a tilapia hybrid *(Oreochromis mossambicus* x *O. urolepis hornorum)*. The narrow-sense heritabilities of CIRC were higher at a constant radius, $CIRC_r$, (representing size-specific growth) than at the margin of the scale, $CIRC_m$, (representing size-at-age). The h^2 estimates from half sib analysis were 0.73 for the average of $CIRC_r$ and negative for $CIRC_m$. The use of CIRC as a criterion for mass selection for size-specific growth is discussed.

Introduction

Annular checks on fish scales have long been used to determine age and growth of fish in seasonal environments (Bagenal and Tesch 1978). Many studies on scale formation have shown that the distance between circuli depends to some extent on growth rate (e.g., Gray and Setna 1931; Bilton 1975). Doyle et al. (1987) and Talbot et al. (this vol.) found a correlation of approximately 0.75 between

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circulus spacing (CIRC) and recent growth in tilapia, and showed that CIRC can be used as an indirect measure of recent growth without reference to annular checks. This information can be used to estimate size-specific growth rates in experimental and aquaculture systems.

One of the principal applications of CIRC measurement is in genetic experiments where it is desired to estimate the genetic component of growth rate variation among individuals and strains. To make efficient use of CIRC as an indicator of growth in genetic experiments, it is necessary to know how much genetic variation exists for the trait *independent* of growth. The objectives of the present study were to estimate heritabilities of various parameters of fish scales, and also of size-specific growth rate as estimated from CIRC, and to compare those heritabilities.

Materials and Methods

Methodology

Heritability estimates and standard errors were determined from a sib analysis (Becker 1984; Falconer 1981). In this procedure, a number of males (sires) are each randomly mated to several females (dams). Data are obtained from the offspring of each family.

The design used in this experiment was a nested ANOVA which included 20 offspring per replicate (cage), 3 replicates per dam, 2 dams per sire and 6 sires.

By using ANOVA, the phenotypic variance was divided into a between-sire component (σ^2_S) which is the covariance of half sibs; a between-dam, within-sire component (σ^2_d) which is the covariance of full sibs; and between-cage (σ^2_r) and within-progenies components (σ^2_w) which are the environmental variance and the remaining genetic components (Table 1).

Sire heritability estimates (h^2_S) and dam heritability estimates (h^2_d) were calculated according to Falconer (1981).

Stocking

The fish used in this experiment were the third generation of the Dalhousie University stock. This stock was established in 1983 when 100 fish were purchased from a local supplier. The fish belong to the "Florida red" strain of tilapia that originated from the hybridization of *Oreochromis mossambicus* and *O. urolepis hornorum*.

Three groups of 20 offspring from each full sib family were randomly chosen and were grown in rearing cages (0.3 x 0.3 x 0.2 m^3) set in two long fiberglass tanks (1.2 x 7.0 x 0.4 m^3). These tanks received water from a common head-tank with the rate of 10 l/min. The water was a mixture of freshwater and seawater with salinity of 4.0-6.0 ppt and temperature range from 24 to 30°C.

The fish were grown for 90 days and fed with an experimental tilapia diet twice daily. Feeding rate was 20% body weight per day in the first month and was

Table 1. Analysis of variance table which was used to determine variance components of heritability estimates in this experiment.

Source	d.f.	Mean square (MS)	Composition of MS
Sires	5	MS_S	$\sigma^2_w+36.31\sigma^2_r+108.93\sigma^2_d+217\sigma^2_s$
Dams within sires	6	MS_D	$\sigma^2_w+36.31\sigma^2_r+108.93\sigma^2_d$
Replicates	24	MS_R	$\sigma^2_w+36.31\sigma^2_r$
Within progenies	1,271	MS_W	σ^2_w

reduced to 10% of body weight per day. The amount of food was adjusted every 15 days. Rearing cages and tanks were cleaned every 15 days.

The experiment ran from June to December 1986.

Scale Collecting

At the end of the experiment, the standard length (L_{90}) was measured on each individual. Four scales were collected from the caudal peduncle starting approximately at the third scale from the last scale and moving forwards. The scales were immediately put in 10% buffered formalin to preserve the fragile outer margin.

Scales were wet-mounted on standard microscope slides and examined using a compound microscope at 400 x magnification. Three measurements were made on each of two scales for each fish (see Doyle et al. 1987) and Talbot et al. (this vol.). The readings for each fish were averaged. The scale parameters were measured as follows (Fig. 1): (1) the radial distance between the center and the anterior margin of the scale "RAD", (2) the width of three circuli at various distances from the center. This measurement is called "$CIRC_r$". For example $CIRC_{10}$ is the width of three circuli at 10 ocular units (350 µm) from the center of the scale. Two $CIRC_r$ measurements were taken, $CIRC_{10}$ and $CIRC_{15}$. In addition, (3) the width of three circuli at the anterior margin of the scale was measured. This is called "$CIRC_m$".

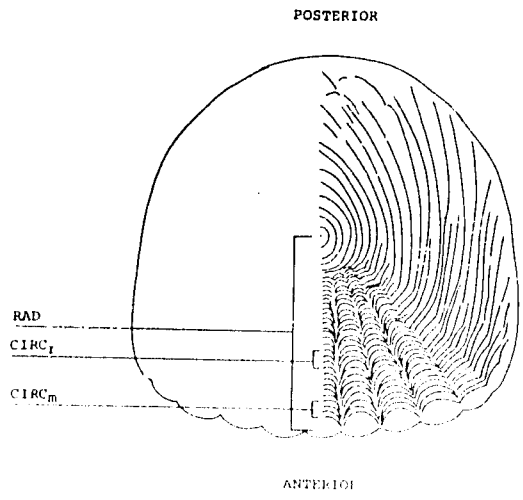

Fig. 1. Scale parameters measured in this study: RAD, the radial distance between the center and the anterior margin of the scale; $CIRC_r$, the width of three circuli at various distances from the center; and $CIRC_m$, the width of three circuli at the anterior margin of the scale.

Results and Discussion

Heritabilities were estimated for $CIRC_{10}$, $CIRC_{15}$, the average of $CIRC_{10}$ and $CIRC_{15}$ (called "MECIRC"), and $CIRC_m$. The estimated heritabilities and statistical analysis of each scale parameter are presented in Table 2. Standard

Table 2. Summary of statistical analysis and heritability estimates of scale parameters

Source	Mean (µm)	S.D.	Unadjusted data F-ratio sire	Unadjusted data F-ratio dam	Unadjusted data h^2 + S.E. sire	Unadjusted data h^2 + S.E. dam	Adjusted data F-ratio sire	Adjusted data F-ratio dam	Adjusted data h^2 + S.E. sire	Adjusted data h^2 + S.E. dam
$CIRC_{10}$	47.08	0.032	0.341^{ns}	23.498^{***}	—	0.79 ± 0.62	3.550^{ns}	1.226^{ns}	0.29 ± 0.66	0.04 ± 0.37
$CIRC_{15}$	54.06	0.060	3.955^{ns}	12.950^{***}	0.62 ± 1.41	0.43 ± 0.70	4.798^{*}	4.329^{**}	0.74 ± 1.52	0.30 ± 0.59
MECIRC	51.57	0.037	3.582^{ns}	12.212^{***}	0.75 ± 1.26	0.39 ± 0.64	8.134^{*}	1.487^{ns}	0.80 ± 1.47	0.07 ± 0.36
$CIRC_m$	60.08	0.059	0.619^{ns}	21.099^{***}	—	1.42 ± 1.12	1.443^{ns}	7.961^{**}	0.04 ± 1.51	1.09 ± 1.64
$CIRCM_{Res}$	0.00	0.047	3.002^{ns}	1.780^{ns}	0.18 ± 0.45	0.08 ± 0.28				
RAD/L90	19.13	1.152	3.057^{ns}	0.930^{ns}	0.29 ± 0.52	—				

*P-value < 0.050
**P-value < 0.005
***P-value << 0.001

errors of heritability estimates were very large because of the small size of the experiment. Although the absolute value of heritability estimates is questionable in the present study because the spawning of half sib families was not simultaneous, comparison is possible because the estimates include the same error due to this effect.

Heritability estimates of CIRC measurement at a constant radius, namely $CIRC_{10}$, $CIRC_{15}$ and MECIRC, are very high especially those of $CIRC_{15}$ and MECIRC (0.73-0.80). The heritability estimate of $CIRC_m$, however, is very low (h^2 was negative in the present experiment) as was the heritability of final size (average growth rate over the 90-day period). We suggest that in both cases the low heritability is due to the intrinsically low repeatability of fish growth measured on a time-specific, rather than on a size-specific basis (Doyle and Talbot, this vol.). This indicates that it may be better to use CIRC at a standard radial distance as a selection criterion, rather than marginal circulus spacing ($CIRC_m$) or size-at-age.

The residuals of the regression of $CIRC_m$ on L_{90} (CSIG) represent the variation in CIRC which is independent of population variation in overall growth up to 90 days. ANOVA showed no significant difference between the sire and dam components. The estimated heritability is 0.29. This trait has a correlation of 0.28 with the relative size of the scale, RAD/L_{90}. The linear relationship between scale radius and individual length is very strong ($R = 0.84$) and the heritability of variation in RAD/L_{90} was nonsignificant ($h^2 = 0.24$) in the present experiments. We suspect that experiments of larger size would show this trait to be partly heritable.

There is a strong linear relationship between scale radius and individual length, and recent growth (Doyle et al. 1987; Doyle and Talbot, this vol.). It can also be assumed that the size of circuli does not change after they are formed (Sire 1986). These correlations were also observed in the present study where the Pearson correlation between scale radius and individual length is 0.92 and the coefficient of multiple correlation between L_{90} and $CIRC_{10}$, $CIRC_{15}$ and $CIRC_m$ range from 0.58 to 0.86. As a result, it may be possible to use $CIRC_r$ as an indirect selection for size-specific growth (Doyle and Talbot, this vol.). This type of selection may otherwise be difficult in practice when no individual identification is available, and fish are not exactly the same age. The CIRC procedure itself is not especially time consuming as 100 g more samples can be handled by a single operator. Despite the large standard error in our experiment, the high estimates of the heritability of the trait (especially when compared with the low estimated h^2 of growth itself; Kamonrat 1987), is very promising. This is consistent with the high phenotypic repeatability of size-specific growth rate (Doyle and Talbot, this vol.). However, indirect selection can only be applied when the genetic correlation of size-specific growth and $CIRC_r$ is high (Falconer 1981). The correlation between $CIRC_r$ and average growth is low because of the low heritability of the latter (Kamonrat 1987). The recommended application is therefore only speculative at present as it depends on the still unknown genetic correlation between $CIRC_r$ and size-specific growth rate.

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Cold Tolerance and Growth of Three Strains of *Oreochromis niloticus**

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Abstract

Progeny of three *Oreochromis niloticus* strains - Egypt (E), Ivory Coast (I) and Ghana (G) - were evaluated for cold tolerance and growth. The mean lower lethal temperature was lower ($P = 0.05$) for E (10.°C) than for I (12.2°C) and G (14.1°C). These differences in cold tolerance were correlated with the geographical origins of the strains. Weight gains for E were greater than for I and G in yield trials in plastic pools, concrete tanks and earthen ponds. Similar rankings (E > I > G) were observed in separately and communally stocked tanks and in communally stocked ponds.

Introduction

Oreochromis niloticus is widely distributed in Africa and has been widely introduced due to its good growth rate (Chimits 1957; Bardach et al. 1972; Shehadeh 1976). Studies have documented yields of *O. niloticus* in different locations with different production systems (Smitherman et al. 1978; Burns and Stickney 1980) but there is no research on the variation which may exist for various culture traits among populations from different origins within its wide geographic range.

The objective of this research was to compare cold tolerance and growth of three *O. niloticus* populations (strains) - Egypt (E), Ghana (G) and Ivory Coast (I) - tested in the same environments.

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Materials and Methods

The study was performed at the Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University, Alabama.

Three strains of *O. niloticus* were used:

Egypt (E) - This strain is derived from fish collected from Ismailia Canal, one of the distributaries of the Nile River, about 75 km northeast of Cairo. The founder stock of 20 males and 66 females was transported to Auburn in May 1982.

Ghana (G) - This strain is derived from fish from the drainage system of Lake Volta in the vicinity of Accra, Ghana, that were sent to Israel in 1978. The Israeli founder stock was 9 females and 2 males. A founder stock of 200 fish was transported from the Fish and Aquaculture Research Station, Dor, Israel, to Auburn in 1982.

Ivory Coast (I) - This strain originated from the tributaries of the Niger River and Lake Volta in the northern part of Côte d'Ivoire (Trewavas 1983; Nugent 1983). The original stock was used in pond culture at Bouaké, Côte d'Ivoire. A small group of fish was shipped to Fortaleza, Brazil, in 1971 (Lovshin et al. 1974). A founder stock of 100 fry was transported to Auburn University from Fortaleza in 1974.

Sixty females and 20 males of each strain were stocked at a 3:1 ratio of females to males in ten 3.05-m^2 plastic pools in July 1982. Their progeny were collected and overwintered indoors until they reached sexual maturity in June 1983. These fish were broodstocks used to produce fish for comparative studies.

Cold tolerance

Progeny of the three *O. niloticus* strains produced during June 1983 were used in an experiment to compare cold tolerance under conditions of ambient cooling from 8 November to 18 December 1983. Fingerlings (17-26 g) from each strain were stocked in three replicate 0.25 x 0.30 x 2.1-m stainless steel troughs that had been divided into three sections. Water flow was 5 l/min and came from a 7-ha reservoir. Forty fish of each strain were randomly assigned to a section of each trough. Fish were fed *ad libitum* daily with 40% protein pelleted feed. Maximum-minimum temperatures were recorded and mortality was observed daily. Mean lower lethal temperatures were determined as described by Chervinski and Lahav (1976).

Growth

Experiment I. This experiment was conducted from 4 August to 16 September 1983 to compare early growth of the three strains in earthen ponds. Four hundred swim-up fry of each of the three strains were each randomly stocked at 20,000/ha in triplicate 0.02-ha earthen ponds. Fry were fed pelleted channel catfish feed (0.2-0.3 mm) containing 32% protein; later, fish were fed pelleted channel catfish feed (0.5 mm). Fish were fed all that they could consume within 60 minutes once a day, 6 days a week for 42 days. Group weights were determined at harvest.

Experiment II. This experiment was conducted from 8 August to 25 September 1984 to compare growth of the three strains in plastic pools. One hundred and fifty-eight swim-up fry from each strain were randomly stocked in each of four replicate 7.2-m^2 circular pools (214,000/ha). Fish were fed catfish crumbles (0.2-0.3 mm), and were later fed pellets (0.5 mm) with 36% protein once a day (all that they could consume within 60 minutes) 6 days a week for 47 days. Group weights were determined at harvest.

Experiment III. The experiment was conducted from 7 June to 17 September 1984 to compare growth to market size. Males of the three *O. niloticus* strains averaging 43-52 g were stocked communally with males of *O. aureus* averaging 47 g in four 0.04-ha earthen ponds. Eighty fish of each of the four groups were stocked in each pond (stocking rate of 8,000/ha). Groups were identified by

dorsal fin marking (Khater 1985). Fish were fed pelleted floating catfish feed (32% protein) once a day (all that they could consume within 60 minutes) 6 days a week for 102 days. Group weights were determined at harvest.

Data were assessed by analysis of variance. Differences among group means were compared using Duncan's Multiple Range Test.

Results and Discussion

Cold tolerance

The three *O. niloticus* strains differed in their cold tolerance. Mean lower lethal temperatures were 10.0°C (E), 12.2°C (I) and 14.1°C (G) (Table 1). E was significantly more cold tolerant than either G or I, which did not differ (P = 0.05). Survival times of G and I were also shorter than that of E. G and I experienced approximately 50% mortality over a 15-day period in November when the minimum water temperature ranged from 11 to 16°C. Cumulative mortality of G and I increased over a 21-day period ending on 13 December, when water temperature dropped to 11°C and all fish died. On the other hand, E mortalities only began when water temperature dropped to 11°C. High mortality of E occurred when the minimum water temperature reached 9°C on 16 December. Differences in cold tolerance among the three strains correlated with their geographical origins: E, 31°N; I, 10°N and G, 6°N.

Growth

Early growth data (Experiments I and II) are presented in Table 2. E grew larger than G and I, which did not differ significantly (P = 0.05). Table 3 presents the data for growth to market size of males of the three strains (Experiment III). Despite a lower initial weight, E grew faster and

Table 1. Cold tolerance of Egypt, Ghana and Ivory Coast strains of *Oreochromis niloticus* at ambient temperature in Auburn, Alabama, 8 November through 18 December 1983. Mean lower lethal temperature (MLLT) is based on minimum daily water temperatures. Means followed by the same letter are not different (P = 0.05; Duncan's Multiple Range Test).

Strain	Survival time (days)		MLLT (°C)	
	Mean	Median	Mean	Range
Egypt	40.5[a]	40.2[a]	10.0[a]	9-11
Ghana	18.6[b]	20.9[b]	14.1[c]	11-16.5
Ivory Coast	20.5[b]	20.2[b]	12.2[b]	11-16.5

Table 2. Mean weights (g) at harvest for Egypt, Ivory Coast and Ghana strains of *Oreochromis niloticus* in two early growth experiments: I (42 days) and II (47 days). The stocking rates of fry were 20,000/ha in earthen ponds (I) and 214,000/ha in plastic pools (II). Means followed by the same letter are not different (P = 0.05; Duncan's Multiple Range Test).

Strain	Experiment I	Experiment II
Egypt	26.3[a]	19.1[a]
Ivory Coast	18.6[b]	14.7[b]
Ghana	16.7[b]	14.6[b]

Table 3. Initial weights and mean weight gains (g) of Egypt, Ivory Coast and Ghana strains of *Oreochromis niloticus* reared communally in ponds and communally or separately in tanks. Means followed by the same letter are not different (P = 0.05; Duncan's Multiple Range Test).

Strain	Initial wt.	Harvest		
		Tanks		Pond
		Sep.	Comm.	Comm.
Egypt	45	364.3a	306.0a	212.1a
Ivory Coast	52	323.8ab	280.3ab	205.9ab
Ghana	48	245.9b	252.0b	185.2b

had higher observed mean weights at harvest under all three conditions. In all growth experiments, survival of the three strains was similar.

Relative rankings of the three strains were the same when stocked separately and when stocked communally. This confirms McGinty's (1984) study which demonstrated that the communal pond concept (Wohlfarth and Moav 1969; Moav and Wohlfarth 1974) is a valid way to assess growth differences among groups of tilapia.

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Progress in Genetic Improvement of Red Hybrid Tilapia in Taiwan

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KUO, H. 1988. Progress in genetic improvement of red hybrid tilapia in Taiwan, p. 219-221. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623 p. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Abstract

The first red tilapia discovered in Taiwan in 1968 were concluded to be an incomplete albino strain of *Oreochromis mossambicus*. Hybridization with *O. niloticus* was done and up to 80% inherited the red color. In 1975 a golden red female tilapia, very fast growing, was found to produce stable colored strains when crossed with male red hybrids. Subsequent hybridization of the F1 progeny females with *O. aureus* and *O. urolepis hornorum* males has led to production and culture of monosex male hybrids growing to 2-3 kg.

Introduction

After World War II, tilapia *(Oreochromis mossambicus)* was distributed to fishfarmers in Taiwan because of its strong viability, ease of reproduction and potential contribution to food supply. Since then, tilapia pond culture in Taiwan has expanded progressively using the following species (date of introduction in brackets): *O. mossambicus* (1946), *Tilapia zillii* (1963), *O. niloticus* (1966), and *O. aureus* (1974), *O. urolepis hornorum* (1981) and monosex (male) pure species and hybrids, including red tilapias and the "Fu-Sou" hybrid *O. niloticus* x *O. mossambicus* developed in 1968. Production now exceeds 50,000 t/year. The annual production of the Fu-Sou hybrid has increased to over 10,000 t.

Our laboratory has concentrated on the development of red tilapias that breed true. This paper describes the history of red tilapia culture in Taiwan.

First Appearance of Red Tilapia in Taiwan

In 1968, several businessmen discovered some red tilapia fry in Tainan and

brought about 100 to my laboratory. They looked like the originally introduced *O. mossambicus* except for their red body color and red eyes. Some of them had red and black spots scattered around their eye sockets. It was concluded that they were an incomplete albino strain of *O. mossambicus*. The lining of their peritoneum was silvery and not pigmented with black (melanin) as in normal *O. mossambicus*. They had the same reproductive and feeding behavior as *O. mossambicus* and their growth was, like *O. mossambicus*, slowed by the low water temperatures seasonally prevalent in Taiwan (down to about 9°C).

Genetic Improvement of Red Tilapia

A hybrid cross was made between albino/red tilapia and *O. niloticus* and the body size variation of different sexes in F1 progeny was studied during 1968-1972. F1 progeny showed two kinds of body color: red (about 30%) and the original black (about 70%). The males generally reached 18-19 cm at the fourth month after hatching, larger than females (16-17 cm). The peritoneum of the red strain was of a clear silver color whereas the black strain kept its pigmented peritoneum. However, there were some black specks on the body surface and peritoneal lining of the red strain. These were approximately symmetrical on both sides. The red strain was judged to be of high potential commercial value especially if its males could be raised in monosex culture.

Further breeding trials using the F1 progeny virtually eliminated the black specks and the probability of red strain inheritance was increased to 80%. However, about 3% of red progeny had a narrow concave (shrunken) abdomen. These fish were of low viability. They were afraid of light and always swam feebly at the bottom. They grew slowly and were unable to resist environmental stress. However, the red strain and was capable of growing to 500-600 g or above within 5 months and up to 1,200 g in 18 months.

During 1975-1979, a female red tilapia, slightly golden-yellow in color and quite similar in appearance to *Chrysophrys major (Sparidae)* was discovered. It grew to over 1,200 g within 18 months. Its female progeny, when crossed with male red hybrids, comprised four groups: original black, red, brownish, and modified white at ratio of 25% each. The brownish strain fish were originally mistaken for the red strain when they were small and were regarded as showing incomplete dominance between red and black strain. The red and modified white strains are very stable. They look very clean without any black specks. The red strain now breeds true and there is a high ratio of red: normal colored offspring in F1 populations, regardless of the species with which it is hybridized.

Production of Monosex Male Red Tilapia

Hybrid crosses between male *O. aureus* and female hybrid red tilapia (descendents of the original red F1 progeny cross with offspring of the golden-yellow tilapia) have been carried out in this laboratory since 1976. A sample of 200 F1 progeny fish comprised 65% red color (all were male) and 35% black color (of which 7-8% were female). All such F1 progeny grew more rapidly and to larger sizes than their parents.

This interspecific hybridization of the red strain with another species *(O. aureus)* has allowed the culture of monosex male hybrids to very large sizes, over 2-3 kg. Similar results have been obtained since 1980 using a cross between female hybrid red tilapia and male *O. urolepis hornorum*, i.e., a 65:35 ratio of red:black progeny and significant heterosis. The F1 progeny from this interspecific cross are consistently all male.

Culture of monosex male red tilapia hybrids is now well established in Taiwan.

However there is much scope for improving the culture performance of the fish and the methods for monosex male production. The red hybrid has been developed into a stable strain over the course of the history summarized here. However, a low-temperature-tolerant strain has not yet been developed, despite studies with interspecific hybrids in this laboratory since 1980.

A list of the papers (all in Chinese) describing these developments follows:

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Genetic Variation in Size and Sexual Maturation of *Oreochromis niloticus* Under Hapa and Cage Culture Conditions

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Abstract

Genetic variation in growth rate and age of female maturation were studied using families of *Oreochromis niloticus*. Broodstock from four cultured strains of *O. niloticus* were stocked at one male and two to six females per breeding pool. Breeding pools were stocked twice with different sets of males and females. Full-sib families were collected from mouth-brooding females and reared together in hapas and cages. Individual fry weights were taken at biweekly intervals from 4 to 14 weeks poststocking. Stocking densities were reduced at 14 weeks

and females were checked for mouth brooding at intervals from 14 to 20 weeks poststocking. Similarities in reproductive patterns between half-sib families were examined. Analysis of variance of size using half-sib families yielded significant effects from common parent, sex (after 10 weeks), growth period and cage. Heritabilities were estimated and ranged from zero to 1.03 ± 0.82 (the latter for 14-week female weight). Despite problems with the experimental design and data analysis, it is tentatively concluded that selection programs for tilapia culture should address early growth and maturation traits.

Introduction

This study grew out of a decision to begin genetic improvement of *Oreochromis niloticus* stocks in the Philippines. Despite the results of Tave and Smitherman (1980) and Hulata et al. (1986) that showed a lack of response of growth rate to selection, we felt that there was enough justification from the success of recent tilapia studies (Jarimopas 1986; Tave, this vol.) and selection programs on other fish (Gjerde 1986) to attempt selective breeding of *O. niloticus* stocks in the Philippines.

Our primary objective was to estimate the genetic variation in growth and reproductive performance and establish broad guidelines for genetic selection programs. Our approach was to combine several strains of *O. niloticus* to create a founder population and estimate genetic variation in growth and reproductive performance by sib-analysis under hapa and cage culture conditions.

Materials and Methods

This study was conducted at the Freshwater Aquaculture Center (FAC) of Central Luzon State University in Muñoz, Nueva Ecija, Philippines.

Four strains of *O. niloticus* were employed as parents to obtain the experimental families. Three of these were obtained from the Philippine Bureau of Fisheries and Aquatic Resources (BFAR) National Freshwater Fisheries Technology Research Center at the same site. The strains were designated as follows:

- TW : imported from Taiwan in 1984, prior history unknown
- IS : origin Israel (Ghana strain)
- SN : obtained from Singapore in 1979; origin Israel (Ghana strain)
- PN : collected from the breeding ponds of FAC.

Breeding was done in 3-m diameter plastic pools (40-50 cm). Two experiments were performed (Table 1). Broodstock

Table 1. Details for strains used, breeding schemes and fry/fingerling feeding rates for two experiments on breeding and growth of *Oreochromis niloticus* families produced by inter-strain and intra-strain crosses: TW = Taiwan strain; IS = Israel strain; SN = Singapore strain, PN = Philippine strain.

Strains used	Breeding scheme/pool	No. of pools (fish)	Mouth brooding inspection interval (days)	Fry/fingerling feeding rates (% biomass/week)		Fingerling cage stocking density (No./m^3)
Experiment 1						
TW, IS, SN	1 ♂ + 2 ♀♀ (one ♀ of same strain as ♂, one different)	24 (8 ♂♂, 16 ♀♀ per strain)	7	weeks 1	100	75
				2	30	
				3	20	
				4	15	
				5 to 14	10	
Experiment 2						
TW, IS, SN, PN	1 ♂ + 4 to 6 ♀♀ (at least one ♀ of each strain)	24 (6 ♂♂, 24 ♀♀ per strain)	10-14	weeks 1	100	60
				2	70	
				3	50	
				4	35	
				5	25	
				6	20	
				7	15	
				8	12	
				9 to 14	10	

weights ranged from 30 to 150 g. Dead fish were replaced by others of the same sex and strain. After breeding, females were either removed to holding tanks or rotated to other pools that had yet to achieve successful spawning by that strain. Eggs and larvae were incubated until completion of yolk absorption.

Fry families were stocked separately in groups of 100 into 1-m^3 small mesh hapas. The water volume varied with pond depth from 0.7 to 0.9 m. Early morning water temperature ranged from 26 to 28°C. All hapas were maintained in one 1,000-m^2 pond, fertilized with a basal application of 1,000 kg/ha chicken manure and a biweekly application of inorganic (50 kg/ha) and organic (1,000 kg/ha) fertilizers. Each hapa received supplemental feed (75% rice bran, 25% fish meal) 6 days/week (Table 1). After 8 weeks, fingerlings were transferred from hapas to 1-m^3 cages.

Hapas with families that could not provide the required stocking numbers were discarded. Fingerlings in cages were fed pellets composed of 30% rice bran, 50% fish meal, 15% copra meal, 5% chick booster feed and 1% vitamin mix.

The mean weight of 20 fry was taken at stocking. From 4 to 14 weeks poststocking fry/fingerlings were sampled biweekly, anesthetized with quinaldine and weighed. Sex was recorded at 12 and 14 weeks.

The growth period was terminated at 14 weeks poststocking and 16 females and 4 males were randomly assigned to each cage for a study of maturation. Cages were gently removed from the pond weekly and females checked for mouth brooding. Any eggs or fry present were counted. Some reproduction occurred in the week prior to the fourteenth week sampling date. These females were included in the results for age and weight at first reproduction, as well as fecundity, but they were removed from the cage and sixteen additional females were stocked in the cage for reproduction. Fecundity refers to eggs and yolk sac fry collected from the mouths of females. This phase of each experiment was terminated at 20 weeks poststocking. Females that had not reproduced by 20 weeks are not represented in the data set. This causes a bias in the data from the exclusion of females that were presumably late maturing and may have reproduced at a later date.

Age, weight and fecundity were recorded for all females that bred with the exception that fecundity data were not collected from females that ejected part or all of their brood from the mouth prior to placement in collecting vessels.

Families sharing a common parent were used to create a data set for half-sib analysis to estimate genetic variation in size and heritability. Six males and one female, in the first experiment, contributed two families to the data set and one female had three spawns included, resulting in 17 families for the half-sib analysis. Ten parents, nine males and one female, contributed two or three families in Experiment 2, for a total of 22 families in the half-sib analysis. Two of the males had three families in the analysis.

Results and Discussion

Thirty-six families were stocked in hapas between 7 February and 15 April 1985. Seven suffered near total mortality in the first 4 weeks, probably due to chemical contamination of the holding basins used in sampling. Twenty-nine cages were stocked from the hapas in the first experiment. All of these reached final sampling at 14 weeks poststocking with an average survival of 95% from 8 to 14 weeks.

In Experiment 2, 37 hapas were stocked during a 5-week period from 23 May to 27 June. Six of the hapas had families with fewer than 60 survivors and were discarded. Thirty-one cages yielded complete growth data and had average survivals of 98% from cage stocking to 14 weeks.

The mean weights obtained for all individuals weighed from all hapas and cages reaching 14 weeks are given in Table 2. At hapa stocking, the fry weights ranged from 6 to 17 mg. By 8 weeks, the

Table 2. Mean, standard deviation and coefficient of variation of weights of *Oreochromis niloticus* fry and fingerlings raised in hapas and cages. For details of feed and fertilizer inputs, see text.

Age (weeks)	Sex	N	Mean (g)	S.E. (g)	C.V. (%)
		Experiment 1 29 Families			
4		580	0.69	0.24	33.86
6		580	1.50	0.36	23.97
8		561	2.75	0.79	28.63
10		594	4.82	1.51	31.35
12	Male	266	8.13	2.34	28.79
	Female	326	7.06	1.97	27.83
14	Male	672	10.75	3.18	29.62
	Female	652	9.09	2.57	28.32
		Experiment 2 32 Families			
4		619	0.77	0.31	40.68
6		621	1.95	0.60	30.56
8		750	3.73	0.98	26.20
10		746	6.37	1.74	27.25
12	Male	379	8.95	2.68	29.90
	Female	389	7.96	1.97	24.77
14	Male	649	11.64	3.38	29.04
	Female	498	10.03	2.51	25.05

range of mean weights was 1.73 to 3.98 g. As a result of the reduction of density from hapas to cage stocking, individuals from those cages with high survivals were available for sexing by dissection and microscopic examination of gonads. The analysis showed no significant difference between the weights of the sexes at that age.

External sexing became possible at 12 weeks for most individuals. By this age, sexual dimorphism in weight was becoming apparent, although the degree differed among the families. The mean weights of the sexes at 14 weeks post-stocking showed less than 2 g difference between males and females in both experiments.

Thirty-nine families from 18 common parents were used in nested ANOVA to examine the contribution of the common parents to variation in size. Table 3 shows the ANOVA on each variable and estimated variance components. At each age, the effect of shared parent and shared cage are significant. It is clear that in Experiment 1, an overwhelming amount of the variance is attributable to the shared cage effect which is composed of the common environmental effect and the effect due to full-sibs, maternal effect and dominance effect. This leads to the variance due to the half-sib parent being estimated as negative in all but one case. This problem also affected Experiment 2, but gave negative half-sib variance components in only three of eight cases.

Sequential growth data were used to study the value of early size as a predictor of adult size. Spearman rank order correlations were calculated for the cage weight means (Table 4). For the first experiment, size at 4 weeks is a poor predictor of later size, and size at 6 weeks is a poor predictor of the families weight at 14 weeks. However, from 8 weeks onwards, the correlations are significant. Thus selection could have occurred at 8 weeks if we

Table 3. Analyses of variance for weight of *Oreochromis niloticus* fry and fingerlings at ages 4 through 14 weeks after stocking. The degrees of freedom (DF), mean square (MS), and estimated variance component (s^2) are given for each source of variation in the model. All treatment mean squares are significant at $P < 0.01$.

Age weeks	Sex	Source of variation	Experiment 1 DF	Experiment 1 MS	Experiment 1 s2	Experiment 2 DF	Experiment 2 MS	Experiment 2 s2
4		Parent	7	1.02	0.001	9	1.35	-0.01
		Cage	9	1.05	0.05	12	1.64	0.08
		Error	323	0.02	0.02	417	0.03	0.03
6		Parent	7	0.58	-0.02	9	2.57	-0.03
		Cage	9	1.51	0.07	12	4.10	0.19
		Error	323	0.09	0.09	418	0.21	0.21
8		Parent	7	4.63	-0.08	9	10.19	0.05
		Cage	8	8.00	0.39	12	7.63	0.28
		Error	304	0.30	0.30	528	0.58	0.58
10		Parent	7	15.79	-0.49	9	39.10	0.30
		Cage	9	36.94	1.77	12	22.68	0.85
		Error	326	1.29	1.29	524	1.50	1.50
12	Male	Parent	7	23.73	-0.93	9	57.50	0.64
		Cage	9	37.70	4.03	12	36.81	3.07
		Error	139	2.77	2.77	237	2.88	2.88
12	Female	Parent	7	13.63	-0.79	9	45.74	0.74
		Cage	9	31.86	2.57	12	21.89	1.68
		Error	184	2.13	2.13	264	1.45	1.45
14	Male	Parent	7	81.75	-2.93	9	107.04	-0.31
		Cage	9	220.79	9.11	12	103.47	5.37
		Error	409	3.82	3.82	420	3.89	3.89
14	Female	Parent	7	85.80	-0.05	9	111.74	2.22
		Cage	9	86.10	3.79	12	29.74	1.82
		Error	362	3.00	3.00	332	2.79	2.79

Table 4. Spearman correlation coefficients between cage mean weights of *Oreochromis niloticus* fingerlings at different ages. Experiment 1 values are above the diagonal; Experiment 2 values are below the diagonal. For full details, see text.

Age	4 weeks	6 weeks	8 weeks	Age 10 weeks	12 weeks	14 weeks Male	14 weeks Female
4 weeks		0.66**	0.35	0.12	0.21	-0.07	0.216
6 weeks	0.60**		0.75**	0.49**	0.38*	0.24	0.27
8 weeks	0.17	0.29		0.74**	0.65**	0.58**	0.53**
10 weeks	0.41*	0.36*	0.68**		0.75**	0.72**	0.63**
12 weeks	0.42*	0.33	0.54**	0.90**		0.80**	0.80**
14 weeks male	0.38*	0.49**	0.49**	0.79**	0.86**		0.79**
14 weeks female	0.40*	0.46**	0.52**	0.79**	0.79**	0.82**	

*$P < 0.05$.
**$P < 0.01$.

assume that the same genes influence growth from fry to 8 weeks as from 8 to 14. Curiously, the 4-week means in Experiment 2 are significantly correlated with the 14-week weights, but the 8-week weights show a low correlation with the early weights. Values from Experiment 2 indicate that the predictive value of early size may be acceptable. It improves at 10 weeks as in Experiment 1.

After 14 weeks, breeding had begun and nearly all females had spawned by 20 weeks. In some cages, reproduction began as late as 18 weeks and a few females had begun breeding before 14 weeks. Tables 5 and 6 summarize the results. It appears that, although growth was faster in Experiment 2, reproduction was delayed and fecundity was lower. The range of reproductive performance by experiment shown in Table 5 is quite interesting. The smallest female to reproduce in Experiment 1 weighed 5.8 g and was brooding 11 eggs. The largest female reproducing in that experiment weighed 34.3 g and brooded 319 eggs. In the second experiment, the smallest was 5.7 g with 9 fry, and the largest weighed 31.9 g and had 222 eggs and fry combined. The highest fecundity was 516 eggs from a 20.3-g female in Experiment 1.

It should be noted that, the variable "age at first spawning" is biased because the experiment was arbitrarily terminated at 20 weeks, the distribution is inappropriate for an ANOVA (see comment on early and late spawners in Materials and Methods). Thus it is not surprising that the variance component for common parents from the ANOVA on age at first spawning is negative and heritability is zero (see Table 6). The analysis of weight at first spawning resulted in significant mean squares and moderately high heritabilities. There is no correlation between female weight at 14 weeks and mean female weight at first spawning. This suggests that the heritability of weight at first spawning is independent of the heritability of female weight at 14 weeks (Table 7).

In most cages, considerably more eggs were collected than yolk sac larvae, but the data were analyzed together. The results of the analysis show that in Experiment 1, the variation attributable to full-sib families and common cage environment is quite low and results in a negative variance component. Zero heritability is estimated for fecundity in Experiment 1. However, Experiment 2 shows significant mean squares for fecundity and the variance components translate into a low heritability.

The experimental design had problems that prevent firm conclusions from the data: environmental effects, including handling stress; lack of replicate cages (which inflates the mean squares for full-sib families); and use of large numbers of individuals which decreases the value of the error mean square and biases the contribution of the full-sib mean squares in the estimation of various components and standard errors of the heritabilities (Becker 1984). We had decided to maximize the number of families and forego replication within families because of the

Table 5. Summary of age, weight and fecundity data for *Oreochromis niloticus* females first spawning between 14 and 20 weeks poststocking in hapas/cages in two experiments.

Variable	Experiment 1 N	Mean	(S.E.)	Experiment 2 N	Mean	(S.E.)
Age (weeks)	104	16.5	(0.16)	169	17.7	(0.13)
Weight (g)	104	15.43	(0.58)	169	16.65	(0.32)
Fecundity (# eggs or fry collected/female)	90	142.2	(9.87)	169	117.6	(6.15)

Table 6. Results of ANOVA on reproductive variables for first spawning of *Oreochromis niloticus*.

Variable	Source of variation	Experiment 1 DF	MS	s2	Experiment 2 DF	MS	s2
Age at first spawning (weeks)	Parent	4	4.13	-0.25	8	8.46**	-0.05
	Cage	5	6.65*	0.59	10	7.57**	0.80
	Error	94	2.45	2.45	150	2.31	2.31
Weight at first spawning (g)	Parent	4	231.62**	6.00	8	73.78**	2.01
	Cage	5	75.30*	6.41	10	32.68**	2.44
	Error	94	24.97	24.97	150	13.06	13.06
Fecundity (no. of eggs/larvae collected from mouth)	Parent	4	17,722.7	1,012.7	8	15,157.3**	153.0
	Cage	5	3,466.1	-791.3	10	11,182.8*	695.1
	Error	80	8,647.7	8,647.7	150	5,597.1	5,597.1

*P < 0.05.
**P < 0.01.

Table 7. Estimated heritabilities for growth and maturation variables for *Oreochromis niloticus* fry and fingerlings raised in hapas and cages to first spawning. Data for weeks 4-10 are for unsexed fish. Standard errors are given in parentheses.

Variable	Experiment 1	Experiment 2
4 week weight	0.06	0
6 week weight	0	0
8 week weight	0	0.21 (0.42)
10 week weight	0	0.46 (0.52)
12 week male weight	0	0.59 (0.67)
12 week female weight	0	0.77 (0.77)
14 week male weight	0	0
14 week female weight	0	1.30 (0.82)
Age at first spawning	0	0
Weight at first spawning	0.64 (0.72)	0.46 (0.44)
Fecundity at first spawning	0	0.09 (0.28)

objectives of parallel studies. Moreover the tilapia growth rates were low throughout, probably because of density effects. Therefore, we can only speculate on the meaning of the correlations obtained between weights at various ages.

The decision to maximize number of families reduced the power of both experiments for estimating the heritability of the traits analyzed. Without replicate cages within full-sib families, much of the variance caused by environmental differences goes into the source of variation that should represent the full-sib effect. The cage parent variance term contains additive and dominance genetic variances, maternal effects, sire x dam interactions, and common environmental variance due to the difference in stocking date of cages (Becker 1984). Therefore no heritability estimates were made from the variance among full-sib groups. Only the variances related to the effect of differences among half-sib families were used.

We took negative variances as being equal to zero (Searle 1971). Thus any negative correlations and variance components lead to zero heritabilities

(Table 7). Clearly the heritabilities estimated for Experiment 2 12- and 14 week female weights are encouraging despite the shortcomings of the experimental design.

The data on reproduction also reflect the flawed nature of Experiment 1 and the need to focus on results from Experiment 2. The way in which the data on age at first reproduction was collected and the, no doubt, non-normal distribution of the values used in this analysis made it unlikely that a standard approach to partitioning the variance would yield useful results. We suspect, however, that the heritability of weight at first spawning is nonzero.

Clearly much more work needs to be done on all these traits but our preliminary conclusion is that further selection for improvement of growth and reproductive traits *O. niloticus* should be attempted.

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Effects of Broodstock Exchange on *Oreochromis niloticus* Egg and Fry Production in Net Enclosures*

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Abstract

Net enclosures *(hapas)* are used as tilapia egg and fry production units. Typically, eggs and fry are harvested and the parents remain in the *hapa* for another spawning cycle. However, removing spent fish after each spawning period and allowing them to recuperate may improve spawning performance. This research tested the effects of broodstock exchange on *Oreochromis niloticus* egg and fry production in *hapas*. Six female and three male *O. niloticus* adults were stocked into twelve 3.3-m^2 net enclosures suspended in 20-m^2 concrete tanks. Treatments tested were: no broodstock exchange; female exchange; and male and female exchange after each 21-day spawning period. Five egg and fry harvests were completed during a 105-day period. Replacement male and female broodstock were held separately in similar *hapas*. Adults were fed a 38% crude protein pelletized trout feed at 2.0% of their body weight daily. Average egg and fry numbers produced per gram of female body weight were 3.1, 3.1 and 3.7 for no exchange, female exchange and male and female exchange, respectively. The difference between the first two and the last treatment is statistically significant as tested by ANOVA ($P < 0.01$).

Introduction

Tilapia seed production in raceways, tanks, pools and *hapas* is becoming widely used as large numbers of same-age fry are required for hormone sex-inversion and commercial grow-out. Increased broodstock density, control of broodstock sex ratio and periodic fry removal from the spawning units have increased seed production per square meter and per female over that achieved by more traditional pond reproduction methods (Berrios-Hernandez 1979; Balarin and Haller 1982; Coche 1982; Behrends 1983; Snow et al. 1983).

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Small mesh (<1.6 mm) *hapas* or net enclosures can be used to spawn mouthbrooding tilapias (Guerrero 1977; Hughes and Behrends 1983). Advantages are: 1) undercapitalized farmers can set up the enclosures for a small initial investment; 2) fry can be harvested from *hapas* with little mortality by crowding them into one end before removal; 3) female broodstock are easily harvested, facilitating egg removal. Periodic egg removal from incubating females shortens the interval between spawns and increases the seed number obtained from each female over the spawning season (Lee 1979).

Disadvantages to spawning tilapia in *hapas* include: 1) the possible need to replace *hapas* every 6 to 12 months if the material is not treated for resistance to water and sunlight; 2) small *hapa* mesh becomes easily clogged with algae which will restrict water exchange and create the potential for low dissolved oxygen in intensively stocked and fed enclosures; 3) phytoplankton turbidity is often high, reducing visibility and making dip-net removal of schooling fry difficult; 4) fry removal from *hapas* in turbid water is best accomplished by concentrating fry in one end of the *hapa*. Broodstock are disturbed during fry crowding and mouthbrooding females often release their eggs which are rarely picked up again. In this case, eggs must be removed and artificially hatched if they are to survive, which requires incubating facilities.

Intensive spawning strategy typically involves partial fry and/or egg harvest. Brooders remain in the spawning unit for successive egg/fry harvests before they are replaced. Guerrero and Guerrero (1985) found that *O. niloticus* broodstock separated by sex and fed a high protein ration before stocking into 100-m^2 concrete tanks yielded peak fry numbers at 19 to 20 days. Fry production decreased during the remaining 30 to 52 days. These results suggested that it might be more efficient to terminate the breeding cycle after 20 days and restock with fresh broodstock. Our research tested the effects of *O. niloticus* broodstock exchange after each partial harvest on egg and fry production in *hapas*.

Materials and Methods

This study was conducted at the Fisheries Research Unit, Alabama Agricultural Experiment Station, Auburn University, Alabama, USA. *O. niloticus* broodstock were obtained from the Centro de Pesquisas Ictiologicas, Pentecoste, Ceara, Brazil, in 1974. The foundation stock for the Pentecoste station originated from the Station de Recherches Piscicoles, Bouaké, Ivory Coast, in 1971 (Tave and Smitherman 1980).

One- and two-year-old male and female broodstock were held in separate concrete tanks and fed a 32% crude protein floating catfish feed *ad libitum* for two weeks before they were stocked into spawning *hapas*. Six females and three males averaging 89 g and 138 g, respectively, were stocked randomly into 3.3-m^2 (2.74 m x 1.22 m) *hapas* suspended in 20-m^2 concrete tanks (3 *hapas*/tank). *Hapas* were constructed of 1.6-mm knotless nylon mesh netting. They were covered with plastic netting to keep fish from escaping. Water depth in the tanks was held at about 80 cm.

Treatments were: no broodstock exchange; female exchange; and male and female exchange after each 21-day spawning period. Each treatment was replicated four times in a completely randomized block design. One replicate from each treatment was placed randomly in each of the four concrete tanks used. All broodstock were removed from the *hapas* at each harvest and weighed to the nearest gram. At this time, incubating females had eggs washed from their mouths. Fry and released eggs were harvested from each *hapa* with a small mesh dip-net. Eggs and fry were preserved in 10% formalin for counting at a later date. Males and females that were not exchanged were immediately restocked

into their respective *hapas*. Replacement broodstock were segregated by sex and held in conditioning *hapas* that were the same as the spawning *hapas*. One male and three female conditioning *hapas* were stocked with about 6 fish/m2. Male and female replacements were randomly selected from the conditioning *hapas*, weighed and stocked into the spawning *hapas*. Spent broodstock were placed into the conditioning *hapas* for at least 21 days before reuse. Five males were lost from the no replacement treatment while one male and one female escaped from both the female-male and female replacement treatments during the experimental period. Losses occurred during harvest and were replaced immediately. Weight of the escaped fish was estimated by averaging the weight of the remaining fish of the same sex in the *hapa*. Five harvests were completed during the 105-day experiment (June 28 to September 21).

Tilapia in spawning and conditioning *hapas* were fed a 38% crude protein sinking trout pellet at 2.0% and 2.5% of their body weight daily, respectively. Fish were not fed on harvest days or when morning dissolved oxygen levels were below 1.5 mg/L. Water was added to the concrete tanks to replace that lost to evaporation and to increase low dissolved oxygen levels. Dissolved oxygen and water temperature in the concrete tanks were monitored in the early morning and afternoon every other day using a Yellow Springs Instrument Co. dissolved oxygen meter (Table 1). Analysis of variance was used to test differences among treatment means.

Results

Average egg and fry numbers/gram female body weight (average of stocking and harvest weight) were 3.1, 3.1, and 3.7 after five harvests (105 days) for no exchange, female exchange and female and male exchange, respectively (Table 2). Male and female exchange gave higher seed numbers/gram female body weight than the other two treatments ($P < 0.01$). There was no difference between female exchange and no broodstock exchange ($P < 0.05$). Initially, stocked tilapia adults had been treated similarly and no exchange had taken place so an analysis of harvests 2 to 5 (84 days) was made. Average seed numbers/gram female body weight were 2.9, 2.9 and 3.5 for no, female, and male and female exchanges, respectively. Statistical results were equal to those found for five harvests.

Table 1. Average and range of water temperatures (T, °C) and dissolved oxygen levels (D.O., mg/l) per spawning period and concrete tank.

Tank no.	Spawning period 1 T	1 D.O.	2 T	2 D.O.	3 T	3 D.O.	4 T	4 D.O.	5 T	5 D.O.
1	27.5 (26-28)	6.4 (4.5-9.5)	28.5 (27-31)	7.3 (3.5-10)	28.8 (28-31)	3.0 (1.0-10)	26.1 (25-23)	2.6 (1.0-5.7)	24.5 (24-26)	3.5 (1.3-4.3)
2	27.5 (26-28)	5.6 (2.5-9.6)	28.5 (27-31)	8.0 (2.6-9.0)	28.8 (28-31)	6.1 (4.0-10)	26.1 (25-28)	2.8 (1.2-4.0)	24.5 (24-26)	3.4 (1.3-3.5)
3	27.5 (26-28)	5.7 (3.6-9.5)	28.5 (27-31)	8.8 (7.5-10)	28.8 (28-31)	3.3 (1.0-9.5)	26.1 (25-28)	1.9 (0.7-3.5)	24.5 (24-26)	4.0 (1.5-4.5)
4	27.5 (26-27)	5.9 (3.9-10)	28.5 (27-31)	8.7 (7.0-10)	28.8 (26-31)	3.4 (1.2-10)	26.1 (25-28)	1.7 (0.7-3.4)	24.5 (24-26)	3.0 (1.0-3.3)

Table 2. Effects of no broodstock exchange (No), female exchange (F) and male and female exchange (M+F) on *Orcochromis niloticus* egg and fry yields from net enclosures.

Treatments	Harvest date 28/06 No	28/06 F	28/06 M+F	19/07 No	19/07 F	19/07 M+F	09/08 No	09/08 F	09/08 M+F	30/08 No	30/08 F	30/08 M+F	21/09 No	21/09 F	21/09 M+F	Average No	Average F	Average M+F
Av. total male[1] wt./hapa, g	444	465	490	552	547	635	593	584	574	642	629	737	696	682	740	585	581	635
Av. total female[1] wt./hapa, g	592	567	558	617	696	705	793	852	828	885	925	915	913	1,100	1,060	771	828	813
Av. egg no.	1,342	1,093	1,171	435	784	979	910	686	545	0	0	0	401	0	0	618	513	539
Av. fry no.	987	1,196	1,173	621	1,011	884	753	1,164	1,079	3,469	2,760	3,914	3,111	4,304	5,250	1,788	2,087	2,460
Av. egg and fry no.	2,329	2,289	2,344	1,056	1,795	1,863	1,663	1,850	1,624	3,469	2,760	3,914	3,512	4,304	5,250	2,406	2,600	2,999
Av. eggs and fry/g female	3.9	4.0	4.2	1.6	2.6	2.6	2.1	2.2	2.0	3.9	3.0	4.3	3.8	3.9	5.0	3.1	3.1	3.7
Av. eggs and fry/g female/day	0.19	0.19	0.2	0.08	0.12	0.12	0.10	0.10	0.10	0.19	0.14	0.20	0.18	0.19	0.24	0.15	0.15	0.18

[1] The average of stocking and harvest weights.

Average individual male and female stocking weights, as indicated by the average total male and female weights per *hapa*, had a greater rate of increase over the experimental period in the replacement treatments than in the no replacement treatments (Table 2). Therefore, it appears that isolating males and females in conditioning *hapas* permitted them to gain more weight than when they were held together in the spawning *hapas*. However, an expected increase in reproductive output/gram female body weight due to broodstock conditioning was not realized in the female replacement treatment.

Initial seed production/*hapa* was intermediate, followed by decreases in harvests two and three (Fig. 1). However, egg and fry numbers rebounded at the fourth harvest followed by the highest seed production obtained during the experimental period at harvest five. Egg and fry increases and decreases are independent of treatments and appear to be influenced by something other than broodstock sex segregation and conditioning.

Discussion

Increases and decreases in seed numbers over the experimental period are similar to patterns observed by Guerrero and Guerrero (1985) working with *O. niloticus* in the Philippines. They noted an initial spawning peak after stocking followed by a decline in fry production over a 49- to 72-day experimental period. Hughes and Behrends (1983) working in the USA also reported an early peak in *O. niloticus* eggs and fry harvested followed by decreasing numbers until late September-early October when highest seed production was obtained at the end of a 70-day reproduction period. The present research, as well as that performed by Hughes and Behrends (1983), was done in a temperate climate with seasonal temperature and photoperiod changes. Would a similar spawning pattern occur in the tropics if brooders were allowed to spawn for 100+ days instead of the 49 to 72 days used by Guerrero and Guerrero (1985)?

Guerrero and Garcia (1983) were able to produce an average of about 0.03 *O.*

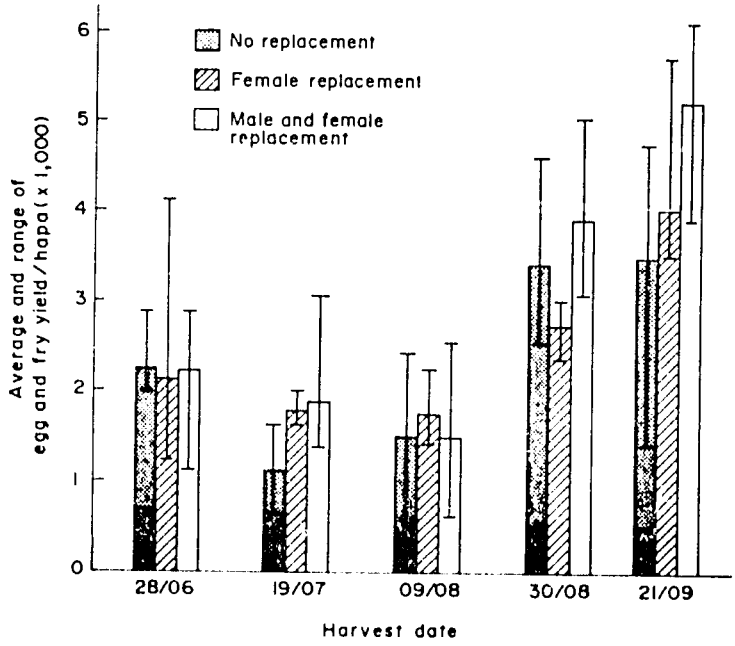

Fig. 1. Relationships between *Oreochromis niloticus* egg and fry yields and broodstock exchange for five harvest dates. Bar lines represent the range of eggs and fry harvested.

niloticus fry/gram female/day in 48-m^2 *hapas* stocked with 192 brooders at a ratio of 1 male to 3 females and partially harvested daily. Hughes and Behrends (1983) collected an average of 0.03 to 0.16 eggs and fry/gram female/day from 3.3-m^2 *hapas* stocked with 5 or 10 broodstock per m^2 at a ratio of 1:3 or 1:2 males to females and harvested every 10 to 18 days. In the present research, average eggs and fry produced/gram of female/day were 0.15, 0.15 and 0.18 for no exchange, female exchange, and male and female exchanges, respectively. Seed yields are higher than those found by Guerrero and Garcia (1983) and equivalent to those obtained in the best treatments tested by Hughes and Behrends (1983). Guerrero and Garcia (1983) harvested only fry, while the authors and Hughes and Behrends (1983) harvested eggs and fry. Removing eggs from incubating females increases the seed produced/female/day (Lee 1979).

Male and female broodstock exchange resulted in a 16% increase in egg and fry numbers/gram female over a 105-day spawning period when compared with female only and no broodstock exchange. Thus, for every kilogram of adult *O. niloticus* females, an increase of about 2,500 eggs and fry/105 days can be obtained with male and female exchange.

Males may have more influence on tilapia seed numbers than was previously thought. No explanation is given for the seed increase obtained with male and female broodstock replacement compared with female only replacement even though females in both treatments were conditioned before exchange. Influence of male broodstock exchange requires further testing.

Application of tilapia broodstock exchange technology will depend upon local socioeconomic conditions and facilities, but will be most practical in reproduction systems where broodstock are easily harvested. To be adopted, increased seed production must compensate for higher costs for additional broodstock, conditioning facilities and labor.

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The Inheritance of Black Pigmentation in Two African Strains of *Oreochromis niloticus*

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Abstract

Oreochromis niloticus broodstocks in Ein Hamifratz derive from founder populations imported from Uganda and Ghana. The Uganda *O. niloticus* produced a few light colored phenotypes (L) which all had a clear peritoneum. They lacked the typical black stripes body color (-) and had a uniform shade of grey which appeared with various degrees of intensity on the dorsal section and tail. All progenies derived from the Ghana stock (G) had a normal wild type coloration (W) and a full display of stripes (+).

Inbreeding between L individuals produced mainly L pink (p) phenotypes but also bright red (r) orange (o) as well as a few albino (a).

Crosses between GW + females Ga- and Gr- males produced only W+ F_1 individuals. In F_2 progenies the L:W ratio was 1:3. In crosses between F_1 W+ females with Lr- and Lo- phenotypes, the ratio was 1:1. Crosses between L- F_2 females W+ x La-) and Lr- or Lo- mainly produced L+ phenotypes but some only had a partial display of stripes. All L+ and L- individuals had a clear peritoneum. Internal and external black pigmentation on the body of *O. niloticus* is controlled by one gene and two alleles, 'B' dominant and 'b' recessive. The striped pattern on dorsal fin and tail is probably controlled by two or more genes which control the production of color and its distribution along the dorsal section and tail. The appearance of partially striped phenotypes in progenies deriving from W+ and L- crosses together with other phenotypes is still not fully understood.

Introduction

Among the tilapias, *Oreochromis niloticus* is considered as one of the best for pond culture in tropical areas. It is usually cultured as a pure species but is sometimes hybridized with *Oreochromis aureus* (Fishelson 1962; Mires 1977, 1983) or with *O.urolepis hornorum* (Lovshin 1982). *O. niloticus* from different geographical regions have similar coloration. Their dorsal region is black, the middle section is olive and the ventral, silvery white. Red coloration mainly around the head can be seen under the black pigmentation. These red areas become brighter during breeding activity especially in males.

Wild type *O. niloticus* (W) typically have vertical stripes on the tail and dorsal fin. Like many other tilapia species, they also have a black peritoneum. While searching for pure strains of tilapia broodersuitable for hybridization, two stocks of *O. niloticus* were imported and bred in the Ein Hamifratz Fish Hatcheries: from Uganda (U) 1969 and from Ghana (G) 1974. All subsequent progenies derived from G and most derived from U had a W coloration but some U progenies had a light coloration (L) with no external or internal black pigmentation. Instead of the typical striped pattern on the dorsal fin and tail (+) most of the L phenotypes had a partial or complete greyish coloration (-) which substituted the striped areas. Only very few such individuals had unpigmented tails and fins. Further inbreeding of L phenotypes produced albino (a), orange (o), red (r) and several other phenotypes with various combinations and intensities of red and orange.

In 1983 it was decided to study the inheritance of color in these *O. niloticus* stocks. A two phase program was prepared: first to study the inheritance of black pigmentation and second that of red and orange. In this paper the results of the first phase are reported.

Our basic assumption was that black pigmentation in *O. niloticus* is controlled by one gene and two alleles, 'B' dominant and 'b' recessive. No theory concerning the genotype of + and - phenotypes was established initially.

Materials and Methods

A few ULp- fry were collected from commercial broodstock and reared to maturity in 1-m^3 tanks. Later these were divided into three groups with 8-10 females and 1-2 males each and stocked in 0.5-m^3 aquaria. Eggs were collected directly from mouth-brooding females, incubated in jars and fry were reared to maturity. Three such generations were produced in similar conditions. Four groups were established, each with 8-10 GW+ females. The first was crossed with two LUa- males; the second with one LUr- male and the third with one Luo- male. The fourth group included UW + females and one LUo- male. The following year, one batch of heterozygous W+ (GW+ x LUa-) females from the first group was crossed with one LUr- male and another with one LUo- male.

A year later F_2 progenies were collected separately from crosses between heterozygous males and females F_1 W+ (GW+ x LUa-) and F_1W+ (GW+ x LUr-). For all crosses, L:W ratio was established as soon as fry began to swim in incubators. A layout of all crosses is given in Fig. 1.

Results

Out of thousands of fry produced by ULp- stocks during three generations, no W+ recombinants were identified. After a nursing period of 5-6 months it was obvious that most individuals inherited Lp- characteristics, that only a few had unpigmented dorsal fin and tail and that out of these fewer still were also r, o, or r/o phenotypes. Crosses between GW+ females and ULa-, ULr-, and ULo-males as well as crosses between UW+ females and ULo- males only produced W+ phenotypes. 97.8% of L F_2 individuals born to W+ heterozygous parents were + phenotypes but 54% out of them only had a partial display of stripes which often only covered the dorsal area of the tail, 2.2% had pigmented tails with various intensities of grey and no stripes at all. None were totally unpigmented. Unfortunately all F_2 W individuals were discarded at an early stage before the ratio between +, - and other intermediate phenotypes was established. All other results are presented in Table 1.

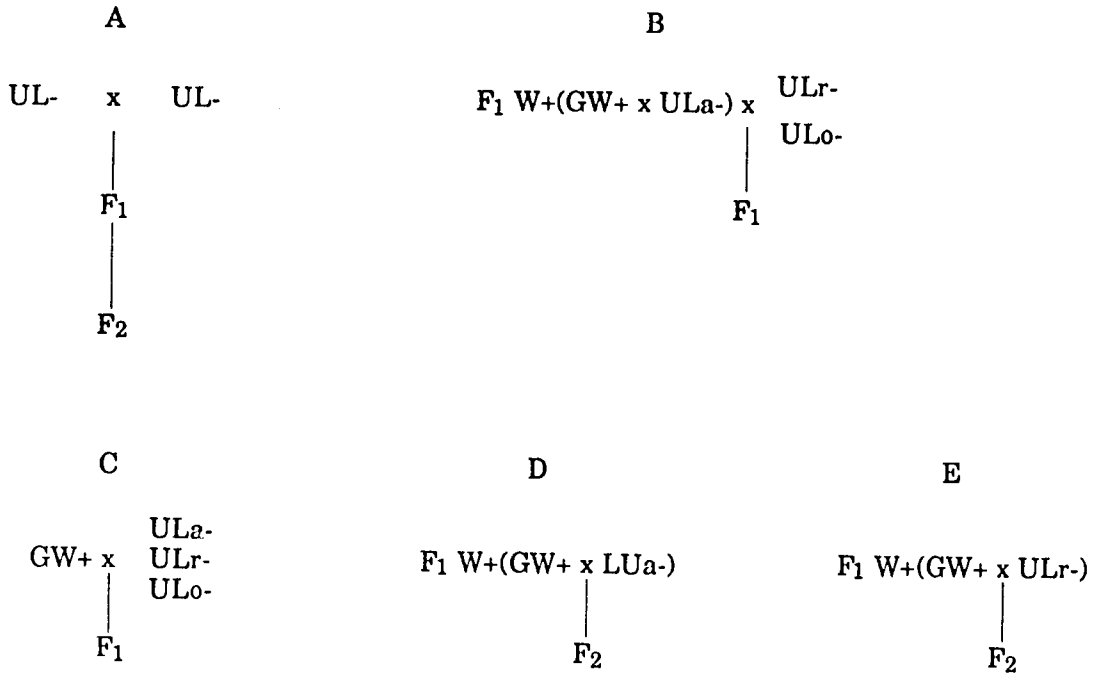

Fig. 1. Layout of crosses between various phenotypes of *Oreochromis niloticus;* G = origin Ghana, U = origin Uganda; W = wild type, L = light colored pigmentation of which, a = albino, p = pink, r = red, o = orange are subcategories. + and - indicate the presence or absence of stripes on dorsal fin and tail. The female parent given first in all crosses.

Discussion and Conclusions

Our findings confirm our initial assumptions pertaining to the genotypes of W and L *O. niloticus* phenotypes.

All L phenotypes are homozygous, recessive genotypes, 'bb' for black pigmentation. All GW+ and most UW+ fish are homozygous 'BB' since all GW+ x UL+ crosses produced only W+ phenotypes which were identified as heterozygous 'Bb' and L:W ratio was 1:3 in all F_2 generations (Fig. 2). Moreover crosses between F_1 heterozygous females and L- phenotypes produced a L:W ratio of 1:1 (Fig. 2). The difference in intensity of pigment on dorsal fin and tail, the apparition of partially striped individuals and the absence of unpigmented phenotypes, and the unusual repartition of phenotypes in F_2 (GW+ x ULr-) progenies imply that probably more than two genes control the + traits, some control the production of pigment and others its repartition into stripes. Further investigations are required to identify the genotype of + and - phenotypes. As the cross UW+ x UL- only produced F_1 W+ phenotypes it seems obvious that the genotype of our U stock is 'BB', identical to our G stock. Nevertheless, some individuals in the original stock were probably heterozygous 'Bb' and were therefore responsible for the appearance of L- phenotypes.

Other L tilapias have also been obtained by crosses between mutant reddish-orange *O. mossambicus* with other species (Behrends et al. 1982) and by inbreeding of L phenotypes produced by these parental stocks. Red mutants of

Table 1. Results of crosses between different colored *Oreochromis niloticus* phenotypes expressed as the phenotypic ratio of progeny: U = origin Uganda, G = origin Ghana, W = wild type, dark pigmentation, L = light colored pigmentation of which a = albino, p = pink, r = red, o = orange are subcategories; + or - indicates the presence or absence of stripes on dorsal fin and tail. The female parent is given first in all crosses. For details of the breeding method, see text.

F1 generation, W+ (GW+ x ULa-) x ULr-

	Progeny phenotypes (%)	
Harvest	W	L
1	566 (49.9)	569 (50.1)
2	659 (50.6)	643 (49.4)

Phenotypic ratio L:W = 1:1 ($p > 0.50$) from a total of 6 spawns.

F1 generation, W+ (GW+ x ULa-) x ULr-

1	846 (49.68)	857 (50.32)

Phenotypic ratio L:W = 1:1 ($p > 0.70$) from a total of 5 spawns

F2 generation from heterozygous females and males F1 W+ (GW+ x ULa-)

1	535 (74.8)	181 (25.2)
2	78 (73.6)	28 (26.4)
3	822 (74.4)	284 (25.6)
4	166 (76.2)	52 (23.8)
5	1170 (75.5)	380 (24.5)

Phenotypic ratio L:W = 1:3 ($p > 0.95$). Exact no. of spawns is unknown.

F2 generation from heterozygous females and males F1 W+ (GW+ x ULr-)

1	78 (76.5)	24 (23.5)
2	256 (77.1)	76 (22.9)
3	488 (73.1)	180 (26.9)
4	502 (74.4)	173 (25.6)

Phenotypic ratio L:W = 1:3 ($p > 0.50$). Exact no. of spawns is unknown.

O. mossambicus are little used in culture but their hybrids are becoming popular (Fitzgerald 1979). Such hybrids have some disadvantages which may be very difficult to overcome. Compared to other pure tilapia strains, their growth rate is poor (Behrends et al. 1982 and pers. comm.). Their genetic background is poorly known. When they are inbred, recombination of wild types can occur and many other individuals may have black patches scattered all over their bodies. There are no such problems with L *O. niloticus*. In growth tests, under various management conditions at the Ein Hamifratz fish farm, these fish have shown very good performance (Mires 1987).

As a recessive trait (bb), the lack of black pigmentation in *O. niloticus* may well offer an elegant and ideal solution to the danger of contamination of these broodstocks (Pullin 1983). With these fish, hatchery operators as well as fish farmers will easily be able to identify and control the purity of their stocks by systematically eliminating all wild types.

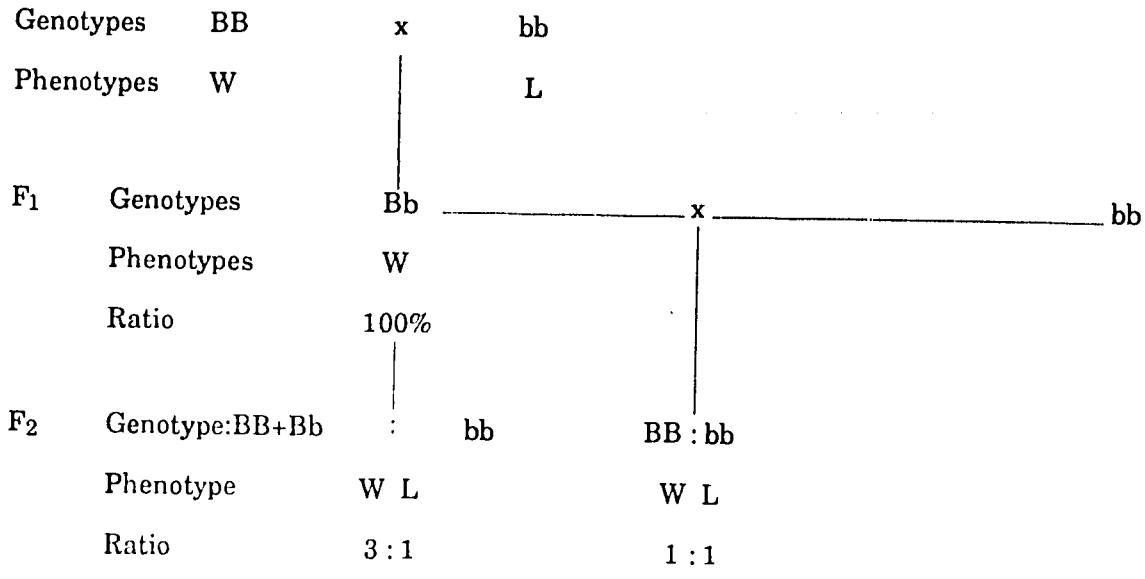

Fig. 2. The genotype and phenotype ratio of crosses between different phenotypes of *Oreochromis niloticus*. W = wild type, L = light colored. Bb= internal and external black pigmentation of which B = dominant and b = recessive. Females given first in all crosses.

Properly documented stocks are essential in modern animal husbandry. Compared to genetic improvements in cattle, poultry and pig husbandry, fish farming is still at a very early stage of development. The development of pure L *O. niloticus* can well be an important contribution towards better management of fish farms in tropical areas.

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Techniques for Producing All-Male and All-Triploid *Oreochromis mossambicus*

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Abstract

Elimination of uncontrolled reproduction is desirable to channel the available energy for the efficient and rapid growth of tilapias. This may be achieved by hormonal treatment and chromosomal manipulation. For *Oreochromis mossambicus*, experiments were designed to evaluate the interactions between feeding rate with a diet containing 17α-methyltestosterone (MT), dose and treatment duration. Complete masculinization of first-feeding fry was obtained by a feeding regime which ensured the uptake of MT of 1.5 µg/g fish/day lasting for 11 days, commencing on the 10th day after hatching. Heat shocking of 2.5-minute old fertilized eggs (42°C for 3 minutes) resulted in the formation of triploids. A technique for ensuring 100% triploidy is described.

Introduction

Control of reproduction in tilapia is possible through monosex culture, which may be achieved by various methods, including manual sexing, hybridization, hormonal sex reversal and chromosomal manipulation to produce all sterile triploids. This paper describes procedures for producing all-male *Oreochromis mossambicus* fry by hormonal sex reversal and for producing 100% triploid progeny by heat shock.

Pandian and Varadaraj (1987) pointed out that some authors have arbitrarily chosen hormone dose, time and duration of treatment. They have also indicated that within the treatment duration, hierarchy is established among the fry fed on hormone-supplemented diet. Consequently, a dominant individual eats and acquires more than the critical minimum dose and may suffer from side effects. A submissive individual eats and acquires less than the critical minimum and hence may become either intersex or remain

female. Therefore, it has become necessary to study the interaction between feeding rate and treatment duration to fix the minimum hormone dose that will ensure 100% reversal.

In several fish species, thermal shock is known to produce triploidy and tetraploidy, but it is not known whether these polyploids are produced due to the inhibition of second meiotic division or due to the retention of second polar body. However, to achieve triploidy or tetraploidy, one has to precisely fix the time and duration as well as the temperature level to effect cold or heat shock: these characteristics appear to vary from species to species.

Materials and Methods

Hormonal sex reversal

Two experiments were made to study the interactions amongst feeding rate, dose of 17α-methyltestosterone (MT) and treatment duration for 6-day old fry (post-hatching) of *O. mossambicus*. The first experiment was designed to study the interaction between dose and ration. The fry were divided into 18 groups of 50 each; 15 groups were divided further into three experimental series containing five groups each and the remaining three groups served as controls for each experimental series (Table 1). In each series, the fry were fed with MT dose of 5, 10, 20, 30 or 40 µg/g diet (pelleted diet composition: blue-green alga *(Spirulina)* 30%; fish meal 25%; rice bran 24%; wheat flour 20%; mineral mix 0.5%; and vitamin mix 0.5%: protein concentration 30%) for a period of 19 days. The three experimental series and their respective controls were fed at the rate of 10, 20 and 30% body weight/day by the same pelleted diet without hormone. In all the feeding regimes, experimental and control fry consumed all the food (we assumed that all the food was eaten to the same extent by all fry). The control and treated fry were examined 30 days after treatment for sex reversal by following the standard gonadal squash technique of Guerrero and Shelton (1974) to estimate the minimum dose required for 100% sex reversal.

To determine the minimum treatment duration required to ensure complete sex reversal, the second experiment was made

Table 1. Hormone uptake, eventual sex-ratio and growth of 6-day old *Oreochromis mossambicus* fry administered with 17α-methyltestosterone (MT) in feed for 19 days and reared for 45 days

Treatment dose MT (µg/g diet)	Feeding rate (% body wt/day)	Hormone consumed* (µg/g fish/day)	Sex ratio ♀	Sex ratio Intersex	Sex ratio ♂	Fish weight (mg/individual) ($\bar{X}$)	Fish weight (mg/individual) (S.D.)
Control	10	0.0	0.43	0.00	0.57	453	193
5	10	0.5	0.30	0.10	0.60	471	217
10	10	1.0	0.10	0.20	0.70	492	262
20	10	2.0	0.00	0.00	1.00	552	279
30	10	3.0	0.00	0.00	1.00	643	301
40	10	4.0	0.00	0.00	0.00	643	301
Control	20	0.0	0.42	0.00	0.58	576	181
5	20	1.0	0.10	0.10	0.80	623	197
10	20	2.0	0.00	0.00	1.00	696	253
20	20	4.0	0.00	0.00	1.00	791	297
30	20	6.0	0.00	0.00	1.00	811	261
40	20	8.0	0.00	0.00	1.00	822	247
Control	30	0.0	0.44	0.00	0.56	802	285
5	30	1.5	0.00	0.00	1.00	1,073	321
10	30	3.0	0.00	0.00	1.00	1,143	530
20	30	6.0	0.00	0.00	1.00	1,121	572
30	30	9.0	0.00	0.00	1.00	1,026	434
40	30	12.0	0.00	0.00	1.00	978	428

*Assuming all the food consumed

administering different doses of the hormone for different durations. Fry were divided into 6-day old, 9-day old and 12-day old. Each series was further divided into two groups, and each of these groups was treated with 5 or 10 µg MT/g diet until the 15th, 20th or 25th day of hatching (Table 2). Forty days after the cessation of treatment, the control and treated fingerlings were weighed and killed for gonadal examination as above.

Each experimental fertilized egg was allowed to waterharden for 2 hours after heat shock, then transferred to incubator and incubated at 27 ± 1°C until hatching. An antibiotic mixture of penicillin and streptomycin sulfate (50 IU/ml and 0.05 mg/ml, respectively) was added once every 2 days to inhibit bacterial infection. Dead eggs were counted and removed daily prior to the antibiotic treatment. To ascertain that the heat shock resulted in triploidy,

Table 2. Effect on sexual differentiation and weight of administration of different doses of 17α-methyltestosterone (MT) for different periods to *Oreochromis mossambicus* fry of different ages.

Age of fry at start and end of treatment (days after hatching)	MT dose (µg/g diet)	MT consumed* (µg/g fish/day)	Treatment duration days	Sex ratio			Fish weight (mg) ($\bar{X}$ ± S.D.)	
				♀	Intersex	♂		
0	0	0	0	0.42	0.00	0.58	586	136
7-15	5	1.5	9	0.28	0.07	0.65	605	115
7-20	5	1.5	14	0.00	0.90	1.00	700	091
7-25	5	1.5	19	0.00	0.00	1.00	816	117
10-15	5	1.5	6	0.30	0.09	0.61	625	123
10-20	5	1.5	11	0.00	0.00	1.00	849	179
10-25	5	1.5	16	0.00	0.00	1.00	1,022	209
13-15	5	1.5	3	0.38	0.04	0.58	598	209
13-20	5	1.5	8	0.31	0.04	0.65	675	097
13-25	5	1.5	13	0.25	0.06	0.69	779	073
7-15	10	3.0	9	0.26	0.07	0.67	674	068
7-20	10	3.0	14	0.00	0.00	1.00	790	074
7-25	10	3.0	19	0.00	0.00	1.00	952	083
10-15	10	3.0	6	0.30	0.66	0.64	713	082
10-20	10	3.0	11	0.00	0.00	1.00	1,068	201
10-25	10	3.0	16	0.00	0.00	1.00	991	129
13-15	10	3.0	3	0.34	0.04	0.61	615	051
13-20	10	3.0	8	0.26	0.08	0.66	769	063
13-25	10	3.0	13	0.20	0.05	0.72	812	086

Production of triploid fish by heat shock

Ripe eggs were stripped from three selected gravid spawners. Testes were removed from two mature males and ground with 12-ml Ringer's solution (composition in g/l: NaCl 7.25; KCl 0.38; $CaCl_2$ 0.16; $MgSO_4$ 0.23; $NaHCO_3$ 1.00; NaH_2PO_4 0.41; Glucose 1.00). Sperm activated and mixed with eggs immediately by stirring with a feather. Broodstock were reared in freshwater at 27 ± 2°C and artificial wet fertilization was done at 27 ± 1°C. The fertilized eggs were maintained at the same temperature. The temperature, time and duration of treatment and the consequent changes are summarized in Table 3.

all the fry from individual experimental and control batches were karyotyped, following the techniques described by Kligerman and Bloom (1977).

Results

Hormonal sex reversal

Table 1 indicates that irrespective of the changes in feeding rate (30% body weight or 10% body weight), the fry that took up 1.5 µg or more MT/g fish/day for a period of 19 days were all male. The minimum dose required for 100% masculinization shifted to lower levels, from 20 to 5 µg/g diet, when the feeding rate was increased from 10 to 30% body

weight/day. The sex ratio generally increased in favor of males with increasing dose. It appears that a dose of 1.5 µg MT/g fish/day is the critical minimum required to ensure 100% sex reversal. The uptake of lower doses resulted in the production of some females and/or intersexes.

Feeding MT to 6- or 9-day old *O. mossambicus* fry for a period of 11, 14, 16 or 19 days induced 100% masculinization (Table 2). Shortening the treatment period to less than 9 days or commencement of the hormone treatment 13 days after hatching failed to induce 100% sex reversal. It was concluded that treatment between the 10th and 20th day following hatching, and uptake of 1.5 µg/g fish/day are the critical minimum age and dose requirements, respectively, to ensure 100% sex reversal.

Production of triploid tilapia by heat shock

Table 3 shows the percentages of triploid, diploid and abnormal fry resulting from the heat shock experiments. Among the treated eggs, a few died before hatching and immediately after hatching; 5-10% of the embryos from heat shocked eggs displayed morphological abnormalities (e.g., short and curved tail; distorted yolk sac). Most of the abnormal embryos hatched successfully but died soon after. The percentage mortality of such abnormal fry appears to depend on the duration of the heat shock and the time after fertilization at which the heat shock is applied. It may be noted that only 2% abnormal fry were present in the control.

The eggs (2.5 minutes postfertilization) subjected to heat shock at 31 or 42°C for 2, 5 or 7 minutes induced triploidy (Table 3). Treatment lasting for longer duration (5 minutes) at 42°C resulted in 100% mortality. There was a sharp decline in survival of the group exposed to heat shock for 5 minutes or longer. The best heat shock treatment based on percentage triploidy and survival was 42°C, for a period of 3 minutes on eggs that were 2.5 minutes old (Table 3). Surviving triploid fry were as viable as diploids and no difficulties were experienced in raising them. Triploidy was confirmed by chromosome counts (Fig. 1). Induction of triploidy through heat shock appeared to be directly related to the duration of heat shock and inversely related to survival.

Discussion

Oral administration of different MT doses induced sex reversal in *O. mossambicus* as found previously by Clemens and Inslee (1968), Nakamura (1975), Guerrero (1979) and Macintosh et al. (1985). However, these authors used higher doses for a longer duration to ensure 100% sex reversal. Nakamura (1975) used 50 µg/g diet to ensure 100% masculinization. We have found that a minimum dose of 5 µg/g diet (equivalent to an uptake of 1.5 µg/g fish/day) is adequate. Clemens and Inslee (1968) claimed that gonadal differentiation took place between the 35th and 48th day following hatching. Conversely, Nakamura and Takahashi (1973) reported that for complete sex reversal, the fry should be subjected to hormone treatment between the 7th and 25th day following hatching. Our study has identified a shorter treatment duration of 11 days from the 10th day following hatching. This is the first report to determine a critical minimum dose and duration required for 100% masculinization in *O. mossambicus*.

The present findings confirm that heat shock induces triploidy in *O. mossambicus*. Among tilapias, triploidy has so far been reported only in *O. niloticus* (Chourrout and Itskovich 1984); the triploidy was induced in 4-minute old (postfertilization) eggs exposed to 40-41.5°C for 2.7 minutes. Our observation on *O. mossambicus* shows that there is good scope for inducing triploidy in other tilapia species also. Valenti (1975) claimed that it has been possible for him to obtain polyploid (tetraploid) *O. aureus* when freshly inseminated eggs were exposed to 4°C for 15 minutes, 11°C for 60 minutes or 38°C

Table 3. Effect of heat shock on survival and induction of triploidy in eggs stripped from *Oreochromis mossambicus*. Each value represents results from a batch of 100 eggs. Initial ambient temperature and incubation temperature = 27 ± 1 °C.

Heat shock		Time after fertilization (minutes)	Viability (%)	Triploid (%)	Diploid (%)	Abnormal fry (%)
Temperature (°C)	Duration (minutes)					
Control	0	0.00	93	0	100	2
35	1	1.0	92	0	100	0
	1	2.5	90	0	100	0
	1	3.5	95	0	100	1
	1	5.0	82	0	100	0
35	2	1.0	97	0	100	0
	2	2.5	92	0	100	0
	2	3.5	90	0	100	0
	2	5.0	93	0	100	2
35	3	1.0	89	0	100	0
	3	2.5	91	0	100	0
	3	3.5	90	0	100	0
	3	5.0	86	0	100	0
35	5	1.0	82	0	100	0
	5	2.5	80	0	100	3
	5	3.5	85	0	100	0
	5	5.0	80	0	100	2
35	7	1.0	80	0	100	3
	7	2.5	85	0	100	5
	7	3.5	79	0	100	1
	7	5.0	77	0	100	7
39	1	1.0	81	0	100	2
	1	2.5	80	0	100	0
	1	3.5	75	0	100	5
	1	5.0	73	0	100	3
39	2	1.0	82	0	100	1
	2	2.5	87	0	100	3
	2	3.5	79	0	100	5
	2	5.0	77	0	100	2
39	3	1.0	75	0	100	0
	3	2.5	79	0	100	3
	3	3.5	75	0	100	2
	3	5.0	77	0	100	5
39	5	1.0	77	0	100	3
	5	2.5	70	10	90	7
	5	3.5	72	0	100	12
	5	5.0	75	0	100	19
39	7	1.0	71	0	100	13
	7	2.5	73	4	96	10
	7	3.5	69	0	100	20
	7	5.0	65	0	100	15
42	1	1.0	68	0	100	5
	1	2.5	66	0	100	13
	1	3.5	76	0	100	19
	1	5.0	69	0	100	17
42	2	1.0	72	0	100	14
	2	2.5	69	27	73	9
	2	3.5	65	3	97	17
	2	5.0	66	0	100	11
42	3	1.0	68	0	100	8
	3	2.5	63	100	0	15
	3	3.5	70	5	95	7
	3	5.0	78	0	100	14
42	5	1.0	0	0	0	0
	5	2.5	0	0	0	0
	5	3.5	0	0	0	0
	5	5.5	0	0	0	0

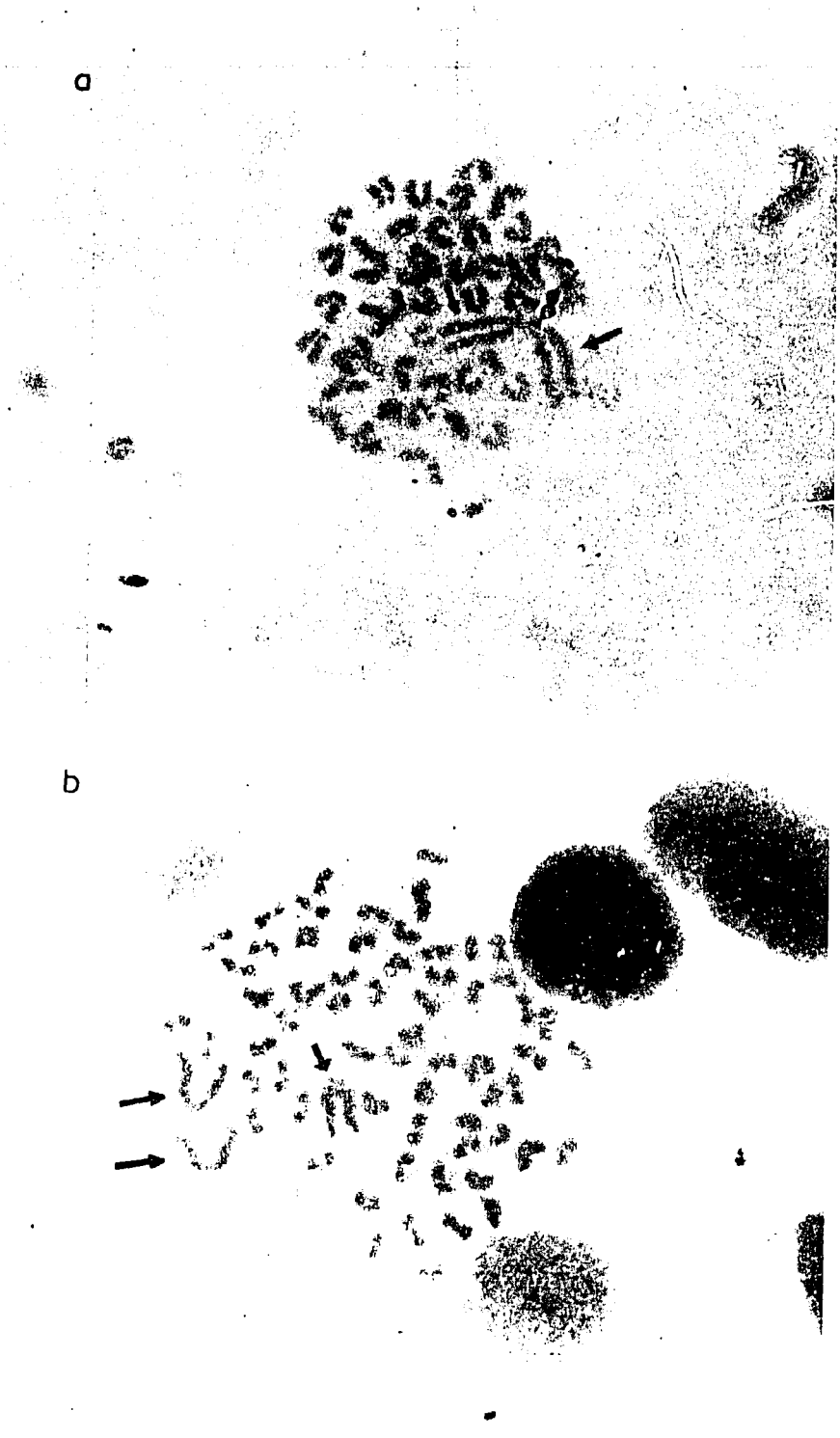

Fig. 1. Karyotypes of a: diploid, 2n = 44; and b: triploid, 3n = 66 gill epithelial cells of *Oreochromis mossambicus*. Arrows indicate marker chromosomes.

for 60 minutes. He adduced the increase in nuclear volume of erythrocytes as the sole evidence for polyploidy. Our report is perhaps the first to determine triploidy in a tilapia by karyotyping.

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A Preliminary Study on the Use of Canonical Discriminant Analysis of Morphometric and Meristic Characters to Identify Cultured Tilapias*

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Abstract

A preliminary study using canonical discriminant analysis of morphometric and meristic characters to attempt to distinguish different populations of cultured Philippine tilapias *(Oreochromis niloticus* and *O. mossambicus)* is described. Analysis of meristic characters separated species but not strains of *O. niloticus* or introgressed hybrids. When both morphometric and meristic characters were used in the analysis, the strains and hybrids were not well discriminated. These results suggest that the typical morphometric and meristic characters used by taxonomists, e.g., body depth, head length, standard length, interorbital width, orbit diameter, dorsal spine count, dorsal fin ray count, anal fin ray count and total gill raker count, offer little promise for differentiating tilapia strains and introgressed hybrids. The paper concludes with some suggestions for further work on morphometric/meristic studies of cultured tilapias as alternatives to electrophoresis.

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Introduction

Tilapia culture is now an important industry in the Philippines (Smith et al. 1985). The preferred species is *Oreochromis niloticus*. However, many culturists face problems of their cultured *O. niloticus* interbreeding with the less desirable species *O. mossambicus* which is present in many water bodies. The admixture of *O. mossambicus* genes is held to reduce growth performance and to promote early sexual maturation, both well-known disadvantageous traits of *O. mossambicus*.

Such introgressive hybridization of cultured tilapias in the Philippines has been demonstrated by Macaranas et al. (1986) and Taniguchi et al. (1985), using gel electrophoresis. This is a powerful tool for establishing the identity of cultured stocks, but expensive and laborious for widespread application by culturists who wish to monitor the quality and identity of their breeders.

To maintain the line purity of their stocks and to attempt to eliminate *O. mossambicus*-like fish, some culturists in the Philippines discard any dark-colored tilapias from their ponds and retain the light-colored ones. Most of them also note the number of caudal fin bars (the presence of which is diagnostic for typical *O. niloticus*) as an important character when selecting broodstock. They believe that the more caudal fin bars there are, the faster the fish will grow and the higher the probability that it is more *O. niloticus*-like. However, just how these pigmentation traits relate to growth performance has not been investigated.

Multivariate statistical analysis of morphometric and meristic characters has been utilized for studying fish stock diversity (e.g., Copeman 1977; Sharp et al. 1978; Winans 1984). Such characters have also been used in tilapia taxonomy but no one has previously attempted to use them in multivariate analysis to differentiate tilapia species and strains.

This paper describes a preliminary study on canonical discriminant analysis of some morphometric and meristic characters of different tilapias cultured in the Philippines.

Materials and Methods

Samples of seven cultured tilapia populations were examined. Table 1

Table 1. Tilapia populations studied, abbreviations used, sample size (n), collection site and collection date. All except R are from the Philippines. I originated in Israel. T was introduced to the Philippines from Taiwan, but probably also originated in Israel.

Tilapia group (*Oreochromis* spp.)	Code	n	Collection site	Collection date
O. mossambicus	M	40	Paombong, Bulacan	30 Jan 1985
O. niloticus	S	38	SPDA[1], Mindanao	31 Mar 1985
O. niloticus (Taiwan)	T	40	BFAR[2], Nueva Ecija	29 Mar 1985
O. niloticus (Ghana strain from Israel)	I	40	BFAR, Nueva Ecija	29 Mar 1985
O. niloticus (Philippines)	P	40	BFAR, Nueva Ecija	29 Mar 1985
Red tilapia	R	13	Thailand	Dec 1984
Red tilapia	L	40	Sampaloc Lake, Laguna	26 Mar 1985

[1] Southern Philippines Development Authority.
[2] Bureau of Fisheries and Aquatic Resources.

describes these populations. *O. niloticus*, Ghana strain (I), was the reference for this species. This strain was introduced into the Philippines in 1979 from Israel. The *O. mossambicus* reference sample (M) was collected from a brackishwater earthen pond in Paombong, Bulacan, Philippines. The other *O. niloticus*-like samples (P, S and T) are previously identified introgressed hybrids (Macaranas et al. 1986). The red tilapias R and L were assumed to be hybrids of *O. mossambicus*, *O. niloticus* and *O. aureus* based on electrophoretic analysis (unpublished data).

Five morphometric and five meristic characters were studied following Trewavas (1983), except for gill raker counts (GRC) which were not according to Trewavas. Total counts for gill rakers were made under a dissecting microscope. Morphometric measurements (body depth, BD; head length, HL; orbit diameter, OD; interorbital width, IOW) were measured to the nearest 0.01 cm using vernier calipers. Standard length (SL) was measured using a ruler.

The meristic characters studied were: dorsal spine count (DSP); dorsal fin ray count (DR); anal fin ray count (AR); caudal fin bar count (CFB) (for *O. niloticus* and *O. niloticus*-like fish); and GRC.

All morphometric measurements were log transformed. Body depth and head length measurements were divided by the standard length for each fish to minimize the effect of size, i.e., BD/SL, HL/SL. Ratios were used despite their tendency to cause nonrandomly distributed variances in the data. This was done because one of the objectives was to find a discriminating index that could be used by culturists in the field. Other methods of removing size effects from measurement data are generally too complicated for field use. Canonical discriminant analysis was performed at the University of Houston-Clear Lake using the SAS statistical package (SAS Institute Inc., Cary, North Carolina 27511).

Results and Discussion

Canonical discriminant analysis was first done on the two reference samples, using only meristic variables to generate standardized canonical coefficients. The meristic characters DSP, DR, AR and GRC were used. The standardized canonical coefficients generated for these were as follows: DSP, 0.578; DR, 0.608; AR, -0.510, GRC, -2.436. These coefficients were subsequently used in an index to classify the rest of the *O. niloticus* and *O. niloticus*-like populations. Index = 0.578 (DSP) + 0.608 (DR) - 0.510 (AR) - 2.436 (GRC). The differences exhibited between these two species, as shown by the value of coefficients, can be ranked as follows: GRC, DR, DSP and AR.

Fig. 1 shows how the scores of *O. niloticus* and *O. niloticus*-like samples were distributed in a frequency bar chart

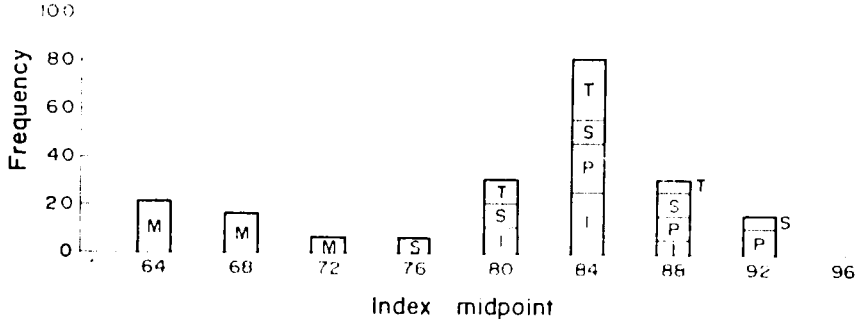

Fig. 1. Frequency bar chart showing classification of *Oreochromis mossambicus* (M), *O. niloticus* (I) and *O. niloticus*-like populations (P, S, T) when standardized canonical coefficient loadings for the meristic characters, DSP, DR, AR and GRC were used in an index of classification. For a full explanation of the sources of samples and the characters used, see Table 1 and text.

based on the discriminant index using only meristic characters. All were evenly distributed around the I mode, whereas the M sample were separated to the left. This clearly indicates that meristic characters are useful to separate *O. niloticus* and *O. mossambicus*.

Canonical discriminant analysis was then performed with all the *O. niloticus*, and *O. niloticus*-like samples using meristic variables (except CFB) to separate these samples. The first axis explains 51.4% of the total variation (Table 3a). Separation between the different *O. niloticus* and *O. niloticus*-like populations was due to variation in AR, which has a high positive loading, and to GRC and DR which have moderate negative and positive loadings, respectively. The second axis explains 29.3% of the total variation. The populations were mainly separated by variation in GRC, indicated by the high positive loading of this character on the second axis. When the first canonical variate was plotted against the second canonical variate, no distinct separation was seen. The high loadings present on these meristic characters, which indicate differences between these samples, are not significant enough to separate *O. niloticus* and *O. niloticus*-like populations.

CFB was then included in an analysis to attempt to separate all the *O. niloticus* and *O. niloticus*-like samples. High positive loading w s generated on CFB and moderate positive loading on AR (Table 3b), indicating differences between the samples. However, when the first canonical variate was plotted against the second from this analysis (Fig. 2), it was clear that the inclusion of CFD did not enhance the separation of the *O. niloticus* and *O. niloticus*-like samples. The red tilapias were well separated from the rest of the *O. niloticus* samples apparently due to absence of CFB.

This implies that using CFB for choosing good broodstock, as practiced by some Philippine culturists, may be groundless. It does not correlate with other characters that separate *O. mossambicus* from *O. niloticus*-like populations and does not improve their separation. The *presence* of caudal fin bars is, however, an important distinguishing character between these species. Thus, meristic variables alone cannot effectively

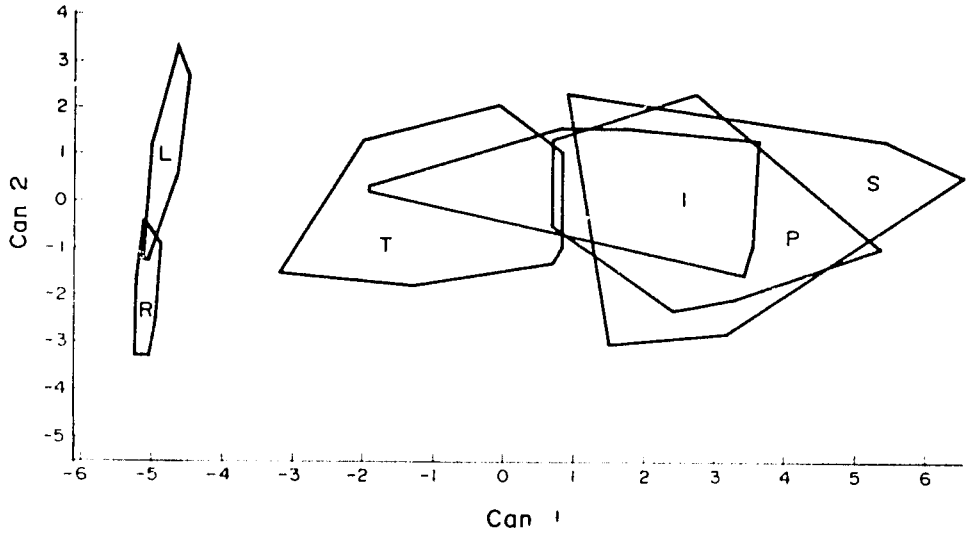

Fig. 2. Plot of first two canonical variates from analysis meristic (DSP, DR, AR, GRC and CFB) characters of tilapias: *Oreochromis niloticus* (I); *O. n* .*icus*-like populations (P, S, T) and red tilapias (L, R). For a full explanation of the sources of samples, characters and abbreviations used, see Tables 1, 2 and text.

distinguish between the *O. niloticus* and *O. niloticus*-like strains and hybrids studied here.

From analyses of the morphometric and meristic data, the first canonical variate generated roughly equal high positive loadings for characters BD/SL, HL/SL, IOW and OD (Table 3c). This implies that the firt canonical variate, which explains 68.6% of the total variation, is a characteristic measurement of size (Pimentel 1979). The second canonical variate which explains 30% of the total variation generated moderate positive loadings for IOW and OD and a high negative loading on BD/SL. Discrimination on the second variate was based on the differences of these characters between the samples.

The plot of the firt two canonical variates (Table 3c) is presented in Fig. 3. Ghana (I) and Taiwan (T) *O. niloticus* were grouped together, (the Taiwanese fish almost certainly came from Israel) whereas the Philippine (P) and SPDA (S) *O. niloticus*-like samples were grouped together. The red tilapias formed two separate clusters: R (red tilapia from Thailand) and L (red tilapia from Sampaloc Lake, Philippines). The overlapping groupings show that discrimination was based mainly on size differences and/or similarities. Where ratios were utilized (BD/SL and HL/SL) in an attempt to standardize the data and to minimize the effect of fish size, their coefficients of variation were still not significantly lowered (Table 2).

This preliminary study suggests that canonical discriminant analysis of some morphometric and meristic characters commonly used in fish taxonomy offers little promise in differentiating tilapia strains and introgressed hybrids. However, size and shape studies offer an interesting alternative or complement to tilapia biochemical genetic stock differentiation. They are cheaper and easier than electrophoresis. The morphometric characters used in this study were too few to delineate fish shape. Humphries et al. (1981) have devised an

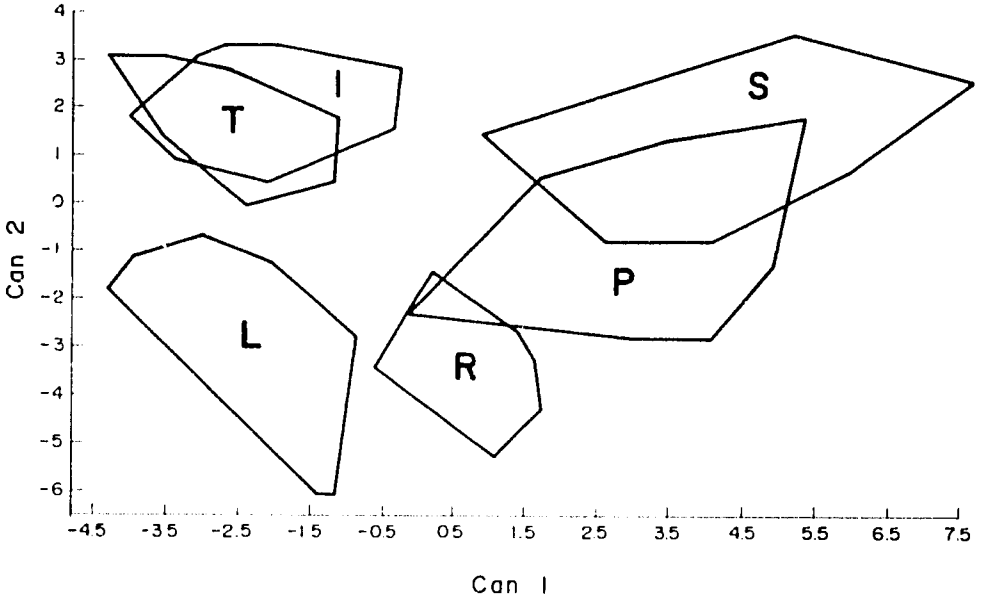

Fig. 3. Plot of the first two canonical variates from analysis of morphometric (BD/SL, HL/SL, IOW and OD) and meristic (DSP, DR, AR and GRC) characters of tilapias: *Oreochromis niloticus* (I); *O. niloticus*-like populations (P, S, T) and red tilapias (L, R). For a full explanation of the sources of samples and the characters used, see Tables 1, 2 and text.

Table 2. Mean and standard deviation (in parenthesis below mean) of all characters (DSP, DR, AR, GRC, CFB, BD/SL, HL/SL, SL, IOW, DD) used in canonical discriminant analyses for *Oreochromis mossambicus*, *O. niloticus* and *O. niloticus*-like populations and red tilapias. See Tables 1 and 2 for sources of samples, abbreviations used and sample size (n).

Character	*O. mossambicus*		*O. niloticus*			Red tilapia	
	M	S	P	T	I	R	L
Dorsal spine count (DSP)	15.92 (0.474)	16.42 (0.642)	16.75 (0.669)	16.68 (0.572)	16.90 (0.632)	17.00 (0.707)	16.80 (0.405)
Dorsal ray count (DR)	11.92 (0.526)	12.55 (0.828)	12.90 (0.709)	12.85 (0.662)	12.78 (0.479)	12.15 (0.899)	12.88 (0.563)
Anal fin ray count (AR)	11.30 (0.608)	10.42 (0.642)	10.55 (0.504)	10.00 (0.599)	10.48 (0.506)	9.31 (0.855)	10.25 (0.494)
Gill raker count (GRC)	22.85 (1.222)	29.47 (2.102)	30.52 (1.485)	29.10 (1.392)	29.08 (1.163)	31.46 (1.330)	29.18 (1.907)
Caudal fin bar (CFB)	0 0	8.12 (1.343)	7.75 (1.171)	5.13 (1.239)	7.40 (1.236)	0 0	0 0
Body depth/Standard length (BD/SL)	56.04 (3.765)	63.68 (2.120)	63.67 (2.170)	51.45 (2.129)	51.62 (1.879)	64.09 (2.460)	58.09 (3.109)
Head length/Standard length (HL/SL)	55.34 (3.499)	62.36 (4.157)	61.58 (2.194)	48.71 (1.501)	50.17 (1.394)	55.61 (1.479)	49.83 (2.428)
Standard length (SL)	13.68 (2.023)	14.84 (1.535)	12.59 (0.993)	12.72 (0.786)	13.06 (1.057)	12.22 (0.706)	7.36 (0.699)
Interorbital width (IOW)	1.54 (0.155)	2.11 (0.223)	1.76 (0.192)	1.29 (0.102)	1.30 (0.156)	1.56 (0.100)	1.04 (0.096)
Orbit diameter (OD)	1.00 (0.099)	1.36 (0.146)	1.23 (0.101)	0.85 (0.052)	0.92 (0.069)	0.98 (0.067)	0.72 (0.065)

Table 3. Characters (DSP, DR, AR, GRC, CFB, BD/SL, HL/SL, IOW and OD) used in canonical discriminant analyses for *Oreochromis niloticus* and *O. niloticus*-like populations and the loadings for each character on canonical axes 1 and 2. The loadings are total canonical structure: a. and b. analyses of meristic characters c. analyses of morphometric and meristic characters.

Character	a.		b.		c.	
	CAN1	CAN2	CAN1	CAN2	CAN1	CAN2
Dorsal spine count (DSP)	−0.207	−0.194	−0.115	−0.053	−0.168	−0.108
Dorsal fin ray count (DR)	0.333	−0.274	0.054	0.447	−0.113	0.026
Anal fin ray count (AR)	0.897	0.282	0.337	0.620	0.165	0.168
Gill raker count (GRC)	−0.418	0.774	0.013	−0.747	0.248	−0.224
Caudal fin bar (CFB)			0.999	−0.028		
Body depth/Standard length (BD/SL)					0.838	−0.520
Head length/Standard length (HL/SL)					0.971	−0.056
Interorbital width (IOW)					0.931	0.261
Orbit diameter (OD)					0.949	0.267
Percentage of variation explained (%)	51.4	29.3	94.7	2.8	63.6	30.0

approach to the analysis of fish shape called the truss network (see also Brzeski and Doyle, this vol.) which gives an even areal coverage of the entire fish form. We hope to use this or a modified version for further studies on multivariate discrimination of shape differences among tilapia strains.

Acknowledgements

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Genetic Improvement of Tilapias: Problems and Prospects*

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Abstract

Tilapias are cultured throughout the tropics and subtropics but little attention has been given to genetic improvement of cultured breeds. The largest tilapia culture industries are in Asia whereas nearly all tilapia genetic resources are in Africa. This paper discusses approaches to tilapia genetic improvement: documentation of genetic resources; evaluation of the culture performance and the use of promising material in breeding programs. Conservation of genetic resources, research methods and prospects for genetic improvement are discussed. The major emphasis is on the most popular cultured species, *Oreochromis niloticus*.

Introduction

Tilapias are cultured throughout the tropics and subtropics and the scope for growth of tilapia culture is vast (Pullin 1985). The most popular species is *Oreochromis niloticus* because of its good growth in freshwater. There is lesser but significant use of *O. aureus*, because of its cold tolerance and suitability for production of monosex male hybrid fry in intensive systems. Several other species are cultured where *O. niloticus* is not available or where they are preferred for various reasons, principally traditional and environmental. Red, orange and other colored tilapias, mostly hybrids, are also produced for a limited market. This paper

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considers the prospects and problems for genetic improvement of cultured tilapias and concentrates on *O. niloticus*.

The Feasibility of Genetic Improvement of Tilapias

Research for the genetic improvement of cultured fish has a short history compared to that for crops and domestic animals. There is now a broad consensus that applied genetics, particularly quantitative genetics, can have tremendous impact on aquaculture (e.g., Gjedrem 1985). However, most tilapia genetics research has been on hybridization and monosex male fry production (Wohlfarth and Hulata 1983). The few studies made on selective breeding and heritability of commercial traits are summarized in Table 1: note the possibilities of bottleneck and/or founder effects for some of the *O. niloticus* populations.

There are other problems with research methodology. For example, in comparative trials, different genotypes should be tagged and co-stocked in the same pond or cage and corrections made for differences in initial length or weight (Wohlfarth and Hulata 1983). Until such

Table 1. Summary of quantitative genetics research on growth performance traits in cultured tilapia (*Oreochromis* spp.); data for length and weight traits at various ages are pooled.

Location	Fish	Type of study, Results	Remarks
Auburn, USA	*O.n. niloticus*[1]	Heritability estimation by half-sib analysis: h^2 not significantly different from zero	Low variability (Tave and Smitherman 1980)
Georgia, USA	*O. aureus*	Bidirectional mass selection: positive response, high lines 7-27% > low lines; $h^2 = 0.21 (\pm 0.07)$	Good response (Bondari et al. 1983)
Auburn, USA	*O.n. niloticus*[1]	$h^2 = -0.16$	(Teichert-Coddington and Smitherman, cited by Hulata et al. 1986)
Philippines	*O. niloticus*[2]	Bidirectional family selection: very slight response	Possibly low variability (Abella et al. 1986)
Israel	*O.n. niloticus* (Ghana strain)	Mass selection: no response	Probably low variability (Hulata et al. 1986)
Thailand	Red tilapia[3]	Mass selection: positive response selected line 10-30% > control; h^2 = 0.17 – 0.19	Good response (Jarimopas 1986)

[1] 50-100 fish (non-native; see text) transferred from Côte d'Ivoire to Brazil, 1971; 100-200 juveniles from 5-10 parents transferred to Auburn from Brazil, 1971; thereafter 150-200 randomly mating pairs maintained.

[2] Experimental founder stock prepared by crossing introduced Israel, Singapore and Taiwanese 'strains'; all probably came via Israel, predominantly Ghana strain.

[3] An *O. niloticus*-*O. mossambicus* hybrid (see Pante et al., in press).

methods are well established, the evaluation of different tilapias and their use in selective breeding schemes will make little progress. Meanwhile, there is no reason to suppose that the potential for culture performance improvement through selective breeding forecast by Gjedrem (1985) for a "wide range of aquatic animals" should not include the tilapias. The short generation time for *O. niloticus* (about four months) and its capacity to breed year-round in the tropics mean that any genetic gains will be rapidly obtainable. However, there are major problems--limited information and availability of tilapia genetic resources.

The largest tilapia culture industries are in Asia; for example, the Philippines (Smith and Pullin 1984) whereas all important natural tilapia genetic resources are in Africa. Genetic improvement research should serve existing and emergent culture industries. Tilapia culture in Africa, with some exceptions, is probably not yet sufficiently developed to interface with such research. Tilapia culture in Asia, at least its more organized sectors, is sufficiently developed for this, but the problems of bringing together the required resources are immense. Good fish genetics research facilities are scarce throughout the tropics. Moreover, genetic resources are a global asset. If African tilapia genetic resources are to be used to improve global tilapia production, then African aquaculture research and development must receive commensurate support so that these can prosper. This will require much greater interregional and international cooperation.

Tilapia Genetic Resources in Africa

The natural distribution of tilapias has been reviewed by Philippart and Ruwet (1982) and Trewavas (1983). For *O. niloticus*, the natural distribution of subspecies, mostly clustered in central and east Africa, is summarized in Fig. 1. Numerous transfers, many undocumented, have been made between and within African nations and, more importantly, between river basins (Philippart and Ruwet 1982). Some recent transfers of *O. niloticus* and *O. aureus* have been made from western universities and Israel to Africa.

O. niloticus now extends beyond its native range in Africa. For example, *O. niloticus* is non-native to the south-flowing rivers west of the Volta in Ghana and Côte d'Ivoire. Its Soudanian form is native to only a few extreme northern, north-flowing streams in the Côte d'Ivoire (Daget and Iltis 1965). However, *O. niloticus* 'Bouaké' strain (a mixture of earlier introductions) is now widely cultured in Ivorian freshwaters. From farms close to the Bia River, it may have colonized the Bia and Tano Rivers which are shared with Ghana. If so, further tranfers to mix these fish with the Volta strain of *O. niloticus* could occur.

Obviously a balanced view is necessary with respect to tilapia transfers. They will continue to be thought acceptable where better fish can significantly improve established aquaculture to benefit needy people. Where risks to important genetic resources outweigh such benefits, transfers are best prevented. Conservation is vital for important native tilapia populations: ideally for all undisturbed riverine and lacustrine populations throughout Africa. Payne and Collinson (1983), describing such populations in Lake Manzala, state "The existing widespread transport of stocks whose original provenance and genetic background are uncertain can lead to breakdown of local species differences and will certainly make the job of the selective fish breeder so much more difficult when the critical hurdle of true domestication is approached."

Tilapia Genetic Resources in Asia

Published information on *O. niloticus* intoductions to and transfers within Asia

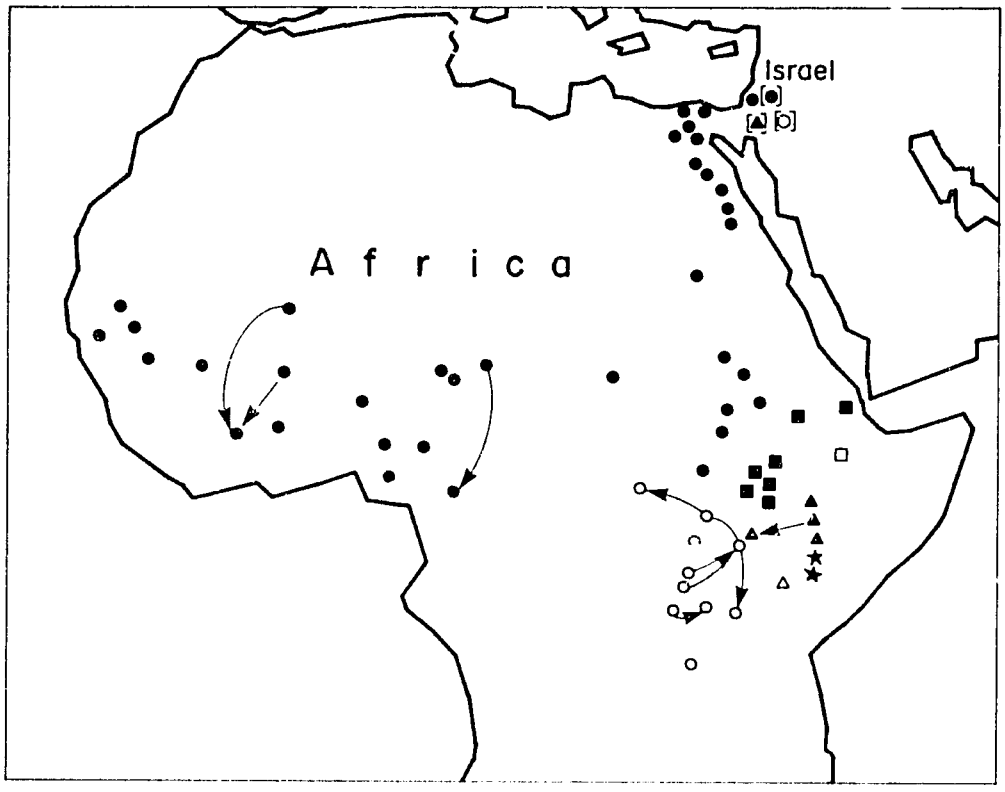

Fig. 1. Distribution of *Oreochromis niloticus* subspecies in Africa (modified after Trewavas 1983) and their transfers within Africa and to Israel for aquaculture and research purposes. Key: ● = *O.n. niloticus*; ○ = *O.n. eduardianus*; ■ = *O.n. cancellatus*; □ = *O.n. filoa*; ▲ = *O.n. vulcani*; △ = *O.n. baringoensis*; ★ = *O.n. sugutae*. Arrows indicate transfers within Africa. Israel has received introductions bracketed [ ]: [●] *O.n. niloticus*, Ghana; [○] *O.n. eduardianus*, Uganda and [▲] *O.n. vulcani*, Kenya.

is summarized in Table 2 and Fig. 2. Many other unrecorded transfers have been made within Asia. However, to our knowledge, no direct introductions have been made other than those from: 1) the Nile River, Sudan, to Hubei Province, People's Republic of China, in 1978--two shipments of fish (27 and 34 fish, with 90% survival during transportation); despite the need to overwinter broodstock here, the fish bred in 1979 and now a tilapia hatchery industry produces 100 million fry/year in this province (He Yukang, pers. comm.); 2) from Cairo, Egypt to Japan: about 200 individuals were shipped and about 120 survived; the exact origin (farm or wild stock) is not known (T. Maruyama, pers. comm.). The Japanese stock seems to have maintained a high genetic variability: observed heterozygosity (H^o) = 0.091 (Basiao and Taniguchi 1984). Fifty fish were sent from Japan to Thailand in 1965 (Chotiyarnwong 1971). However, the number that survived to breed (the founder stock) in Thailand is unclear. This stock is called the Chitralada strain. The fish in the palace pond have been kept well-isolated from other tilapias. A sample of 20 Chitralada strain fish from the Asian Institute of Technology (AIT) examined in 1984 at 21 protein loci had H^o = 0.014 (ICLARM and the University of the Philippines, unpublished data), which indicates that a bottleneck has occurred at some stage. However, the Chitralada

Table 2. Introductions of *Oreochromis niloticus* to and subsequent transfers within Asia: summarized from Welcomme (1981) and Guerrero (1985). For additional information see Fig. 2.

Date	To	From	Date	To	From
1962	Japan	Egypt	1974	Bangladesh	Thailand
1965	Thailand	Japan	1978	China	Sudan
1969	Indonesia	Taiwan	Late 1970s	Sri Lanka	Israel
1972	Philippines	Israel[1]	1979	Philippines	Israel[2]
1972	Philippines	Israel	1979	Philippines	Singapore[3]
1972	Philippines	Thailand	1984	Philippines	Taiwan
1972	Hong Kong	Taiwan			

[1] Uganda strain — current status unclear.
[2] Ghana strain.
[3] Origin Israel, Ghana strain.

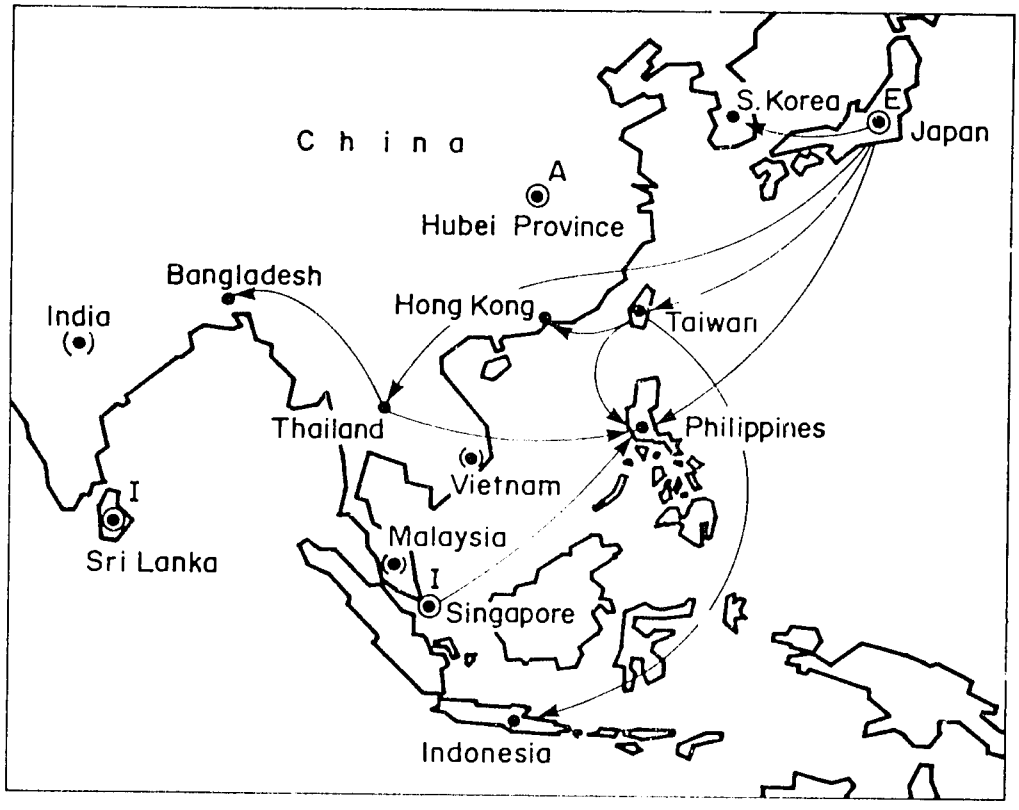

Fig. 2. Introductions of *Oreochromis niloticus niloticus* to Asia and some subsequent transfers between Asian countries based on Welcomme (1981), Guerrero (1985) and authors' unpublished observations. Entries bracketed (●) indicate presence of stocks transferred from undocumented sources within Asia. ◉ = primary introductions to Asia from origins A (Sudan); E (Egypt); I (Israel, Ghana strain); ● = transfers within Asia. Many recent transfers within Asia are omitted for clarity and/or insufficient information (see also Table 1).

strain has performed very well in Thai aquaculture to date.

The other Asian *O. niloticus* populations have all come via Israel. Few details are published of the founder stocks but their impact has been tremendous; for example 50,000 t/year production in the Philippines (Smith and Pullin 1984). The relevant original introductions of *O. niloticus* from Africa to Israel were: 1) *O.n. eduardianus* from Kajansi Station, Uganda (origin Lake George), 120 fry in 1969 and a further sample in 1970 (Pruginin et al. 1975); 2) nine females and two males from the Volta, Ghana, in 1974 (Hulata et al. 1986).

The general conclusion is clear. The genetic diversity of cultured *O. niloticus* in Asia is probably low and a poor base from which to attempt selective breeding. Moreover, *O. mossambicus* is present in many Asian waters. Introgressive hybridization between such fish and *O. niloticus* affects culturists and researchers (Macaranas et al. 1986). However, based on our unpublished observations and on electrophoretic analysis of a sample of 20 fish from Pathum Thani Province (Macaranas et al. 1986), *O. niloticus* in central Thailand seem relatively unaffected. To sustain and hopefully improve Asian tilapia culture there is a strong case for new introductions from Africa. But which of the African populations are of most interest?

Moreau et al. (1986) recommend ϕ' (= $\log_{10} K + 2 \log_{10} L_\infty$; where K and L_∞ are parameters of the von Bertalanffy growth equation) for tilapia growth comparisons. For *O.n. niloticus* from many different waters (native and introduced populations) ϕ' ranged from 2.36 to 3.11. However, values from Lake Kainji (ϕ' = 3.11) and Lake Nasser (ϕ' = 3.07) fish were markedly higher than the remainder (ϕ' = 2.36 to 2.77). *O.n. eduardianus*, (Lake Albert) had a high value of ϕ' = 2.88 (Moreau et al. 1986). For the Lake George fish, Lowe-McConnell (1958), cited by Trewavas (1983), estimated that a 23-cm total length (TL) individual would grow 9 to 10 cm in a year and that the average TL at reaching sexual maturity was 28 cm, reached in the second year.

O.n. vulcani has been cultured at Dor, Israel in semi-intensive polyculture (Yashouv and Halevy 1972): its daily growth increments were 2.9 g (spring) and 3.4 g (summer). In Lake Turkana, Worthington and Ricardo (1936) and Lowe-McConnell (1958), cited by Trewavas (1983), estimated its maximum TL as 64 cm. However, the weight for length (condition) was the same as for Lake George fish. Stunted fish were recorded from Crater Lake C., Ferguson Spit and Loiengalani. *O.n. baringoensis* has a low maximum TL of 36 cm and matures at 18 cm (Ssentongo and Mann 1971), cited by Trewavas (1983).

There is little additional information and much of that cited here may be of limited use as an indicator of culture potential because of the tremendous plasticity of tilapias with respect to growth and reproduction in different environments (Lowe-McConnell 1982). However, the possession of a high ϕ' value is probably a good indicator of high growth potential in a suitable culture environment (see Pauly et al., this vol.). It is clear that much more work is needed to investigate the variability of different *O. niloticus* stocks for commercial traits. For example, those at the extremes of the geographical range (such as Egypt) and those in adverse environments (such as higher elevations) may be of particular interest for subtropical culture.

Future Research: Documentation and Conservation of Tilapia Genetic Resources; Comparative Evaluation and Breeding Programs

It is clear that tilapia genetic resources are poorly documented. A major effort is needed to survey these and to enact conservation measures for important wild populations and their habitats. Similar recommendations for

other fish have been widely published (FAO/UNEP 1981; Ryman 1981; Meffe 1986). Their execution faces enormous financial, logistical and political problems. However, something must be done. It should be possible to assess the status of at least some of the more important populations and to conserve some material in the wild and in culture collections.

This raises the possibility of gene banks. Unlike crops for which germplasm is easily stored--for example the International Rice Research Institute germplasm banks (duplicated between the Philippines and the USA) comprised 61,000 Asian cultivars, 2,575 African rices, 1,100 wild rices and 680 testers in 1982 (Chang 1983)--and farm livestock for which cryogenic storage of semen and embryos is widely practiced (FAO/UNEP 1984; Smith 1984), the technology available for fish gene banks is restricted to the maintenance of live fish collections and cryopreservation of spermatozoa. Live fish collections are expensive to maintain and require very careful management. Replication at different locations is essential. Sperm banks, recommended by Harvey (1987) are potentially a useful means of conserving and distributing material, but monosex haploid gene banks have obvious limitations. They also require rigorous quality control standards and database management. Future documentation and conservation work on tilapia genetic resources may, therefore, involve three approaches: conservation of natural populations, live fish collections and sperm banks.

Further research on the estimation of genetic parameters, comparative evaluation of different tilapias for culture performance and breeding schemes to produce genetically improved breeds should be undertaken in close cooperation with farmers. Just as for crops and livestock, there are elements here of on-station and on-farm research which are highly interactive. The approaches used successfully in salmonid culture, particularly in Norway (Gjedrem 1985), could be repeated for tilapias, provided that the required support and climate of international cooperation are forthcoming.

Given the growing importance of Asian tilapia culture and renewed interest in African aquaculture we are convinced that a major program to document, conserve, evaluate and utilize tilapia genetic resources is urgently needed and we are optimistic that it will attract the necessary sustained support.

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Electrophoretic Studies on Induced Gynogenetic Diploids and Triploids in Tilapia (*Oreochromis niloticus* and *O. aureus*)

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Abstract

Preliminary electrophoretic screening of laboratory maintained tilapia stocks revealed enzyme polymorphism at three co-dominantly inherited loci--adenosine deaminase *(Ada)*, aminopeptidase *(Ap)* and malic enzyme (*Me*-2). *O. niloticus* and *O. aureus* broodstock used in artificial gynogenesis and polyploidy experiments were genetically tagged with these biochemical markers. Results of manipulations to induce diploid gynogenetic and triploid broods from heterozygous females were determined by genetic analysis.

Segregation ratios in the control broods confirmed Mendelian inheritance at the *Me*-2 and *Ada* loci. Genetic analysis of enzyme polymorphism in gynogenetic broods, produced from ova fertilized with genetically inert sperm and heat-shocked 5 minutes after fertilization indicated diploidy restoration by second polar body retention. Diploidisation of gynogenomes by suppression of first cleavage of mitosis in the zygote, attempted by heat shocking eggs 20-45 minutes after fertilization with UV-treated sperm, proved effective in one brood (heat shock at 45 min. after fertilization) in which a high incidence (~100%) of individuals homozygous for *Me*-2, was observed. Finally, electrophoretic analysis of triploids revealed banding patterns different from those observed in normal and gynogenetic diploids. Such banding phenotypes, peculiar only to triploids, denoted success in triploidy induction which was achieved here with the fusion of the paternal pronucleus and the maternal genome made double by suppression of meiosis II.

Introduction

Numerous attempts at inducing gynogenesis and polyploidy in fish have been made and only a few have been unsuccessful. Since the mechanisms behind spontaneous diploid gynogenesis and natural triploidy have been elucidated, and methods for ploidy manipulation have been discovered, the number of bisexual fish species found capable of producing viable diploid gynogenetic and polyploid broods through artificial means, has risen (Stanley and Sneed 1974, listed 17 species) and is still expected to increase.

Gynogenesis or all-maternal inheritance, which requires (a) the fertilization of mature ova with genetically inert sperm (or with foreign sperm as in the case of "hybrid" gynogenesis--Thorgaard 1983) and (b) the diploidisation of the female chromosome complement in the resulting embryo (Cherfas 1981), is induced in a variety of ways. Inactivation of the sperm DNA is possible by exposing sperm material to radiation (e.g., x-rays, δ -rays, ultraviolet light) or to chemical mutagens (e.g., toluidine blue, dimethylsulfate, or tryplavine) whilst diploidisation of maternally-derived genomes via suppression of cell divisions (blockage of meiosis II for meiotic gynogenetics) or of mitosis I for mitotic gynogenetics) is achieved by subjecting fertilized eggs to thermal shocks (heat or cold shock), pressure shocks (hydrostatic pressure) or chemical treatments (cytochalasin B, colchicine, polyethylene glycol, etc.) (Cherfas 1981; Thorgaard 1983). Ploidy manipulation on the other hand, which necessitates but the blocking of an early meiotic division in the fertilized ova (Thorgaard 1983), employs the same physical and chemical treatments used in the diploidisation phase of gynogenesis (Purdom 1972; Cassani et al. 1985).

The chromosome engineering techniques just described are applicable to nearly all fish species. However, these methods are believed to vary among the different species with respect to effective treatment concentrations, timing, and duration of application. Hence, it is important to determine and follow treatment levels, times, etc. appropriate for each species if only to ensure the success of gynogenesis and triploidy induction. Equally important to establishing such standard techniques is the identification of chromosomally manipulated individuals. Proper identification of gynogenetic and polyploid individuals enables one to gauge the success of artificially producing them. For induced gynogenetic diploids, genetic markers are used in providing proof of all maternal inheritance. Genetic analysis of marker loci, e.g., color, morphological or biochemical markers, can confirm the success of gynogenetic induction as well as define the mechanism by which diploidisation in induced gynogenesis is achieved. This has been demonstrated in studies on gynogenetic plaice (Thompson et al. 1981), rainbow trout, zebrafish and carp (reviewed, Cherfas 1981; Thorgaard 1983). While genetic markers, particularly biochemical markers, allow identification of gynogenetics, they also permit detection of polyploids. Aside from this method, erythrocyte volume measurement, nucleolar counting, morphological examination, chromosome counting and cellular DNA content determination can also be applied (Thorgaard 1983).

The work reported here is a study on presumed "meiotic" and "mitotic" gynogenetic diploid and triploid Tilapia produced from chromosome manipulation experiments. Through the genetic analysis of polymorphic enzyme loci (biochemical markers), attempts were made to confirm the exact nature of the broods and assess the effectiveness of the chromosome manipulation techniques used to produce them.

Experimental Procedure

Materials

Laboratory stocks of *Oreochromis aureus*, *O. niloticus* and *O. mossambicus* were initially screened for protein polymorphism. The first two species originated from Lake Manzala, Egypt. Enzyme proteins, found to be the most variable lot among an array of protein systems already examined in Tilapia (McAndrew and Majumdar 1983), were used as biochemical markers. Of the 29 fish analyzed (fourteen *O. niloticus*--2 males, 12 females; twelve *O. aureus*--3 males, 9 females; and three *O. mossambicus*), at least six were used to produce diploid gynogenetics and triploids aside from the normal diploids.

The artificial gynogenetic *Oreochromis* species produced at the University College of Swansea's Genetics Department Tilapia

laboratory, were from heat shocked eggs previously inseminated with UV irradiated sperm (1 minute at 1.2-3.0 cm distance from a UVS-11 lamp) using the procedure of Chourrout and Itskovich (1983). Heat shock (41°-41.5°C for 3 min. 30 sec.-4 min.) was applied 5 min. post-fertilization to produce "meiotic" diploid gynogenetic broods. "Mitotic" gynogenetic broods were similarly obtained with heat shock applied at a much later time--20-45 min. post-fertilization. Triploids were produced by the same method except that normal sperms were used to inseminate ova heat shocked immediately after fertilization.

Methods

Fish muscle and blood samples were tested. Skeletal muscle samples from live fish were collected following the muscle biopsy technique (McAndrew 1981). Blood samples, drawn from the caudal end of anaesthetized fish using heparin-lined syringes, were centrifuged, and clotted red blood cells were kept for analysis. Horizontal starch gel electrophoresis using buffer schemes CTC (continuous tris-citrate) and TEB (tris-EDTA-borate) was employed throughout (McAndrew and Majumdar 1983). Since a wider range in enzymes exhibited maximal activity in skeletal muscle tissues than in red blood cells, only muscle samples were taken and electrophoresed. Fourteen specific enzyme stains which revealed twenty loci, were initially examined. Loci found polymorphic were then used to genotypically identify the stocks.

Results

In this paper, reference has been made to the work done by McAndrew and Majumdar (1983) on the identification of nine tilapia species using interspecific electrophoretic variation since it gives an exhaustive account of enzymes observed particularly in *O. niloticus* and *O. aureus*. The different enzyme loci found either variable or invariable by McAndrew and Majumdar (1983) are outlined in Table 1 and alongside it are the results from the present investigation. McAndrew and Majumdar (1983) noted six of 18 loci to be polymorphic and these were-- $\propto$ *Gpdh*-2, *Me*-2, *Sod*, *Est*-2, *Ada* and *Pgi*-1. Loci identified polymorphic here were *Ada*, *Ap*, and *Me*-2. With a substantial knowledge of banding patterns expected from each of the three variable loci, the broodstock were genetically tagged. Analysis of samples from *O. aureus* broodstock indicate that of the total 12, nine were heterozygous for the *Ada* locus, two homozygous for the *Ada* fast (F) allele, and one homozygous for the *Ada* slow (S) allele (*p = 0.542, q = 0.458 where *p = frequency of the F allele and q = frequency of the S allele) whilst *Ap* and *Me*-2 were found to be invariable. Of the 15 *O. niloticus* broodstock examined, three were *Ada* heterozygotes, two *Ap* heterozygotes, and ten *Me*-2 heterozygotes. Four fish were heterozygotes at more than one locus and two were homozygous for all three loci. Allelic frequencies were calculated to be p = 0.1, q = 0.9 for *Ada*; p = 0.857, q = 0.143 for *Ap* and p = 0.5, q = 0.5 for *Me*-2.

After allozyme tagging of the parental stock, control broods and chromosomally manipulated broods from parents found heterozygous for one or two loci were screened and genotype frequencies were recorded (for a summary of results, see Table 2).

Discussion

"Meiotic" diploid gynogenesis

Diploidisation in induced meiotic gynogenetics is caused by the retention of the second polar body when, in oogenesis, meiosis II is blocked. In the production of artificial meiotic diploid gynogenetics, the resulting segregation ratios are expected to be equal proportions of homozygotes and a proportion of heterozygotes (from recombination) ranging from 0 to 100% of

Table 1. List of enzyme loci studied for *Oreochromis aureus* and *O. niloticus* and the allelic frequencies scored at each variant locus by McAndrew and Majumdar (1983) and this study. In this study allele frequencies were estimated using the observed genotype frequencies in broodstock randomly sampled from the population.

	McAndrew and Majumdar (1983)			This study		
Enzyme locus	Allelic variant	*Oreochromis aureus* (n = 35)	*Oreochromis niloticus* (n = 50)	Allelic variant	*Oreochromis aureus* (n = 12)	*Oreochromis niloticus* (n = 15)
Gpdh-1	100	1.0	1.0	100	1.0	1.0
Gpdh-2	100	0.971	1.0	100	1.0	1.0
	76	0.029			0	
Mdh-1	100	1.0	1.0	100	1.0	1.0
Mdh-2	100	1.0	1.0	100	1.0	1.0
Me-1	100	1.0	1.0	100	1.0	1.0
Me-2	110		0.222	110		0.5
	106		0.222			0
	100		0.556	100		0.5
Sod	100		1.0	100		1.0
	52	1.0		52	1.0	
Ck-1	100	1.0	1.0	100	1.0	1.0
Ak	100	1.0	1.0	100	1.0	1.0
Pqm	100	1.0	1.0	100	1.0	1.0
Est-1	100	1.0	1.0	100	1.0	1.0
Est-2	109	0.086*		109	1.0*	
	100	0.914	0.910	100	1	nsbs
	93		0.090	93		
Ap	100	1.0	1.0*	100	1.0	0.857*
				79		0.143
Ada	141	0.50		141	0	
	128	0.20		128	0.542	
	122	0.30		122	0.458	
	118		0.48	118		0.1
	108		0.52	108		0.9
Pgi-1	100	1.0	0.95	100	1.0	1.0
	33		0.05	33		0
Pgi-2	100	1.0	1.0	100	1.0	1.0

= allele frequencies based on n = 4.

nsbs = not seen in the broodstock; variation noted in one brood examined in the latter part of the experiment.

= allele frequencies based on n = 7.

* = Discrepancies between the results of this study and those of McAndrew and Majumdar (1983) cannot be explained adequately. The small sample size for this study may explain the discrepancy for Ap, but the results are very divergent for Est-2.

Table 2. Segregation ratios in the gynogenetic and triploid broods screened for this study (for details, see text).

P_1 (♀x♂)		F_1 (batch code)	Treatment	Locus	N	Observed ratios	Expected ratios
O. aur.	007 x 151	B64	Control	*Ada*	30	8S/S:15F/S:7F/F	1S/S:2F/S:1F/F
	het het	B49	Meiotic gynogenesis		35	1S/S:32F/S:2F/F	equal percentages
		B50	Meiotic gynogenesis		31	1S/S:30F/S:0F/F	of homozygotes plus some % heterozygotes from recombination
O. nil.	012 x RT	B52	Control	*Me-2*	35	0S/S:19F/S:16F/F	1F/S:1F/F
	het homo F	B56	Meiotic gynogenesis		24	6S/S:10F/S:8F/F	equal percentages of
		B55	Triploidy		15	6FS/S:2FF/S:7FF/F	homozygotes plus some % heterozygotes from recombination
O. nil.	012 x ?	$UVHS_5$	Meiotic gynogenesis HS applied 5 min postfertilization	*Me-2*	6	1S/S:5F/S:0F/F	1:1 plus % heterozygotes
	het	$UVHS_{30}$	Mitotic gynogenesis HS applied 30 min. postfertilization		5	2S/S:0F/S:3F/F	100% homozygotes
O. nil.	118 x ?	$UVHS_{20}$	Mitotic gynogenesis	*Me-2*	4	0S/S:2F/S:2F/F	100% homozygotes
	het		HS applied 20 min.	*Ap*		1S/S:1F/S:2F/F	100% homozygotes
			postfertilization	*Me-2* & *Ap*		50% homozygotes*	100% homozygotes
		$UVHS_{25}$	Mitotic gynogenesis	*Me-2*	8	2S/S:4F/S:2F/F	100% homozygotes
			HS applied 25 min.	*Ap*		0S/S:3F/S:5F/F	100% homozygotes
			postfertilization	*Me-2* & *Ap*		25% homozygotes*	100% homozygotes
		$UVHS_{34\frac{1}{2}}$	Mitotic gynogenesis	*Me-2*	23	15S/S:4F/S:4F/F	100% homozygotes
			HS applied 34½ min.	*Ap*		6S/S:10F/S:7F/F	100% homozygotes
			postfertilization	*Me-2* & *Ap*		43% homozygotes*	100% homozygotes
		$UVHS_{45}$	Mitotic gynogenesis	*Me-2*	7	2S/S:0F/S:5F/F	100% homozygotes
			HS applied 45 min.	*Ap*		1S/S:2F/S:5F/F	100% homozygotes
			postfertilization	*Me-2* & *Ap*		71.4% homozygotes*	100% homozygotes

Legend:

- P_1 = parental stock
- F_1 = offsprings
- het = heterozygote
- homo = homozygote
- F = fast allele
- S = slow allele
- HS = heat shock
- ? = unknown; unrecorded
- * = total frequency of homozygotes
  = (nFFff + nFFss + nSSff + nSSss)
  where n = frequency
  F = fast *Me*-2 allele
  S = slow *Me*-2 allele
  f = fast *Ap* allele
  s = slow *Ap* allele

the total progeny depending on the gene-centromere distance. For genes near the centromere, F_1-s are predominantly homozygotes. For genes found distant from the centromere, the proportion of heterozygotes increases accordingly (Thorgaard 1983). Genotypic distribution data in gynogenetics therefore allow one to map genes. Since each heterozygote produced represents one crossover chromosome pair, the gene-centromere distance can be calculated by taking half of the crossover frequency (map length = 1/2 x crossover frequency x 100) (Thompson et al. 1981).

When the control brood and two presumably "meiotic" diploid gynogenetic broods from female 007 *O. aureus* were examined (see Table 2). Chi-square analysis on the genotypic ratio of the control brood B64 (produced from a cross of individuals heterozygous for *Ada*) suggested typical Mendelian inheritance at this locus (H_0 = 1S/S:2F/S:1F/F X^2 = 0.0667 d_f = 2 p > .95). Chi-square tests made on the Ada segregation ratios in gynogenetic broods B49 and B50 against the 1:2:1 expected ratio (assuming that gynogenesis failed and a normal cross took place) gave highly significant results (B49: X^2 = 24.085 d_f = 2 p << 0.001 and B50: X^2 = 30 d_f = 2 p << 0.001). This means that though broods B49 and B50 had a low level of homozygosity at the *Ada* locus, these presumed "meiotic" gynogenetic broods are indeed gynogenetic. The unusually high incidence of heterozygotes may be explained by the *Ada* locus having a map length close to 50 map units (map length *Ada* = 1/2 x 62/65 x 100% = 47.7), the maximum distance between a locus and a centromere that can be detected.

In the control and presumed "meiotic" diploid gynogenetic *O. niloticus* brood from 012 on the other hand, Chi-square analysis on the control brood B52 data confirms a segregation ratio of 1F/S:1F/F expected from normal diploid crosses as thus (X^2 = 0.257 d_f = 1 0.70 > p > 0.50). A contingency test between the control group and the gynogenetic B56 found them to be significantly different from each other (X^2 = 9.75 d_f = 2 0.01 > p > 0.001). This suggests that B56 is likewise a meiotic gynogenetic. The *Me*-2 locus was mapped at a distance greater than or equal to 20.83 map units (map length *Me*-2 = 1/2 x 10/24 x 100 = 20.83) from the centromere.

"Mitotic" diploid gynogenesis

In contrast to "meiotic" gynogenesis, oogenesis in "mitotic" gynogenesis is undisturbed. In "mitotic" gynogenetics, 100% homozygosity is observed amongst offsprings irrespective of whether the parent is homozygous or not (Purdom and Lincoln 1973).

When $UVHS_5$ "meiotic" and $UVHS_{30}$ 'mitotic"" gynogenetic broods from *Me*-2 heterozygous ♀ 012 were examined, direct inference from the small-sized data indicated 100% homozygosity (5/5) in the $UVHS_{30}$ brood compared to 16.7% homozygosity (1/6) in brood $UVHS_5$. This could mean that mitotic gynogenesis was successfully induced in ♀ 012 eggs when heat shock was applied 30 min. postfertilization.

In another phase of the study, four gynogenetic broods from ♀ 118 *O. niloticus* heterozygous for *Ap* and *Me*-2 loci, were screened (see Table 2). "Mitotic" gynogenesis seems to have occurred when four batches of eggs were each subjected to heat shock later than the usual 5 min. recommended for meiotic gynogenesis. Based on the results of the *Me*-2 and *Ap* allozyme segregations, I conclude that mitotic gynogenetics can be produced from ova treated with heat shock more or less 45 min. postfertilization. The presence of more heterozygotes in the first three 'early' heat shock application batches (20, 25 and 34 1/2 min.) indicate that the treatment was less or partially effective in these broods. Considering the *Me*-2 locus alone, homozygosity seems to be positively correlated with the time delay in heat shock application.

Triploidy induction

A brood (B55) confirmed triploid through karyological investigation (D.J. Penman, pers. comm.) was examined for segregation at *Me*-2. Electrophoretic observations likewise proved that the individuals in the brood are triploid as banding patterns peculiar to triploids were noted. For a tetrameric protein like malic enzyme, in diploids one expects to find three *Me*-2 banding patterns--a five banded phenotype for heterozygotes, and two one-banded phenotypes for homozygotes, one for each type (see Fig. 1). There are four possible genotypes from the crosses S/S x F/S; F/S x F/S; and F/F x F/S. Four banding patterns were observed--two one-banded phenotypes, one for each type of homozygote (FF/F and SS/S) and two seemingly three-banded phenotypes, each characteristic of the heterozygote it represents (FF/S bands having a faster mobility than FS/S bands; see Fig. 2).

Actual banding patterns observed do not show distinctly the separate bands expected for the heterozygous phenotypes from this locus; nevertheless, the different genotypes were identified from the relative mobility of the bands obtained. Zymograms from 012 triploids gave three types of banding patterns reflective only of the FF/F, FF/S and FS/S genotypes (see Fig. 2). Results agree with what is expected from a cross between a male *Me*-2 (F) homozygote and an *Me*-2 heterozygote. Scoring the different genotypes gave a segregation ratio of 6 FS/S:2FF/S:7FF/F.

Production of triploids involves basically the same method as in meiotic gynogenesis; only, in triploidy induction, the eggs are inseminated by normal sperm. Hence for brood B55, one ought to find frequencies of FS/S and FF/F genotypes equal to S/S and F/F genotype frequencies in gynogenetic broods. The FF/S proportion should also be close to the number of F/S heterozygotes obtained in diploid gynogenesis. A contingency X2 test between gynogenetics and triploids found no significant difference in the *Me*-2 genotypic distribution. Excess of heterozygotes in triploids examined here might be due to heterosis (heterozygote advantage) or to linkage disequilibrium (Kirpichnikov 1981). However owing to the limited data available for analysis, such inferences could not be fully supported here.

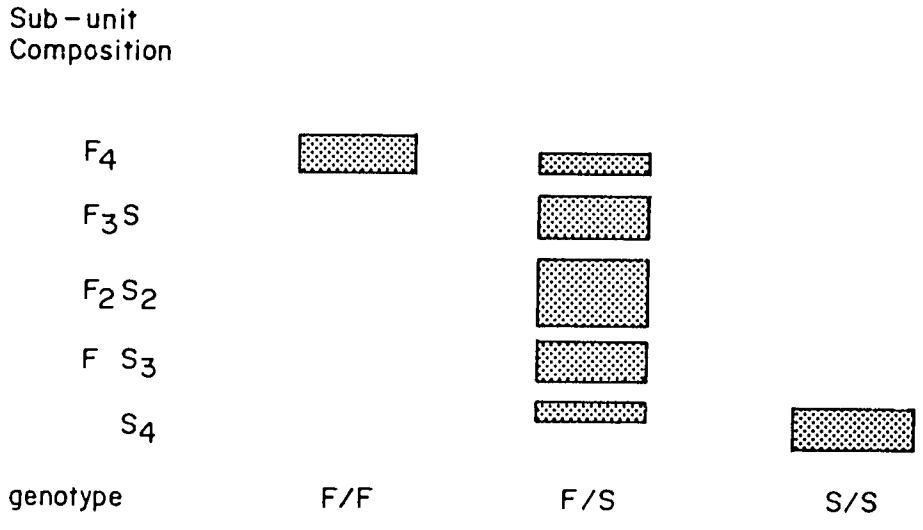

Fig. 1. Expected banding patterns at the Me-2 locus in diploids.

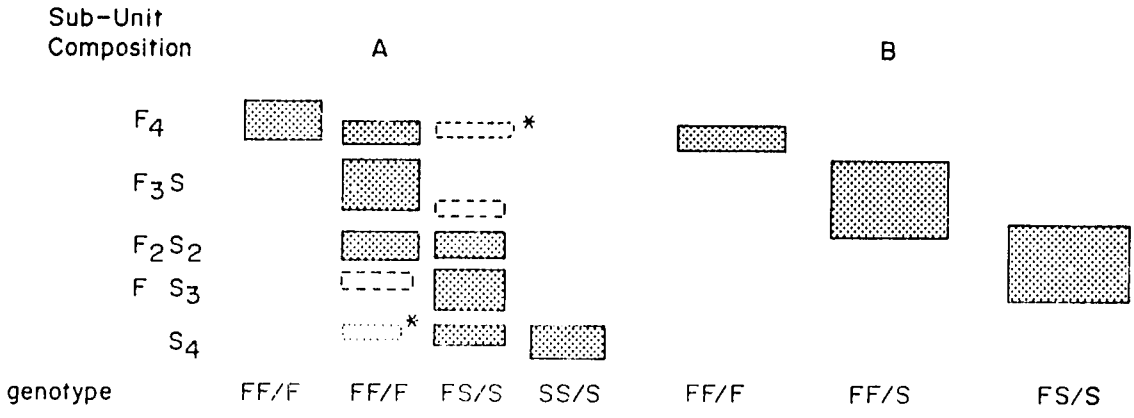

Fig. 2. Banding patterns at the Me-2 locus in triploids (* = not observed, see Fig. 1) A. expected pattern. B. observed pattern.

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Synchronous Spawning of Nile Tilapia through Hypophysation and Temperature Manipulation

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Abstract

This study was an attempt to improve synchronization of spawning of *Oreochromis niloticus* broodstock. Chinese carp pituitary gland (PG) at 0.10, 0.25, 0.50 mg PG/100 g breeder and human chorionic gonadotropin (HCG) at 10, 25, 50 and 100 IU HCG/100 g breeder were administered. Hypophysation generally failed to induce spawning. There was only partial success with the 25 IU and 50 IU HCG/100 g breeder treatments and in most experiments natural spawning (controls) was equal or greater. This is discussed in relation to previous work. Exposing experimental fish to cool water (22.0 ± 1.5°C) for long periods of 1, 2 or 3 weeks and subsequent return to ambient temperature (29°C-30°C) did not induce tilapia to spawn within 4 weeks of observation. However, treatments using short-term exposure (6-24 hours) to cool water showed a significantly improved spawning frequency (10-25% above controls: P = 0.01). The use of cool treatments with ice-blocks may be appropriate for rural hatcheries.

Introduction

As tilapia breed asynchronously year-round in the tropics with relatively small number of eggs produced per spawning, large numbers of broodstock have to be kept to produce the fry required for cost effective sex-reversal operations. Synchronous spawning within a population would improve broodstock productivity

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through: increased total egg production over a set time period; increased fecundity of individual fish and control of the timing and level of egg production. This study was initiated to investigate whether the spawning activities of Nile tilapia (*Oreochromis niloticus*) could be manipulated and synchronized through hypophysation with Chinese carp pituitary gland (PG) or human chorionic gonadotropin (HCG) or by exposure to cool temperature followed by return to normal ambient temperature.

Materials and Methods

This study was divided into two parts.

1. Hypophysation in Nile tilapia, using Chinese carp pituitary glands (PG) and human chorionic gonadotropin (HCG)

Oreochromis niloticus broodfish were obtained from experimental ponds at the Asian Institute of Technology (AIT), near Bangkok. A hundred and twenty females, 100-300 g in weight, were selected for sexual maturity on the basis of external morphological characteristics such as swollen belly and protruding genital papillae, which were red or pink in color. The fish were held in two cages (2 x 2 x 1 m) suspended in a cement tank (6 x 3 x 2 m) at a stocking density of 60 fish/cage. One hundred and twenty males were similarly selected and similarly caged. Thirty per cent of the tank water was changed weekly. The fish were fed twice a day, with commercial catfish pellets (28.5% crude protein) at a rate of 3% body weight/day. Fish selected for hypophysation were transferred to experimental holding tanks (1 x 3 x 0.75 m) prior to treatment.

PG was made from Chinese carp (bighead carp, *Aristichthys nobilis* and silver carp, *Hypophthalmichthys molitrix*) pituitaries stored in acetone and kept in a refrigerator at 4°C prior to experimentation. When required, the pituitaries were dried at room temperature for 3 minutes, weighed on an analytical balance to the exact required weight, ground with a tissue homogenizer until completely pulverized. Normal saline solution (0.9%) was then added and thoroughly mixed to the desired dilution.

HCG (Profasi; Union Medical, Thailand Co., Ltd., P.O. Box 3-50, Bangkok 10300) was used. Normal saline solution (0.9%) was used for dilution. Injections were given at the base of the pectoral fin. Only females were injected. Injection volume was 1 ml per kilogram of recipient.

Circular cement tanks (1.5 m diameter and 45 cm water depth) were used as breeding tanks. Each tank was divided into three parts with three bricks placed on the floor of the tank. Water in the tanks was not changed although aeration was provided by an air pump during the observation period. Three injected females and an equal number of males were placed in each tank. Spawning activity was monitored by looking for eggs in the female mouths on days 3 and 7 after injection. The experimental procedures adopted in this series of experiments are outlined in Table 1 (Experiments 1-7).

2. Effect of temperature manipulation on spawning activities of Nile tilapia

This part of the study was conducted in two series of experiments.

(a) Long-term exposure to cool temperature (Experiment 8)

Sixty female fish (100-200 g) were selected as described in 1 above, and stocked in a 2 x 2 x 1 m cage suspended in an earth pond for 1 week before being transferred to the experimental tank. The tank was made of plywood (5 x 1.5 x 0.7 m) lined with heavy duty plastic and water depth was kept at 60 cm. The water was recirculated through a mechanical and biological filter containing varying sizes of plastic caps. The tank was located within an air-conditioned room. Experi-

Table 1. Experimental procedures adopted in hypophysation experiments with *Oreochromis niloticus* using carp pituitary gland (PG) and human chorionic gonadotropin (HCG). Only females were injected.

Experiments	Treatments	Replication	Observation period
1	0.10 mg PG/100 g breeder 0.25 mg PG/100 g breeder 0.50 mg PG/100 g breeder	Each treatment was in triplicates of 3 fish each	3 days after injection
2 & 3	As in Experiment 1	As in Experiment 1	3 and 7 days after injection
4	10 IU HCG/100 g breeder 25 IU HCG/100 g breeder 50 IU HCG/100 g breeder	As in Experiment 1	3 days after injection
5	25 IU HCG/100 g breeder 50 IU HCG/100 g breeder 100 IU HCG/100 g breeder	As in Experiment 1	3 and 7 days after injection
3	50 IU HCG/100 g breeder 50 IU HCG + 0.25 mg PG/100 g breeder	As in Experiment 1	3 and 7 days after injection
7	50 IU HCG + 0.25 mg PG/100 g breeder in 3 equal portion injections at 6 hours intervals	As in Experiment 1	3 and 7 days after injection

*In all experiments, two control treatments were included:
1. natural spawning with no hypophysation
2. normal saline solution (0.9%) injection

mental fish from the holding cage were transferred to the experimental tank containing water at a temperature of 25°C. At the start of the experiment, three fish were sampled to determine the stage of gonad maturity by the measuring the gonadosomatic index (GSI) and percentage of mature eggs by histology.

The remaining fish were acclimatized in the experimental tank (25°C) and cooling of the water commenced by means of the air-conditioner. The desired temperature of 22.0 ± 1.5°C was attained in 2 days. Subsequently, at weekly intervals, twelve fish were sampled, three for determination of gonad maturity, and the remaining nine were used for spawning activities, in three replicates of three fish each. For each replicate, experimental females were stocked with an equal number of males in the breeding tanks (as described in 1. above) and spawning activity monitored every 3 days for 4 weeks.

(b) Short-term exposure to cool temperature (Experiments 9-12)

A hundred female and 100 male fish of 30-50 g were selected as described in 1. above and stocked in two separate holding cages (5 x 10 x 1 m) suspended in an earth pond. The fish were fed twice a day with catfish pellets at a rate of 3% body weight/day. The experimental tank as described in Experiment 8 (2a. above) was used, where a cage (2 x 2 x 1 m) was placed to hold fish to facilitate the transfer fish after exposure to cool temperature. The tank had a recirculating water system

maintained at 22.0 ± 1.5°C by means of an air-conditioner. After treatment, experimental fish were allowed to spawn in breeding cages (2 x 3 x 1 m) suspended in the same earth pond as the holding cages for the broodstock.

Experiment 9

Thirty-three female fish were selected. At the start of the experiment, nine were sampled, three fish to measure per cent egg maturity, the remaining six for spawning activities in breeding cages in two replicates of three fish, each serving as a control treatment. The remaining 24 fish were transferred and placed immediately in the experimental tank with water at 22.0 ± 1.5°C. Subsequently, at 3, 6, 12 and 24-hour intervals, six experimental fish were sampled and transferred to two breeding cages with three fish each. Equal number of males were stocked. Spawning activity was monitored by looking for eggs in female mouths on the 3rd and 7th day after transfer to breeding cages.

Experiment 10

In this experiment, Experiment 9 was repeated following the same experimental procedures previously outlined with an increased number of replicates to three for each exposure time.

Experiment 11

Experiments 9 and 10 showed that exposing fish to six or more hours of cool water slightly improved the spawning success within the treatment groups. Therefore, a 6-hour cool exposure experiment was repeated with a larger number of fish (10) per duplicate treatment. Forty-three fish were selected and at the start of the experiment three were sampled for determination of gonad maturity. Twenty fish were used as a control treatment where they were allowed to spawn in cages (5 x 10 x 1 m) suspended in the same earth pond as the broodstock holding cages. The other twenty fish were transferred to the experimental tank, holding water at 22 ± 1.5°C for 6 hours and then transferred to breeding cages. The spawning activity was monitored on days 3 and 7 after transfer to breeding cages.

Experiment 12

Results from Experiments 9, 10 and 11 showed that exposing ripe females to cool temperature for a period of 6 hours significantly increases the number of females spawning within treatment groups. However, as the techniques were being developed to benefit small-scale farmers, cooling of water by air-conditioners was not deemed appropriate. Hence Experiment 12 was designed to test whether cooling by ice-blocks could be used instead. Preliminary studies showed that 12 ice-blocks (20 x 26 x 54 cm) placed in the experimental tank holding 4 m^3 of water reduced the water temperature from 29°C to 21°C in 1 hour. After 6 hours, the water temperature increased to 22°C and was 23°C after 24 hours. Experimental procedures similar to Experiment 11 were adopted.

Data were analyzed by ANOVA and Duncan's Multiple Range Test.

Results and Discussion

Hypophysation with PG

Results from PG hypophysation are presented in Tables 2, 3 and 4. It was clear that most hypophysation at 0.1, 0.25 and 0.50 mg PG/100 g breeder treatments had lower numbers of spawning fish than natural spawning. These results are contrary to the results of hypophysation using PG in Chinese carps, Indian carps and other species of fish. It is a routine technique in fish culture. Probable reasons to explain the low success rate in inducing fish to spawn through hypophysation in tilapia include low potency of the PG used,

Table 2. Numbers* of *Oreochromis niloticus* spawning on day 3 after hypophysation with Chinese carp pituitary gland (PG): Experiment 1. For details, see Table 1 and text.

Treatment	Day 3			
	**r-1	r-2	r-3	Total
Natural spawning	0(3)	1(3)	2(3)	3(9)
Saline injection	0(3)	0(3)	0(3)	0(9)
0.10 mg PG/100 g breeder	0(3)	0(3)	0(3)	0(9)
0.25 mg PG/100 g breeder	1(3)	1(3)	0(3)	2(9)
0.50 mg PG/100 g breeder	1(3)	0(3)	0(3)	1(9)

*The number indicates number of spawned fish, the number in parentheses indicate the total number of tested fish.

**r indicates replicate treatment.

Table 3. Numbers* of *Oreochromis niloticus* spawning on days 3 and 7 after hypophysation with Chinese carp pituitary gland (PG): Experiment 2. For details, see Table 1 and text.

Treatment	Day 3			
	**r-1	r-2	r-3	Total
Natural spawning	1(3)	0(3)	0(3)	1(9)
Saline injection	0(3)	0(3)	1(3)	1(9)
0.10 mg PG/100 g breeder	0(3)	0(3)	0(3)	0(9)
0.25 mg PG/100 g breeder	0(3)	0(3)	0(3)	0(9)
0.50 mg PG/100 g breeder	0(3)	1(3)	1(9)	1(9)

Treatment	Day 7			
	r-1	r-2	r-3	Total
Natural spawning	1(3)	0(3)	1(3)	2(9)
Saline injection	0(3)	0(3)	1(3)	1(9)
0.10 mg PG/100 g breeder	1(3)	1(3)	0(3)	2(9)
0.25 mg PG/100 g breeder	0(3)	0(3)	0(3)	0(9)
0.50 mg PG/100 g breeder	0(3)	0(3)	1(3)	1(9)

*The number indicates number of spawned fish, the number in parentheses indicate the total number of tested fish.

**r indicates replicate treatment.

phylogenic differences between donor and recipient PG's, and poor selection of sexually mature females. Such selection was conducted on the basis of external characteristics from a pool of preselected mature females. Some difficulties were experienced during the selection exercise because belly swelling in sexually mature tilapia is not as distinctive in carps. Attempts were made to ensure consistency in the selection of sexually mature females and males for every experiment but it is possible, faced with such difficulties, that there be bias and error in evaluating sexual maturity; hence the inconsistent results.

Table 4. Numbers* of *Oreochromis niloticus* spawning on days 3 and 7 after hypophysation with Chinese carp pituitary gland (PG): Experiment 3. For details, see Table 1 and text.

Treatment	Day 3			
	**r-1	r-2	r-3	Total
Natural spawning	1(3)	2(3)	0(3)	1(9)
Saline injection	1(3)	0(3)	1(3)	2(9)
0.10 mg PG/100 g breeder	1(3)	1(3)	0(3)	2(9)
0.25 mg PG/100 g breeder	0(3)	0(3)	1(3)	1(9)
0.50 mg PG/100 g breeder	1(3)	0(3)	1(3)	1(9)

Treatment	Day 7			
	r-1	r-2	r-3	Total
Natural spawning	1(3)	2(3)	0(3)	3(9)
Saline injection	1(3)	0(3)	1(3)	2(9)
0.10 mg PG/100 g breeder	1(3)	1(3)	0(3)	2(9)
0.25 mg PG/100 g breeder	0(3)	1(3)	1(3)	2(9)
0.50 mg PG/100 g breeder	1(3)	0(3)	1(3)	2(9)

*The number indicates number of spawned fish, the number in parentheses indicate the total number of tested fish.

**r indicates replicate treatment.

Hypophysation with HCG

Results presented in Tables 5 and 6 indicated that HCG hypophysation at 25 and 50 IU/100 g breeder induced spawning in some fish in Experiment 5. Increasing the dosage to 100 IU HCG/100 g breeder did not improve the success rate as compared to the lower dose. Hence, it can be concluded that at the dosages used in this study, HCG was only marginally effective.

Dadzie (1970) succeeded in inducing *Oreochromis aureus* to spawn by hypophysation with 0.25 mg PG plus 50 IU HCG/100 g breeder with more than two injections and all his injected fish spawned within 1 day. We repeated his experiment with *Oreochromis niloticus* in Experiment 6 and the results showed that the natural spawning treatment had a higher number of fish spawning (3 out of 9) than the 0.25 mg PG plus 50 IU HCG/

Table 5. Numbers* of *Oreochromis niloticus* spawning on days 3 and 7 after hypophysation with human chorionic gonadotropin (HCG): Experiment 4. For details, see Table 1 and text.

Treatment	Day 3			
	**r-1	r-2	r-3	Total
Natural spawning	0(3)	0(3)	0(3)	0(9)
Saline injection	0(3)	0(3)	0(3)	0(9)
10 IU HCG/100 g breeder	0(3)	0(3)	0(3)	0(9)
25 IU HCG/100 g breeder	1(3)	1(3)	0(3)	2(9)
50 IU HCG/100 g breeder	0(3)	0(3)	0(3)	0(9)

*The number indicates number of spawned fish, the number in parentheses indicate the total number of tested fish.

**r indicates replicate treatment.

Table 6. Numbers* of *Oreochromis niloticus* spawning on days 3 and 7 after hypophysation with human chorionic gonadotropin (HCG): Experiment 5. For details, see Table 1 and text.

Treatment	Day 3			
	**r-1	r-2	r-3	Total
Natural spawning	0(3)	0(3)	1(3)	1(9)
Saline injection	0(3)	0(3)	2(3)	2(9)
25 IU HCG/100 g breeder	1(3)	1(3)	1(3)	3(9)
50 IU HCG/100 g breeder	1(3)	3(3)	0(3)	4(9)
100 IU HCG/100 g breeder	1(3)	0(3)	0(9)	1(9)

Treatment	Day 7			
	r-1	r-2	r-3	Total
Natural spawning	0(3)	0(3)	1(3)	1(9)
Saline injection	0(3)	0(3)	2(3)	2(9)
25 IU HCG/100 g breeder	1(3)	1(3)	1(3)	3(9)
50 IU HCG/100 g breeder	1(3)	3(3)	0(3)	4(9)
100 IU HCG/100 g breeder	1(3)	0(3)	0(3)	1(9)

*The number indicates number of spawned fish, the number in parentheses indicate the total number of tested fish.

**r indicates replicate treatment.

100 g breeder (1 out of 9) treatment (Table 7) did not induce spawning (Table 8). The result was similar to studies in ayu where it was found that repeated injections of HCG affected the fish and egg quality in terms of stress and low fertilization and hatching rate (Hirose 1980).

Here, natural spawning almost always gave a higher number of spawning fish than hypophysation and saline or water injected controls. The observation time was only 7 days after the injection and this may not be adequate to recover from the trauma and to spawn. However, useful hypophysation is required to act in a matter of hours.

Effect of temperature manipulation on Nile tilapia spawning

The aim of the long-term cool temperature exposure experiment was to investigate whether it would be possible to

Table 7. Numbers* of *Oreochromis niloticus* spawning on day 3 after Chinese carp pituitary gland (PG)/human chorionic gonadotropin (HCG) hypophysation: Experiment 6. For details, see Table 1 and text.

Treatment	Day 3			
	**r-1	r-2	r-3	Total
Natural spawning	0(3)	2(3)	1(3)	3(9)
Saline injection	0(3)	0(3)	0(3)	0(9)
50 IU HCG/100 g breeder	0(3)	0(3)	0(3)	0(9)
50 IU HC & 0.25 mg PG/100 g breeder	0(3)	1(3)	0(3)	1(9)

*The number indicates number of spawned fish, the number in parentheses indicate the total number of tested fish.

**r indicates replicate treatment.

Table 8. Numbers* of *Oreochromis niloticus* spawning on days 3 and 7 after hypophysation using three injections of Chinese carp pituitary gland (PG) + human chorionic gonadotropin (HCG): Experiment 7. For details, see Table 1 and text.

Treatment	Day 3		
	**r-1	r-2	Total
Natural spawning	0(3)	0(3)	0(6)
Distilled water	0(3)	1(3)	1(6)
0.25 mg PG & 50 g IU HCG/100 g breeder	0(3)	0(3)	0(6)

Treatment	Day 7		
	r-1	r-2	Total
Natural spawning	0(3)	0(3)	0(6)
Distilled water	0(3)	1(3)	1(6)
0.25 mg PG & 50 g IU HCG/100 g breeder	0(3)	0(3)	0(6)

*The number indicates number of spawned fish, the number in parentheses indicate the total number of tested fish.

**r indicates replicate treatment.

manipulate the rate of development of oocytes within the ovaries such that they would all mature at the same rate. It was hypothesized that exposure to temperatures lower than normal ambient temperature would result in the arrest of egg development and atresia of all vitellogenic oocytes, thus reducing oogenesis within individual fish as well as within a population, to a uniform state after which subsequent exposure to normal high temperature would trigger rapid and synchronous oocyte maturation and ovulation. After 1 week at 22.0 ± 1.5°C, there was an increase in the percentage of mature eggs although the GSI remained relatively unchanged (Table 9). However, over the subsequent 2 weeks, the percentage of mature eggs as well as the GSI dropped dramatically to nil. The fish were not feeding and the mean body weight decreased from an initial of 163.4 g to 141.5 g after 3 weeks. Presumably the eggs were resorbed to compensate the lack of feeding and used for routine metabolic processes. During continued observation for 4 weeks on return to ambient temperature of 29°C in the breeding tanks, the fish did not spawn. It is obvious that they require longer to recover from the trauma and to regain condition conducive to spawning. Hence, this experiment was redesigned to expose experimental fish to short cool periods, with the view that perhaps the on and off shock of cool temperature may trigger ovulation.

The short-term cool treatment gave encouraging results. Exposure to 22.0 ± 1.5°C for 6 hours or more consistently induced a larger number of fish to spawn than controls about 10-25% more in Experiments 9, 10, 11 and 12 (Tables 10, 11, 12 and 13). The total numbers of fish spawning 7 days after treatment were

Table 9. Per cent mature oocytes and gonadosomatic index (GSI) in female *Oreochromis niloticus* broodstock exposed to cool temperature (22°C) for long periods: Experiment 8. For details, see text.

Cold treatment time (week)	Mean body wt (g)	Mature oocytes (%)			GSI (%)		
		Mean	Min	Max	Mean	Min	Max
0 (Control)	163.4	17.0	0.0	28.4	2.6	0.9	3.9
1	158.5	24.8	19.9	27.8	2.2	1.2	3.8
2	154.4	0.5	0.0	1.7	1.1	0.6	2.0
3	141.6	0.0	0.0	0.0	0.8	0.7	1.0

Table 10. Numbers* of *Oreochromis niloticus* spawning on days 3 and 7 after cool treatment (22°C for 3-24 hours): Experiment 9. For details, see text.

Cold treatment time (hours)	Day 3 **r-1	r-2	Total
0 (Control)	2(3)	1(3)	3(6)
3	1(3)	1(3)	2(6)
6	2(3)	1(3)	3(6)
12	2(3)	2(3)	4(6)
24	1(3)	1(3)	2(6)

Cold treatment time (hours)	Day 7 r-1	r-2	Total
0 (Control)	3(3)	1(3)	4(6)
3	3(3)	1(3)	4(6)
6	3(3)	2(3)	5(6)
12	3(3)	3(3)	6(6)
24	2(3)	2(3)	4(6)

*The number indicates number of spawned fish, the number in parentheses indicate the total number of tested fish.
**r indicates replicate treatment.

Table 11. Numbers* of *Oreochromis niloticus* spawning on days 3 and 7 after cool treatment (22°C for 3-24 hours). Experiment 10. For details, see text.

Cold treatment time (hours)	Day 3 **r-1	r-2	r-3	Total
0 (Control)	0(3)	0(3)	0(3)	0(9)
3	0(3)	0(3)	0(3)	0(9)
6	1(3)	1(3)	0(3)	2(9)
12	2(3)	0(3)	1(3)	3(9)
24	1(3)	1(3)	1(3)	3(9)

Cold treatment time (hours)	Day 7 r-1	r-2	r-3	Total
0 (Control)	0(3)	1(3)	1(3)	2(9)
3	1(3)	1(3)	1(3)	3(9)
6	2(3)	2(3)	1(3)	5(9)
12	2(3)	1(3)	2(3)	5(9)
24	2(3)	2(3)	1(3)	5(9)

*The number indicates number of spawned fish, the number in parentheses indicate the total number of tested fish.
**r indicates replicate treatment.

Table 12. Numbers* of *Oreochromis niloticus* spawning on days 3 and 7 after cool treatment (22°C for six hours): Experiment 11. For details, see text.

Cold treatment time (hours)		Day 3	
	**r-1	r-2	Total
0 (Control)	4(10)	3(10)	7(20)
6	5(10)	6(10)	11(20)

Cold treatment time (hours)		Day 7	
	r-1	r-2	Total
0 (Control)	6(10)	5(10)	11(20)
6	8(10)	7(10)	15(20)

*The number indicates number of spawned fish, the number in parentheses indicate the total number of tested fish.
**r indicates replicate treatment.

Table 13. Numbers* of *Oreochromis niloticus* spawning on days 3 and 7 after cool treatment (22°C for 6 hours, achieved with ice-blocks): Experiment 12. For details, see text.

Cold treatment time (hours)		Day 3	
	**r-1	r-2	Total
0 (Control)	5(10)	5(10)	10(20)
6	9(10)	4(10)	13(20)

Cold treatment time (hours)		Day 7	
	r-1	r-2	Total
0 (Control)	7(10)	6(10)	13(20)
6	10(10)	8(10)	18(20)

*The number indicates number of spawned fish, the number in parentheses indicate the total number of tested fish.
**r indicates replicate treatment.

significantly higher (P = 0.01) than those on day 3 in all experiments conducted in this series; hence the spawning activity of fish should be checked 7 days after treatment. Furthermore, work using ice-blocks confirmed the earlier results and are potentially a more appropriate means of temperature adjustment. This could have implications for small-scale fish farmers operating a hatchery, as electricity may not always be available in remote areas to run air-conditioner units whilst ice-blocks can be obtained usually.

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Genetics and Breeding of Tilapia: A Review*

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Abstract

Genetic and breeding studies in *Oreochromis aureus, O. mossambicus; O. niloticus* and *O. urolepis hornorum* are reviewed. The saddleback phenotype in *O. aureus* is controlled by a dominant lethal autosomal gene. The stumpbody phenotype in *O. aureus* is a nonheritable congenital defect. Genetics of sex and body color are not completely understood. Strain differences for pigmentation, cold tolerance, gro fecundity, and success in reproducing with other species were found for *O. niloticus*. There have been thre election programs to improve early growth in *O. niloticus*. Two were unsuccessful. The third was successful; realized heritability for weight was 0.19 after three generations of selection. On generation of selection improv l growth by 10.7 to 15.5% in *O. aureus*. One generation of selection improved weight gain in *O. mossambicus* by 7.0%. Proposals to improve efficiency of selection for growth have included indirect selection, family selection, and weight-specific selection. Crossbreeding between strains of *O. niloticus* improved growth by as r uch as 36%. Cold tolerance has been improved by interspecific hybridization, and this trait was transferred from *O. aureus* to a cold-sensitive population of red tilapia by backcrossing. An inbreeding study in *O. mossambicus* showed that the inbred group had lower survival and growth rates than crossbred controls. Triploid, tetraploid, and gynogenetic tilapias have been produced by chromosomal manipulation.

Introduction

This review of genetic and breeding research in tilapia is limited to the four important mouth-brooding tilapia used in aquaculture: *Oreochromis aureus, O. mossambicus, O. niloticus* and *O. urolepis hornorum*. It will cover research on: the inheritance of qualitative phenotypes; strain evaluations; heritabilities; selection experiments; inbreeding; intraspecific crossbreeding; interspecific hybridization; environmental factors that influence genetic studies; and manipulation of chromosome number. Research on interspecific hybridization to produce all-male populations was reviewed by Wohlfarth and Hulata (1983) and Schwartz (1983). Research on biochemical genetics was reviewed by Brummett et al. (this vol.).

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Qualitative Phenotypes

Sex determination

The presumed mechanism of sex determination in tilapia is that sex is determined by sex chromosomes. This was first proposed by Chen (1969) to explain the production of all-male F_1 interspecific hybrids. Since then, sex reversal studies (Jalabert et al. 1974; Guerrero 1975; Shelton et al. 1978; Calhoun and Shelton 1983) have suggested that sex chromosomes play a major role in sex determination. *O. aureus* (Guerrero 1975) and *O. urolepis hornorum* (Chen 1969) have the WZ sex-determining system. *O. mossambicus* (Chen 1969) and *O. niloticus* (Jalabert et al. 1974) have the XY sex-determining system.

According to most authors, karyological examinations do not reveal morphologically distinct sex chromosomes in these species (Natarajan and Subrahmanyam 1968; Kornfield et al. 1979; Thompson 1981). Nijjhar et al. (1983), on the other hand, claimed that morphologically distinct sex chromosomes of the WZ sex-determining system could be seen in *O. niloticus*. The validity of this is questionable. It contradicts evidence gathered from sex reversal (Jalabert et al. 1974; Shelton et al. 1978; Tayamen and Shelton 1978; Calhoun and Shelton 1983) and hybridization studies (Jalabert et al. 1971; Pruginin et al. 1975) that support the XY sex-determining system in *O. niloticus*. If Nijjhar et al.'s (1983) interpretation were correct, the presumed sex-determining system for the other three species would also have to be changed in order to explain sex ratios in F_1 interspecific hybrids.

Sex determination is also controlled by autosomal sex-influencing or sex-modifying genes. Shelton et al. (1983) examined family sex ratios in both *O. aureus* and *O. niloticus* and found that the sex ratios ranged widely around the expected 1:1 ratio. Some families of *O. aureus* were 100% male, whereas others were 100% female. In *O. niloticus*, the percentage of males ranged from 31% to 77%. Repeatability of family sex ratio was high (r = 0.65). These data strongly suggest that autosomal genes play a major role in sex determination.

Two theories about autosomal sex genes have been proposed to help explain sex determination in tilapia. Avtalion and Hammerman (1978) proposed that sex in F_1 hybrids is influenced by two nonliked genes. This theory explains some of the unusual sex ratios that have been observed in hybridization studies, but some sex ratios cannot be explained; additionally, this system proposes sex ratios that have never been observed. Hammerman and Avtalion (1979) modified their theory by assigning different "strengths" to the sex-determining loci. This modification explains observed sex ratios that cannot be explained by the original model. Moav (unpublished, cited in Wohlfarth and Hulata 1983) proposed that sex is determined by sex chromosomes and by a single autosomal sex-determining gene that has multiple alleles.

The existence of autosomal sex-influencing or sex-modifying genes can be a major source of frustration when trying to produce all-male populations by interspecific hybridization. However, the female-producing autosomal alleles can be eliminated by a breeding program called reciprocal recurrent selection. One such program was described by Hulata et al. (1983).

Saddleback

The saddleback phenotype in the Auburn University strain of *O. aureus* was the first qualitative phenotype whose genetic basis was determined (Tave et al. 1983). This phenotype is produced by a dominant lethal autosomal gene: the *S* gene. Homozygous recessive individuals (++) are normal; heterozygous individuals (S+) are saddlebacks, and homozygous dominant individuals (*SS*) are aborted. The *S* allele exhibits variable expressivity, and saddlebacks are subviable and more susceptible to disease. Because the saddleback phenotype is produced by a

dominant allele, all *S* alleles can be eliminated from a population by a single generation of selection against the saddleback phenotype. This was done in the Auburn population, and it now breeds true and produces no saddlebacks (Tave et al. 1983).

Stumpbody

The stumpbody phenotype (a form of dwarfism) was the second qualitative phenotype that was discovered in the Auburn University strain of *O. aureus*. This phenotype proved to be a nonheritable congenital defect. It has no genetic basis (Tave et al. 1982).

Body color

Commercial interest in red and other light-colored tilapia has prompted several studies on the genetics of body color. Three different genetic mechanisms have been proposed for body coloration in tilapia.

McGinty (1983) proposed that red coloration in an *O. niloticus-O. mossambicus* hybrid population is controlled by a single gene. He felt that normally pigmented and white (pink) fish were homozygous and that red fish were heterozygous.

Halstrom (1984) proposed that body coloration in an *O. mossambicus-O. urolepis hornorum* hybrid population is controlled by two genes with recessive epistasis: the *R* and *M* genes. He felt that the *R* locus controls red pigmentation and that the *M* locus controls melanin production and is the epistatic locus.

Behrends (L.L. Behrends, pers. comm.) and Behrends and Smitherman (in press) proposed that body coloration in a commercial hybrid population was controlled by two independent genes, each of which controls a separate phenotype and each of which exhibits complete dominance. In this system, the *M* gene produces melanin and the *R* gene produces red pigmentation. The simultaneous and independent expression of the genes produces either normally pigmented, red, pink, or white tilapia.

Many red tilapia have melanistic patches (Halstrom 1984; Behrends and Smitherman, in press). Fitzgerald (1979) reported that tilapia culturists in Taiwan had reduced the incidence of melanistic patches in red tilapia by selective breeding, which suggests that these patches were controlled by a modifier gene or genes.

No studies have been performed on the population genetics of body color in wild populations of tilapias, many of which exhibit wide ranges of melanism and also have color variants.

Quantitative Phenotypes

Strain evaluations

Khater (1985), Khater and Smitherman (this vol.) and Jayaprakas et al. (this vol.) evaluated Auburn University-Egypt, Ghana, and Ivory Coast strains of *O. niloticus*. They found that the Egypt strain was the most cold tolerant, had the fastest growth rate, had more red pigmentation and had more isozymic variability than the other two strains. The Ghana strain was the most fecund. Uraiwan and Phanitchai (1986) found that Chitralada strain of *O. niloticus* (origin: Egypt, via Japan) grew faster than an Israeli strain (origin: Ghana) in Thailand. Hulata et al. (1985) found that Ghana strain females had greater spawning success than Ivory Coast females when hybridized with both *O. aureus* and *O. urolepis hornorum*. These data demonstrate that growth, success in reproducing with another species, and cold tolerance can be improved by choosing an appropriate strain.

Heritabilities and selection experiments

Heritabilities (h^2) in tilapia are listed in Table 1. Heritabilities for early growth

Table 1. Heritabilities (h^2) (± standard error) for various phenotypes in tilapia (* indicates realized heritability).

Phenotypic trait	h2 (SE)	Reference
A. *Oreochromis niloticus*		
1. Ivory Coast strain		
45-day length	0.10(0.19)	Tave and Smitherman (1980)
58-day increased length*	-0.10(0.02)	Teichert-Coddington (1983)
90-day length	0.06(0.06)	Tave and Smitherman (1980)
45-day weight	0.04(0.14)	Tave and Smitherman (1980)
90-day weight	0.04(0.06)	Tave and Smitherman (1980)
Dorsal fin ray number		
hard	0.24(0.27)	Tave (1986)
soft	0.23(0.26)	Tave (1986)
total	0.67(0.38)	Tave (1986)
Pelvic fin ray number		
hard	0.0 (0.0)	Tave (1986)
soft	-0.01(0.04)	Tave (1986)
total	-0.01(0.04)	Tave (1986)
Pectoral fin ray number		
total soft rays	0.36(0.25)	Tave (1986)
Anal fin ray number		
hard	0.0 (0.10)	Tave (1986)
soft	0.59(0.31)	Tave (1986)
total	0.61(0.30)	Tave (1986)
Caudal fin ray number		
upper branched rays	-0.05(0.08)	Tave (1986)
lower branched rays	0.15(0.10)	Tave (1986)
total branched rays	0.04(0.09)	Tave (1986)
Number of gill rakers	0.21(0.20)	Tave (1986)
Gill raker density	-0.22(0.30)	Tave (1986)
Number of scales on lateral line		
upper right	0.25(0.29)	Tave (1986)
lower right	0.00(0.11)	Tave (1986)
upper left	0.41(0.23)	Tave (1986)
lower left	0.21(0.18)	Tave (1986)
2. Ghana strain		
4-month increased weight*	zero	Hulata et al. (1986)
3. Population in Thailand		
12-week weight*	0.19	Jarimopas (unpublished, cited in Doyle and Talbot 1986)
B. *Oreochromis aureus*		
at Tifton, Georgia		
49-week increased weight, ♀*	0.38(0.08)	Bondari et al. (1983)
49-week increased weight, ♂*	0.20(0.09)	Bondari et al. (1983)
49-week increased weight, ♀ + ♂*	0.24(0.07)	Bondari et al. (1983)
49-week increased length, ♀*	0.87(0.20)	Bondari et al. (1983)
49-week increased length, ♂*	0.40(0.19)	Bondari et al. (1983)
49-week increased length, ♀ + ♂*	0.53(0.24)	Bondari et al. (1983)
at Auburn, Alabama		
49-week weight, ♀*	0.10(0.06)	Bondari et al. (1983)
49-week weight, ♂*	0.27(0.07)	Bondari et al. (1983)
49-week weight ♀ + ♂*	0.23(0.05)	Bondari et al. (1983)
C. *Oreochromis mossambicus*		
5-month increased weight, ♀*	0.01 to 0.36	Ch'ang (1971a)
5-month increased weight, ♂*	0.10 to 0.76	Ch'ang (1971a)
5-month increased weight, ♀ + ♂*	-0.01 to 0.33	Ch'ang (1971a)

(45 days-4 months) in the Ivory Coast and Ghana strains of *O. niloticus* studied by Tave and Smitherman (1980), Teichert-Coddington (1983) and Hulata et al. (1986) were low. These small h^2's may result from genetic drift that occurred because of bottlenecks in these populations (Tave and Smitherman 1980; Hulata et al. 1986).

There have been three selective breeding experiments in *O. niloticus*. Teichert-Coddington (1983) was unable to improve growth rate by selecting for increased 58-day length in the Auburn University-Ivory Coast strain. Hulata et al. (1986) were unable to improve weight at 4 months in the Ghana strain in Israel. On the other hand, Jarimopas (unpublished, cited in Doyle and Talbot 1986) improved weight gain at 12 weeks in a population of *O. niloticus* in Thailand by selection; after three generations, realized h^2 was 0.19.

Heritabilities for meristic phenotypes of *O. niloticus* (Tave 1986) show that moderate to large amounts of exploitable variance exist for some phenotypes, but that no exploitable variance exists for others.

Growth in *O. niloticus* can be improved by selecting for either weight or length, because the genetic correlations between these phenotypes are essentially 1.0 (Tave and Smitherman 1980). The correlations between length and weight in the other species should be similar to those found in *O. niloticus*.

Uraiwan and Doyle (1986) compared the theoretical efficiency of mass selection, within-family selection and between-family selection as ways to improve growth rate in *O. niloticus*. They felt that family selection may be more efficient than mass selection and suggested that within-family selection may be more efficient than between-family selection.

Growth rate has been improved by selection in *O. aureus*. Bondari et al. (1983) selected the largest 8% of the males and females, and after one generation they significantly increased 49-week body length and weight. Selected and control (unselected) lines were evaluated at Tifton, Georgia and Auburn, Alabama. Weight gain was improved by 15.5% at Tifton and 10.7% at Auburn.

Growth rate has also been improved by selection in *O. mossambicus*. Ch'ang (1971a) selected the largest 11% of the males and females in a single full-sib family and improved weight gain at 5 months by 7.0%. The improvement is remarkable, because the selection was performed on a highly inbred population that had an effective breeding number of ≤ 2. Although growth rate was improved in this population, the general applicability of these results to fish farming is uncertain because the fish were grown in aquaria.

Villegas and Doyle (1986) found a high correlation between duration of early morning feeding and growth in *O. mossambicus* (r = 0.80-0.83). They felt that these correlations suggest that selection for duration of early morning feeding would be an efficient way to improve growth via indirect selection, especially in populations where fish are of different ages. The general applicability of these data is unknown, because the study was conducted in aquaria and the sample was very small.

Males grow faster than females in many tilapias; for examples: *O. aureus* (Pagan 1970; Suwanasart 1972; Galbreath 1979; Behrends 1983; McGinty 1984); *O. mossambicus* (Brown 1971; Guerrero 1973; Guerrero and Guerrero 1975; Behrends 1983); *O. niloticus* (Micha 1973; Stone 1980; Behrends 1983; McGinty 1984) and *O. urolepis hornorum* (Behrends 1983; McGinty 1984). Even when reproduction is prevented, males grow faster than females (Pagan 1970; Guerrero 1973; Stone 1980). This suggests that the differential growth rates have a genetic basis. Sexual dimorphism in tilapia is so pronounced, that if selection is done simply by size, the selected population will be all or nearly all male. Consequently, selection for increased growth should be done independently in males and females.

Intraspecific crossbreeding and interspecific hybridization

There have been three intraspecific crossbreeding experiments with *O. niloticus*. Khater (1985) made all possible F_1 hybrids among the Auburn University-Egypt, Ivory Coast and Ghana strains of *O. niloticus*, and compared their growth rates in a 47-day yield trial in plastic pools. Heterosis for the Egypt-Ghana, Egypt-Ivory Coast and Ghana-Ivory Coast F_1 hybrids were 11.6%, 3.0% and 5.8%, respectively. Although the hybrids exhibited heterosis, no hybrid was better than the Egypt strain.

Jayaprakas et al. (this vol.) also hybridized the Auburn University-Egypt and Ivory Coast strains of *O. niloticus* and compared first-year growth in hapas. Heterosis for F_1 hybrids was 9.5% and 28.3% for length and weight, respectively. In addition, both reciprocal F_1 hybrids were significantly larger than both parental strains. Furthermore, Egypt-Ivory Coast F_2 and backcross hybrids were made. F_2 and backcross hybrids were larger than F_1 hybrids. Heterosis for F_2 and backcross hybrids were 11.8% and 11.3% for length and 36.6% and 32.1% for weight, respectively. Maternal heterosis was responsible for the increased heterosis in the F_2 and backcross hybrids. Backcross hybrids produced by backcrossing F_1 hybrid females to Egypt strain were significantly larger than those produced by backcrossing F_1 hybrid females to Ivory Coast strain.

Uraiwan and Phanitchai (1986) hybridized the Chitralada and an Israeli strain of *O. niloticus*. Heterosis for growth was -28.6%.

Lee (1979) found that *O. aureus* was more cold tolerant than both *O. urolepis hornorum* and *O. niloticus*. He also found that *O. aureus* hybrids were more cold tolerant than other hybrids and also *O. urolepis hornorum* and *O. niloticus*. Behrends and Smitherman (1984) used this information to improve cold tolerance in a population of red tilapia by backcrossing *O. aureus* to it. Some of the backcrossed red tilapia were as cold-tolerant as *O. aureus*.

Inbreeding

There has been only one inbreeding study in tilapia. Ch'ang (1971b) compared progeny produced from one generation of brother-sister matings (inbreeding = 25%) to crossbred controls (inbreeding = 0%) in *O. mossambicus*. The level of inbreeding in this experiment was actually greater, because the population had been inbred for at least three previous generations; the amount of inbreeding prior to the experiment was not reported. Ch'ang (1971b) found that the progeny produced by the brother-sister matings had lower survival and growth rates during the first 2 months of life than the crossbred fish. Because the inbred fish were compared to crossbreeds, the actual inbreeding depression cannot be determined.

Environmental sources of variance

Moav and Wohlfarth (1968, 1974) and Wohlfarth and Moav (1969) developed the communal pond concept to evaluate different groups in the same environment and to circumvent the need for large numbers of replicate ponds. In a communal pond, the different groups are stocked together rather than in separate ponds. McGinty (1984) and Khater (1985) found that the communal pond concept was a valid technique that could be used to evaluate growth of different groups of tilapia in plastic pools, concrete tanks and earthen ponds.

Age differences among families are a source of environmental variance that can confound genetic differences. Circumventing the problem of age-related size differences has been a major goal in tilapia breeding studies, because tilapia are asynchronous spawners. Several different approaches have been used: Tave and Smitherman (1980) sampled each family when it was at a certain age, instead of sampling all families on a single day. Teichert-Coddington (1983) used only fry that were within a 1-mm size range. By choosing fry that fell within this size range, he felt that the fish could be

considered as being of the same age. Hulata et al. (1986) removed eggs from females 2-3 days after spawning and artificially incubated them. They pooled fish into groups that were produced over a 3-day period, and growth was evaluated separately within each group. Uraiwan and Doyle (1986) felt that within-family selection was a way to circumvent age differences when trying to improve growth. They proposed that each family should be grown in a separate pond and that the best fish should be chosen from each family. Doyle and Talbot (1986) proposed weight-specific selection as a way to eliminate age-related size variation. In weight-specific selection, phenotypic variation is reduced drastically at a predetermined age by culling the upper and lower portions of the population distribution. Only those fish that are near the mean are kept, and they are, theoretically, of a similar age.

Siraj et al. (1983) examined the influence of female age and size on reproductive performance, egg size, fry size and early growth in *O. niloticus*. Female age and size were positively correlated with spawning rate, egg length, hatchability and sac fry length and were negatively correlated with number of eggs/kg female. Although they found a strong maternal influence on egg and sac fry length, this influence was not evident by day 20. These data suggest that female age and size could confound genetic differences when evaluating reproductive performance. Consequently, selection programs for egg production, reproductive success, or hatchability must be done within age classes and on fish of similar size.

Chromosomal Manipulation

Triploid tilapia were produced by heat-shocking fertilized eggs (Valenti 1975; Chorrout and Itskovich 1983). Valenti (1975) found that triploid *O. aureus* were larger than diploids at 14 weeks, but his sample was only 6 triploids. Chourrout and Itskovich (1983) found that heat-shocking zygotes at 40.5-41.0°C for 2-7 minutes, 4 minutes after fertilization, produced all or nearly all triploid *O. niloticus;* 68% of the triploids survived to fry. Chourrout and Itskovich (1983) also produced haploid and diploid gynogenetic *O. niloticus*. Haploid fish were produced by activating eggs with sperm whose genetic material had been destroyed by ultraviolet (UV) irradiation. No haploid gynogenetic embryos survived past hatching. Gynogenetic diploids were produced by heat-shocking eggs that had been activated by UV-treated sperm at 40.5-41.3°C for 3.5-5 minutes, 5 minutes after the eggs had been activated. Hatching rate of the gynogenetic embryos was 22-40%.

Myers (1986) produced tetraploid *O. mossambicus, O. niloticus* and *O. mossambicus* x *O. niloticus* hybrids by applying 7,000-7,500 psi pressure at 7.5°C, minutes prior to first cleavage. Production of tetraploids was low, and none survived past the sac fry stage.

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Direct and Indirect Responses to Selection for Age at First Maturation of *Oreochromis niloticus**

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URAIWAN, S. 1988. Direct and indirect responses to selection for age at first maturation of *Oreochromis niloticus*, p. 295-300. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623 p. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Abstract

Selection for growth of tilapia will be more efficient if the relationships between growth and other correlated traits are fully understood. Age and size at first maturation are genetically correlated with fish growth. Two-way selection for age at first maturation was performed over two generations of *Oreochromis niloticus* (Linnaeus) at the National Inland Fisheries Institute (NIFI), Bangkok, and at Bangsai, Thailand. Heritabilities of age and size at first maturation were estimated by full-sib analysis. Average heritabilities (± S.D.) of age and size at maturity of male parents were 0.1 ± 0.04 and 0.01 ± 0.03, respectively. Direct response to divergence selection for age at maturation was significantly obtained in the first generation. There were significant indirect responses in size at 390 days and pre- and post-maturity growth rates in both generations.

Introduction

Age and size at first maturation are important traits to be considered while selecting for increased fish growth (Alm 1959; Gjerde 1981; Naevdal 1983; Mckay et al. 1986). Hulata et al. (1986) found different selection responses in lines of *Oreochromis niloticus* which had been mass selected for growth rate at different ages. Slow growth or negative response was obtained from the juvenile-selected line, whereas no genetic gain was obtained from the post-maturation selected line. McKay et al. (1986) have mentioned that physiological status with respect to maturation is related to size and affects fish growth.

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The present study deals with the relationship between growth rate, and age and size at first maturation of the Chitralada strain of *O. niloticus* (McAndrew 1981). Two generations of two-way selection for age at maturation of tilapia have been completed and analyzed for direct and indirect responses to selection. The selection program started in 1983 and is now continuing at the National Inland Fisheries Institute (NIFI), Bangkok, and in Bangsai District, Ayuthaya Province, Thailand.

Materials and Methods

Length and weight measurements were taken every two weeks, and the age and size at first maturity were recorded. Mature fish were grouped according to age at first maturation. Sexual maturity was determined by the extrusion of gametes following gentle abdominal pressure, checked biweekly. Age at first sexual maturation of individual fish was coded according to maturation group as follows:

	Maturation group	Age (weeks)
Early	1,2	22-24
Medium	3,4	25-27
Late	5,6 and >6	>27

Each maturation group was branded with a hot wire (Bernard and Van der Veen 1974). Since the early, medium and late groups were identified relative to each other, any easily recognizable stage in the gonad maturation process could have been used for comparative purposes. We chose to use the extrusion of gametes upon gentle abdominal pressure because it could be performed quickly and reliably in the field without damaging the fish.

Selection Procedures

Parent generation (P_o)

Five pairs were spawned in separate 6-m^2 concrete ponds to start the selection experiment. The offspring were kept separately in identical ponds for 1.5 months, and then 150 fish per family were transferred into ten 1.2-m^2 cages to form the parent generation. The cages were set up in 1,600-m^2 and 19,200-m^2 earthen ponds at NIFI and Bangsai, respectively.

First and second generations (F_1 and F_2)

The parent generation was kept in cages until they were 390 days old at which time early, medium and late maturation groups were separated from within each family. Thereafter, selected fish from the five families were pooled according to early or late maturation classification; the medium group was discarded. Then three selection groups were established: early, late and an unselected or control group which included all maturation groups. Each selection group contained individuals from all five parental families. Twenty-five pairs of brooders from each selected group were spawned in a 25-m^2 concrete pond at NIFI. Fry were collected and reared for six weeks in six 25-m^2 concrete ponds (3 selected groups by 2 replicates), and then reared in 6 cages at Bangsai.

The second line of the selection program was obtained from a repeat spawning of the same selected parents. It therefore does not constitute a complete repeat of the whole selection program. The first line of the first generation was separated into two replicate cages, the other groups in successive generations were replicated 3 times. The selection procedures in the second generation followed those of the first generation. The procedure is summarized in Fig. 1.

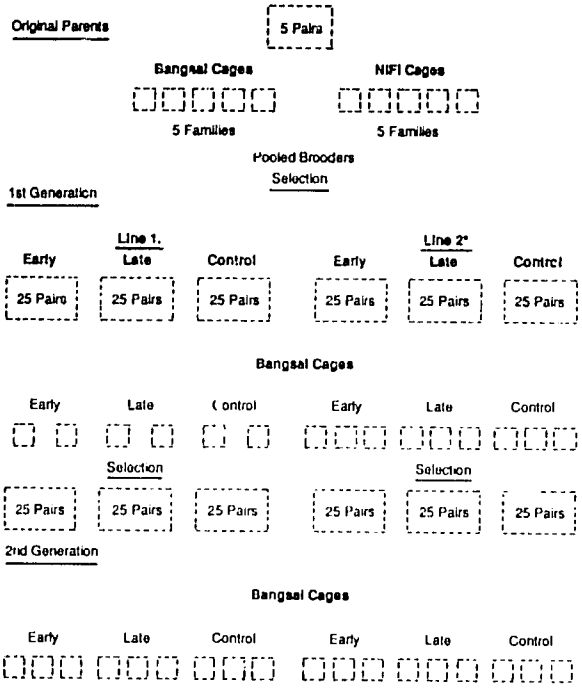

Fig. 1. Two-way selection for age at maturity of *Oreochromis niloticus*. Line 2* in the 1st generation is the repeated spawning of parents in line 1.

Estimation of Genetic Parameters

Parent generation

Average age and size at maturation for full-sib families were analyzed as random effects in a two-way ANOVA (Sokal and Rohlf 1981), and the variance components were divided into genotype, environment and genotype-environment interactions. The heritabilities of age and size at maturation were estimated by ANOVA following Falconer (1981) and Becker (1984). The calculations were performed separately for males and females.

First and second generations of selection

The direct and indirect responses to selection in the F_1 and F_2 generations were calculated. Age at maturation represents the direct response whereas weight at maturation, weight at 390 days and pre-maturation and post-maturation growth rates represent indirect responses. Pre-maturation growth rate was calculated as ln (maturity wt)/(age at maturity), and post-maturation growth rate as [ln wt at 390 days - ln maturity wt]/time.

Results

In the parent generation, genotype, environment and genotype-environment interactions were significant for age- and size at maturation (Table 1). Using full-sib analysis, estimated heritabilities (± S.D.) of age and weight at maturity were 0.10 ± 0.04 and 0.01 ± 0.03 for males and were 0.0 and 0.02 ± 0.00 for females.

In the first generation of selection, the direct response to selection was significant. The fish selected for early maturation matured on average 11 to 14 days earlier than those selected for late maturation. However, responses to selection in the second generation were not consistent (Table 2). Selection for age at maturation significantly improved size at 390 days (Table 3). Early maturing selected fish were 22 to 24% and 19 to 26% larger than late maturing selected fish in the first and second generations, respectively. The same was true of pre- and post-maturation growth rate (Table 4). Early maturing selected fish grew on average 5 to 9% faster than late maturing selected fish before maturation. After maturation, the superiority of the early line was reduced to 1% above the late line.

Discussion

Two generations of mass selection for age at maturation in the present study resulted indirectly in a genetic growth gain. The early maturation selected line was significantly larger and grew faster than the late maturation selected line

Table 1. Two-way analysis of variance on age and weight at maturation of *Oreochromis niloticus* in the parent generation.

1.1 Age (days) at maturation

Sources	Male df.	Male S.S.	Male % Variance components	Female df.	Female S.S.	Female % Variance components
1. Environment (places)	1	41,155**	23.0	1	49,999**	45.0
2. Families	4	36,698**	7.0	4	11,342**	0.0
3. Environment x families	4	17,601**	13.0	4	12,925**	14.0
4. Error	978	185,692	57.0	648	84,911	41.0

1.2 Weight (grams) at maturation

Sources	Male df.	Male S.S.	Male % Variance components	Female df.	Female S.S.	Female % Variance components
1. Environment (places)	1	140**	0.5	1	32**	0.0
2. Families	4	1,282**	7.0	4	668**	1.0
3. Environment x families	4	515**	8.0	4	136**	3.9
4. Error	978	11,501	84.0	648	5.548	85.0

*P < 0.05
**P < 0.01 significantly different at these levels.

Table 2. Difference between late and early selected lines (late-early) in mean age and weight at maturity of *Oreochromis niloticus*.

	(Late-early) Age at maturity (days) F_1	(Late-early) Age at maturity (days) F_2	(Late-early) Weight at maturity (g) F_1	(Late-early) Weight at maturity (g) F_2	(Late-early) ln weight at maturity (g) F_1	(Late-early) ln weight at maturity (g) F_2
Male						
Line 1	9.15*	−2.39	1.71	−7.90*	0.10	−0.15*
Line 2	19.29*	1.91	3.44**	6.32	0.10**	0.11
Female						
Line 1	10.82**	−2.09**	0.30	−3.63	0.02	−0.12
Line 2	2.26**	−2.09**	−3.11**	2.09	−0.12**	0.05

*P < 0.05
**P < 0.01 significantly different at these levels (ANOVA).

Table 3. Difference between late and early lines (early-late) in weight at 390 days of *Oreochromis niloticus*.

	Early-late Mean weight at 390 days (g)		Early-late Mean ln weight at 390 days (g)	
	F_1	F_2	F_1	F_2
Male				
Line 1	65.74**	180.49**	0.23**	0.27**
Line 2	74.97**	16.61	0.21**	0.03
Mean	70.36	98.55	0.22	0.15
Female				
Line 1	57.26**	64.12**	0.22**	0.13**
Line 2	51.32**	78.21**	0.18**	0.23*
Mean	54.29	71.17	0.20	0.18

*P < 0.05
**P < 0.01 significantly different at these levels (ANOVA).

Table 4. Difference between late and early lines (early-late) in pre-maturation and post-maturation growth rates of *Oreochromis niloticus*.

	Early-late Mean growth rates (%/day)			
	Pre-maturity		Post-maturity	
	F_1	F_2	F_1	F_2
Male				
Line 1	0.03**	0.06**	0.07*	0.08
Line 2	0.11**	−0.04**	−0.02*	0.04**
Mean	0.07	0.01	0.03	0.06
Female				
Line 1	0.11**	0.05**	0.03	0.04**
Line 2	0.16**	0.01	0.00	0.11*
Mean	0.14	0.03	0.02	0.14

*P < 0.05
**P < 0.01 significantly different at these levels (ANOVA).

(size 19-26% larger and growth 5-9% faster). These results confirm the genetic relationship that exists between growth rate, age and size at maturity in tilapia, as Alm (1959) and McKay et al. (1986) have reported in salmonids. To make the selection more efficient, selection for age at maturation should be considered together with selection for increasing fish growth.

Environment and genotype-environment interactions are important factors to be considered in selection for growth and maturation (Table 1). Similar to growth variation, most of variation in age and size at maturation was due to environment and genotype-environment interactions. High environmental effects led to low estimated heritabilities and inconsistent genetic gains. The present results, which are preliminary, show low estimates of heritability in age and size at maturation in *O. niloticus* (0.1). For comparison, using half-sib analysis and a 2 x 2 mating design, Gjerde (cited by Naevdal 1983) and Mckay et al. (1986) estimated heritabilities of age at maturation of rainbow trout to be 0.14 and 0.21 ± 0.14, respectively.

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SESSION IV: NUTRITION

Protein Biosynthesis in Circulated Fishponds

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Abstract

One of the problems involved with single cell protein (SCP) production is the need to harvest and to process the microorganisms grown in the system. This problem is avoided if microorganisms are grown and utilized in a fishpond. Continually mixed and aerated/circulated fishponds are effectively a very efficient mixed bed bioreactor, suitable for the production of SCP.

Tilapia *(Oreochromis aureus)* were grown in tanks in which the water was circulated continually using air lift systems. Feed treatments consisted of (a) control, commercial pellets containing 25% protein, mainly from fish and soybean meals, (b) protein-poor feed, pellets made of wheat and sorghum meals and (c) bacterial SCP diet, protein-poor pellets supplemented by daily addition of ammonium sulfate and cellulose. The added level for (c) was adjusted to replace the protein added with the commercial high protein pellets.

It is anticipated that bacteria, getting their energy from the cellulose, will take up ammonium from the water and produce bacterial protein that will be utilized by the fish.

Growth of fish receiving most of their protein as SCP (0.43 g/day) was somewhat lower than those receiving commercial pellets (0.75 g/day) but higher than that of fish grown on the protein-poor pellets alone (0.17 g/day).

Protein and fat contents of fish grown on the SCP diet were similar to those fish grown with the commercial pellets. Tilapia can utilize bacterial SCP. It seems therefore possible to replace, at least partially, expensive protein sources by cheaper carbon and nitrogen sources.

Introduction

The supply of feed materials is a major expense in aquaculture operations. The most essential and expensive components of feeds are the proteins. One possibility of reducing the feeding cost is to produce protein feed materials in the pond by microbial protein synthesis. Schroeder (1978) has discussed the heterotrophic food web as a source of feed for fish through microbial decomposition of manure or other organic residues added to the pond. The central process in this heterotrophic food web is the production of microbial protein.

Production and utilization of microbial proteins, such as single cell protein (SCP), have been studied extensively during the last few decades. One of the problems involved in an economically sound utilization of this approach is that usually dehydration and processing of the product are necessary. However, we propose *in situ* production and utilization of microbial proteins highly accessible to fish thus avoiding the high processing costs.

One obvious problem is how small a particle can be taken up by the fish. Schroeder (1978) reports that the silver carp can filter out particles larger than 20-50 microns. Yet, it was found by Odum (1968) that *Mugil cephalus* preferentially take up particles smaller than 10 microns. An interesting observation made by Taghon (1982) was that benthic invertebrates took up microscopic glass beads only when these were coated with protein, showing that in addition to the particle size, its chemical nature is important for capture by the biological filters. An additional factor favoring the uptake by fish of the microbial cells is the flocculation of those cells and the formation of relatively large clusters (Harris and Mitchel 1973; Avnimelech et al. 1982).

In preliminary laboratory experiments we found high rates of ammonium incorporation through the metabolism of cellulose by microorganisms in a model circulated pond (Avnimelech et al. 1986). The first order rate constant (Weber 1985) was 0.34 l/day. It was estimated that the process studied is fast enough to supply the protein needed by a dense culture of fish. The practical application of this approach requires ponds where the oxygen consumed by the microbial activity will not be a limitation to fish growth and survival in the system. Such demands are satisfied in newly developed circulated ponds (Avnimelech et al. 1986). These ponds, where water is continually circulated and aerated, are typified by very efficient microbial activity and should maintain adequate oxygen for fish if properly aerated. This paper describes work to study the feasibility of such an approach.

Materials and Methods

Tilapia *(Oreochromis aureus)* were grown in plastic 1-m^3 tanks. Aeration was provided by an air lift system. Each tank was equipped with four S-shaped air lifts ensuring circular movement of the water at both the bottom and the surface of the water column. The oxygen concentration in the water was maintained at values above 5 mg/l. Some malfunction of the air lift system occurred toward the end of the experiment in the SCP treatment, where the added cellulose suspension caused some clogging of the tubes.

The bottoms of the containers were sloped slightly towards the center. This, and the continual circular movement lead to the concentration of the heavy nonsuspended particles at the center of the bank bottom, from where they were drained daily.

Three feeding treatments were tested:

(1) conventional feeding with pellets containing 25% protein (20% fish meal, 24% soy meal, 10% wheat and 46% sorghum meals) serving as a positive control;

(2) a negative control treatment where fish were fed with pellets made out of cereal meal (18% wheat and 82% sorghum) containing vitamin concentrate approximating the vitamins found in the fish meal in the conventional pellets. Oil (2%) was added to the cereal pellets before feeding.

The pellets were added for the first two treatments at a rate of 2% of the fish body weight per day during the first 57 days of the experiment and 2.5% later.

(3) a treatment consisting of feeding with the cereal pellets plus a daily addition of cellulose suspension (cellulose paper ground with a Waring blender) and ammonium sulfate. The pellets were supplied at a rate of 1.1% of the fish body weight during the first 57 days of the experiment and then at a rate of 1.38% per day. The reduced level as compared to the other two treatments was given taking in account a possible energetic contribution of the raw cellulose and the SCP

derived from it. Ammoniacal nitrogen was added as ammonium sulfate at a rate equal to the amount of nitrogen added with the protein in the conventional feed treatment (from the 63rd day of the experiment, ammoniacal nitrogen was added at a rate of 1.5 times the nitrogen added with the protein). The cellulose was added at a rate of 30 times the amount of nitrogen. The C:N ratio of the added ammonium and cellulose was 15, calculated to give, considering a 40% carbon conversion efficiency, bacterial biomass with a C:N ratio of about 6.

Seven fingerlings, having an average weight of 137 ± 6 g were introduced into each tank. Fish were weighed every 10 days. All fish were netted and fish weighed individually. Three fish from each treatment were sampled at the end of the experiment, ground, dried in a vacuum oven and analyzed for ash content, gravimetrically following ashing at 600°C (AOAC 1980); protein by Kjeldahl; and total lipids according to Folch et al. (1957).

Oxygen concentration in the water was monitored daily. Water was sampled twice a week for analysis of ammonium, nitrate and nitrites by colorimetry with an autoanalyzer (USEPA 1974). Total nitrogen was determined following an oxidation with persulphate (Raveh and Avnimelech 1979) and organic carbon determined potentiometrically following an oxidation with dichromate (Raveh and Avnimelech 1972). The rate of ammonia uptake in the tanks was followed twice during the experiment by frequent samplings. On one occasion the ammonium rations of four days were given in advance and ammonium concentration followed along a 2-day period. On the second occasion the rate of ammonium uptake was followed by frequent sampling during a 24-hour period after a normal application of ammonium.

Results

The buildup of ammonium concentrations is a major problem in intensive aquaculture systems. In the present experiment, ammonium was added at a daily rate equivalent to 0.5-1.5 mg N/l. A fast incorporation of the ammonium is therefore essential for the maintenance of such a system. The ammonium concentrations (averaged for each treatment) throughout the experimental period are given in Fig. 1. Ammonium concentrations were in the range of 1-2 mg N/l during the first week, after which the concentration in all treatments were low, in the range of 0.1 mg N/l, until the 60th day of the experiment. At that time the feeding rate was raised to 2.5% of the fish biomass and the ammonium concentration rose. The ammonium concentrations in the ammonium-enriched tanks were in the same range as those in the other tanks, indicating that ammonium removal was fast. Free ammonium concentration was low due to the neutral pH of the water. An appreciable amount of the nitrogen in the water was recovered as nitrate nitrogen (Fig. 2). Nitrate concentration started at a level of about 5 mg N/l (similar to nitrate concentration in the supply water) and fluctuated along the experimental period in the range of 1-8 mg N/l. The nitrate concentration was lowest all along the experimental period in the control treatment, highest in the tanks fed with the commercial pellets and intermediate for those tanks receiving cellulose and ammonium.

Another indication for the incorporation of the added ammonium is obtained through the analysis of the total nitrogen in the water (Fig. 3). It can be seen that total nitrogen, comprising mostly of organic nitrogen, is lowest for the negative control treatment. Total nitrogen for the conventional feed and the SCP treatments is practically the same and higher than that of the control. The rate of ammonium incorporation was studied by measuring ammonium disappearance on two occasions. The first assay was performed on 14 August, i.e., about 6 weeks after the start of the experiment, when the ammonium ration of 4 days was given in advance.

The initial ammonium concentration of about 3 mg N/l dropped during 2 days to about 0.2 mg N/l. The rate of ammonium uptake following a normal ammonium

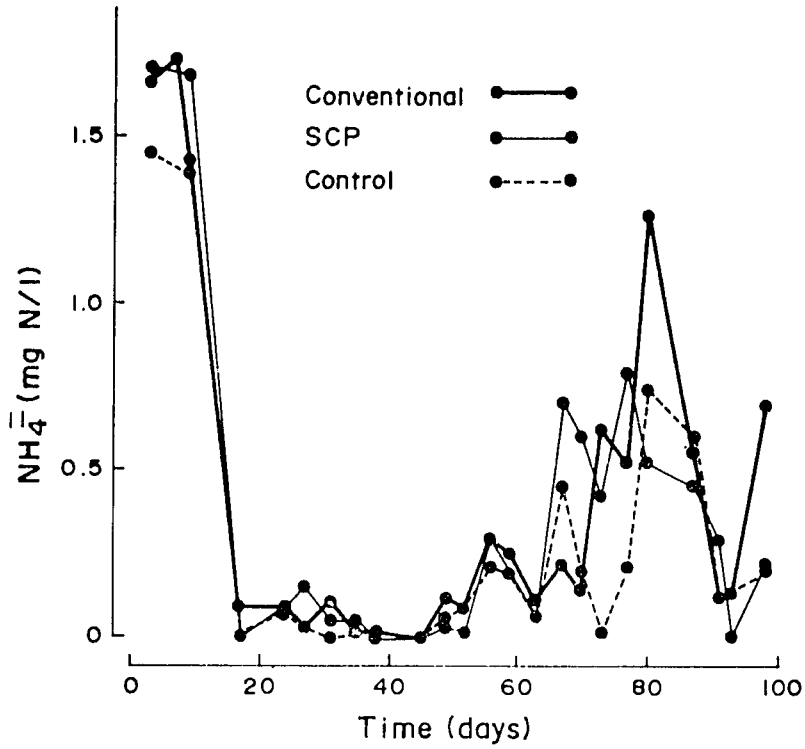

Fig. 1. Ammonium concentrations (averages of 3 replicates) as a function of time in intensive culture of tilapia *(Oreochromis aureus)* using three different feeding treatments: 1. conventional pellets containing 25% protein; 2. SCP-generating microbial protein *in situ* by adding cellulose and ammonium to match the N added in treatment 1; 3. control fed with protein poor pellets made of cereal. For details, see text.

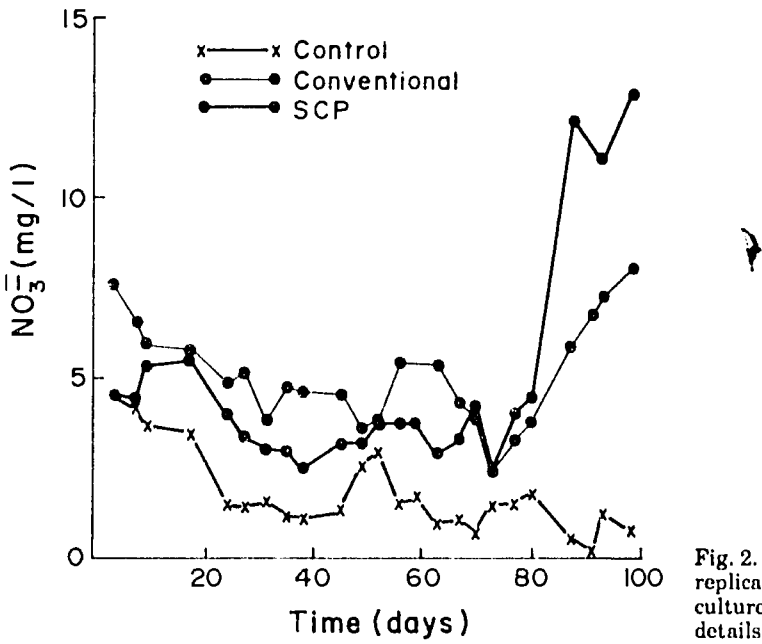

Fig. 2. Nitrate concentrations (averages of 3 replicates) as a function of time in intensive culture of tilapia *(Oreochromis aureus)*. For details of treatments, see Fig. 1.

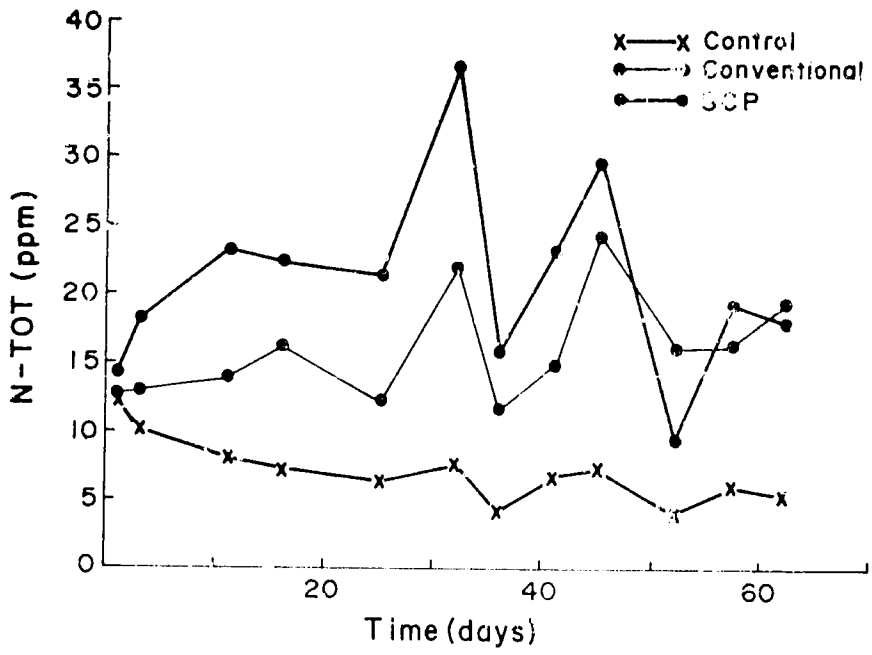

Fig. 3. Total nitrogen concentrations (averages of 3 replicates) as a function of time in intensive culture of tilapia *(Oreochromis aureus)*. For details of treatments, see Fig. 1.

addition was tested a week later. A rapid drop in the ammonium concentration was found in both assays. Plotting the logarithm of the ammonium concentrations at a given time (t in days) (CT) for both assays, relative to the initial concentration (Co) versus time, a straight line was obtained. The correlation of the line

(1) $\text{Log } Ct/Co = -0.019 - 0.586\ t$
is highly significantly ($r = 0.956$).

Fish growing conditions seemed to be good. Fish recovery was above 90%, with just a few cases of fish mortality. Growth rates were linear throughout the experimental period. The growth equations, calculated from all data points of the individual tanks are:

(2) Control treatment:
$W_t = 136.1 + 0.165\ t \qquad r = 0.520$

(3) Conventional feed:
$W_t = 135.4 + 0.754\ t \qquad r = 0.919$

and

(4) SCP fed fish:
$W_t = 126.2 + 0.426\ t \qquad r = 0.907$

where W_t is the average fish weight (grams) at time t, t is the time in days and r is the correlation coefficient.

The growth of the control fish, fed only with the low protein pellets was very poor, 0.16 g/fish/day. Moreover, the growth was not uniform, as reflected by the low correlation coefficient for equation (2). The growth of the fish fed with conventional, protein-rich pellets was fairly good for a tank experiment, 0.75 g/fish/day and was uniform throughout the period. The fish fed with low-protein diet, supplemented with cellulose and ammonium, grew at a rate intermediate between the two former treatments, at a daily rate of 0.426 g/fish. Fish growth was again uniform through the period.

Fish body composition was determined twice during the experiment, the first time on 2 September, about 8 weeks after the start of the experiment and again, at the end of the experiment, on 7 October. Fish composition is presented in Table 1. The

Table 1. Fish body composition in relation to dietary treatment in intensive culture of tilapia *(Oreochromis aureus)* using three different feeding treatments: 1. conventional pellets containing 25% protein; 2. control fed with protein poor pellets made of cereal; 3. SCP-generating microbial protein *in situ* by adding cellulose and ammonium to match the N added in treatment 1. For details, see text.

Treatment	Protein %	Fat %	Ash %
	September 2 Sampling		
Conventional	16.1	4.81	5.13
Protein poor	14.9	5.96	5.49
SCP	16.0	3.71	5.47
	October 7 Sampling		
Conventional	15.5	4.17	4.57
Protein poor	14.2	4.33	5.91
SCP	15.9	2.55	6.65

protein content of the fish is lowest in the fish fed with protein-poor diet, 14.2-14.9%. The protein percentages in the fish fed with the conventional protein-rich pellets and those fed with the protein-poor pellets, supplemented with cellulose and ammonium are practically the same, i.e., 15.5-16.1%. The percentage of fat is highest for the fish fed with protein-poor diet and significantly lower for the fish fed with single-cell proteins.

Discussion

The demands for a practical system of SCP production in a fishpond are: a) efficient conversion of ammonium and carbonaceous materials to SCP, b) harvesting of the SCP particles by the fish and c) proper utilization of the SCP by the fish.

The rate of the SCP formation, as followed here using the measurement of ammonium immobilization rates, is high. The reaction can be described as a first order reaction relative to the ammonium concentration. It is possible that this will be different in systems limited in the supply of the carbonaceous component instead of the ammonium limitation in our experimental system. The rate constant for the reaction was found to be 0.586/day, somewhat higher than the value found in the laboratory models, 0.34/day (Weber 1985). The high rate of the ammonium incorporation reaction is of significance, both as to the specific topic of this work as well as to some general conclusions. In ponds controlled by the above-mentioned rate constant, ammonium concentrations will be reduced to 1/10 within a period of 41.7 hours. This is an effective rate for the production of microbial protein, even in densely stocked ponds. If ammonium is added daily to a level of 3 mg N/l, similar to our assay, the potential daily rate of protein production will be about 14 g protein/m^3. In addition, it seems that such a process could be utilized for the control of excessive inorganic nitrogen in intensive ponds. In such a case the addition of carbonaceous material will lead to a reduction of the inorganic nitrogen due to its incorporation as organic nitrogen. If properly done, the rate of such removal could be similar to that obtained in the system reported here.

The efficiency of the bioconversion of the added ammonia to crude protein was followed also through the monitoring of the different nitrogenous components. Ammonium was added daily at a rate of 0.5-1.5 mg N/l. Yet, ammonium concentration in the SCP fed tanks was in the same range as for the conventionally fed ones and not above 2 mg N/l. The non-utilized mineral nitrogen was nitrified and accumulated as nitrate nitrogen. The levels of

nitrate nitrogen in the SCP fed tanks was lower than that in the conventionally fed ones, till the last phase of the experiment, when the level of ammonium relative to carbon was raised. Lastly, the concentrations of total nitrogen, comprising mostly of the organic nitrogen fractions, was practically the same for the conventionally and the SCP fed tanks, thus demonstrating that the added ammonium was effectively converted to organic nitrogen.

The harvesting and utilization of the microbial protein were followed here through the fish yield response and through the effect of the different diets on the fish composition. The growth rate of the fish fed with SCP was about three times as high compared with that of the control treatment, indicating a clear response to the SCP. Yet, the growth rate of the tested treatment was less than that obtained with the conventional protein rich diet. There could be several reasons for this. One quite obvious reason is oxygen limitation in the SCP treatment. Average oxygen levels in the tanks is given in Fig. 4. It can be seen that the control and the conventionally fed treatments had been all along well aerated. The aeration in the SCP fed treatment was erratic and at times low oxygen levels were found. The amount of feed and thus the oxygen demand in that treatment were higher than in the other treatments. In addition, the aeration tubings in the SCP fed tanks were often clogged by mucilaginous cellulose. Another reason for the reduced growth in the SCP fed treatment may be the probable food limitation in that treatment. Crude bacterial protein contains about 30% of nucleic acids (Dostalek and Molin 1975; Gow et al.

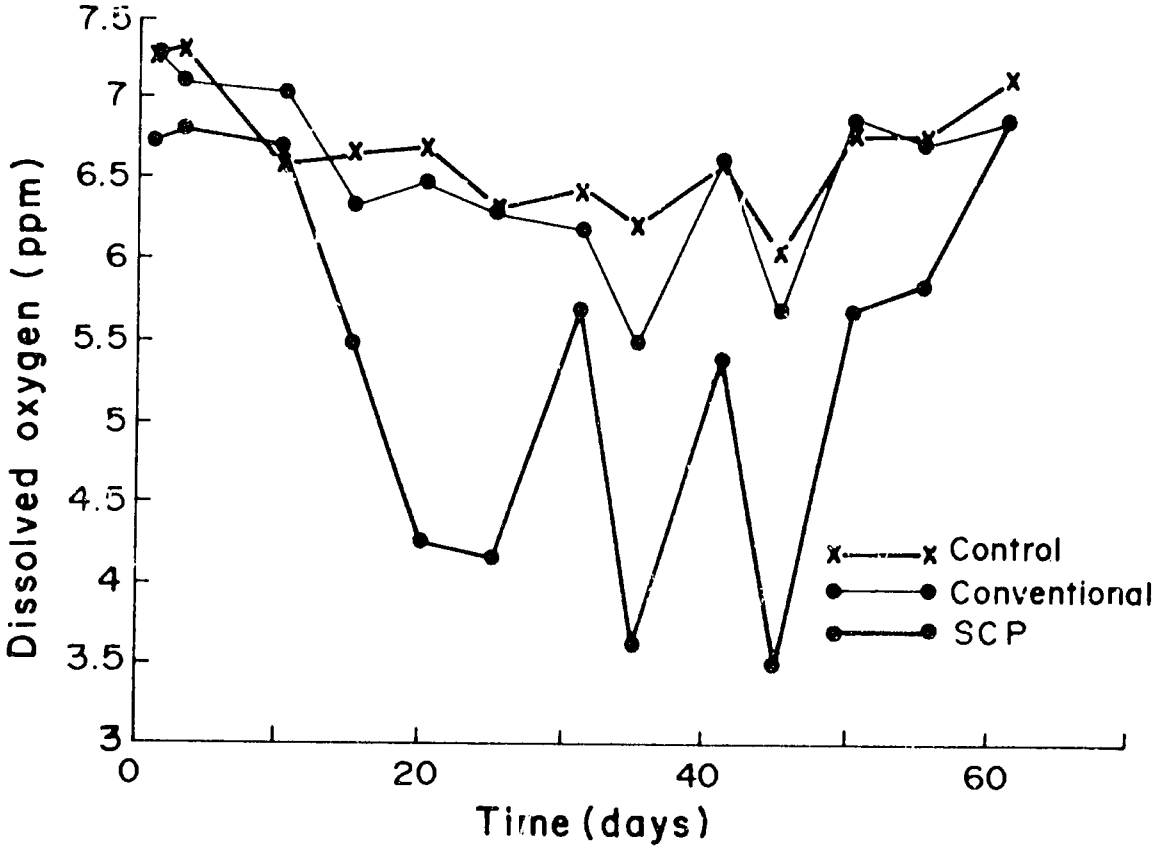

Fig. 4. Dissolved oxygen (averages of 3 replicates) as a function of time in intensive culture of tilapia *(Oreochromis aureus)*. For details of treatments see Fig. 1.

1975). This leads to a reduction in the nutritive value of the product as well as, in cases, to toxicity. However, we do not think that the presence of nucleic acids in the quantities expected here could pose a serious problem in fish nutrition. Tacon and Cooke (1980) found that feeding trout with nucleic acid extract amounting up to 5% of their diet did not have any deleterious effect. A 10% level did lead to damage. Dabrowski (1982) found that feeding carp larvae with SCP was less effective than feeding with zooplankton and suggested that this effect was due to a deficiency of calcium and ascorbic acid in the SCP. Atack et al. (1979) and Atack and Matty (1979), found that feeding carp or trout with SCP was as good as feeding with fish meal.

The present experiments were designed to test the hypothesis that *in situ*-produced SCP could be a source of protein in fish culture under the most stringent conditions. Thus, the added ammoniacal nitrogen was, during most of the experiment, equal to the nitrogen added with the proteinous component in the conventional fish feed. Under such conditions, only two-thirds of the SCP is potentially available since one-third of the crude protein is made of nucleic acids.

The fish fed with SCP contained the same, or possibly somewhat higher, protein percentage as that in fish fed with conventional feed. The main difference between those two groups is the appreciably lower fat contents in the SCP fed fish. This seems to be indicative of a relative deficiency in energy sources to those fish. The fish fed with SCP got in the pelleted food less energy than the other groups, assuming that all the added ammonium will be converted after reaction with the cellulose to available protein, thus supplying the lacking energy. However, if only about two-thirds of the crude SCP was available then the fish in the SCP fed group got less protein and less energy as compared with those obtaining the conventional food. It is possible that the fish fed with SCP needed more energy while their protein ration was satisfied.

Single cell protein (SCP) seems to be a potential source of protein to fish. More research is needed both in respect to the nutritional and to the management points of view.

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The Function of Microbranchiospines in Tilapias

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Abstract

The structure and distribution of microbranchiospines among the tilapias is briefly reviewed and experimental and anatomical evidence is presented in an attempt to elucidate their possible roles in sensory detection of suspended solids, protection of gill filaments and filter feeding.

Introduction

Microbranchiospines are small, bony, rib-like structures, bearing fine lateral spines, which occur in a single row on both faces of the second, third and fourth gill arches of tilapias. They were first described in *Oreochromis niloticus* and *Tilapia zillii* by Gosse (1956), who stated that they were formed by projections of the gill arch skin and that the upper side, which was separated from the gill arch, formed the external part of a gutter running the entire length of the arch. Gosse further hypothesized that the microbranchiospines pressed on the faces of neighboring gill arches, so forming a passive seive supplementary to the gill rakers. Small particles could thus be retained, being swept backwards towards the pharynx by a reversal of the respiratory current flow.

Later writers, such as Whitehead (1959), Campbell (1981) and Drenner et al. (1984) have tended to concur with this view, and Fryer and Iles (1972) elaborated on the theory by postulating that the spines probably originated in a benthic-feeding common ancestor. However, Trewavas (1983) has commented on the lack of any hard, supportive evidence.

In this paper we briefly review the structure and occurrence of microbranchiospines among the tilapias before evaluating their possible roles in sensory detection of suspended particles, protection of the gills, feeding or respiration.

The Structure and Occurrence of Microbranchiospines

The microbranchiospines occur predominantly on the external faces of the gill arches, where they form a single, continuous row of toothed projections lying between the gill rakers and gill filaments (Fig. 1). As the fish and thus the gill arch grows, the microbranchiospines divide so that in an adult fish there may be several hundred on a gill arch. The main body consists of a thin, bony plate, covered with a thin layer of ridged epithelial tissue, which sits 50-100 µm above the gill arch on a base of epithelial and connective tissue, underlain by well-vascularized dermal tissue (Fig. 2). Each microbranchiospine bears numerous, lateral, tusk-like denticles, largely covered in ridged epithelium, which project forward towards the gill rakers and inwards towards the gill arch surface. The denticles consist of a base of bone stem cells which connect the denticle to the main body of the microbranchiospines, a partially hollow, bony mid-section, and a hard, solid tip of indeterminate composition (Fig. 3) (Beveridge et al., unpublished data).

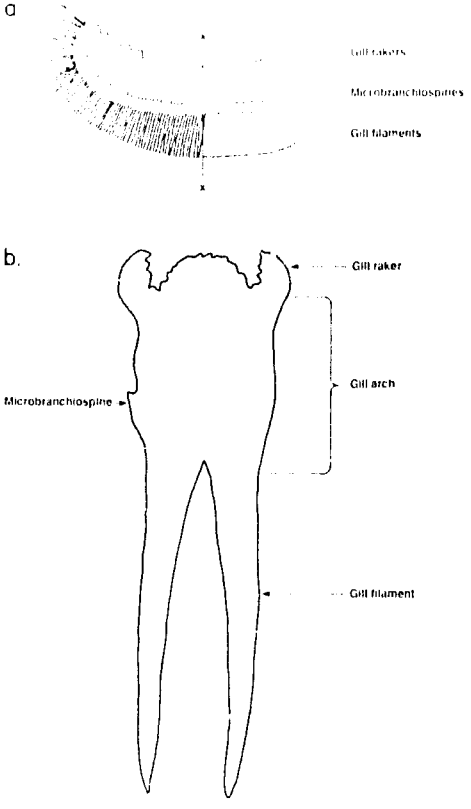

Fig. 1. Diagram of location of microbranchiospines on a tilapia gill arch. a. general view of gill arch bearing gill rakers, row of microbranchiospines and gill filaments. b. cross-section of gill arch taken at x - x (see a). The microbranchiospine spine row shows as a single projection on the external face of the gill arch (modified from Goose 1956; Fryer and Iles 1972).

Fig. 2. Transverse section through microbranchiospine from a 146-g fish. H & E (x 200). pl. plate, d. denticle, se. surface epithelium, c. connective tissue, f. collagen fibers.

Fig. 3. Longitudinal section through microbranchiospines from a 146-g fish. H & E (x 400). b. base, m. mid-section, t. tip.

The dimensions of the microbranchiospines vary with species and size but are approximately 100-400 µm long, with lateral denticles, 20-40 µm length. Distances between adjacent spines are also highly variable but are generally in the range of 0-25 µm (Beveridge et al., unpublished data).

Microbranchiospines have been found to occur in almost all tilapia species that have been examined, the exceptions being two subgenera of the genus *Oreochromis*, *Alcolapia* and *Vallicola*, and members of the genus *Danakilia* (Trewavas 1983). However, the subgenus *Alcolapia* contains only one species, *Oreochromis alicalus*, with two subspecies, *alicalus* and *grahami*, whilst *Vallicola* has only one species, *Oreochromis amphimelas*. There is also only one species of *Danakilia*, *D. franchetti*.

The Role of Microbranchiospines in Feeding

The gill rakers in filter-feeding species of tilapias such as *O. niloticus* and *O. aureus* are small when compared with the gill rakers of many other filter-feeding fish species, such as the silver carp *Hypophthalmichthys molitrix* (Hyatt 1979). Moreover the gill raker spacings in these tilapia species are around 200 µm and thus it was supposed that the fish would be unable to trap particles of dimensions smaller than this. However, it has been demonstrated from field and laboratory data that much smaller particles can be ingested (Greenwood 1953; Drenner et al. 1984) and thus it seemed likely that another mechanism, most probably the microbranchiospines, in view of their size, structure, and distribution on the gill arches, was also involved in food entrapment.

In order to assess the possible role of the microbranchiospines in filter feeding, 2 g of water-insoluble, Sudan black dye (Sigma chemicals, practical grade), of particle size determined to be between 2 and 25 µm, was introduced into a heated (27°C) aquarium filled with 10 l of water, thoroughly mixed by hand, and kept in suspension by vigorous aeration. Two 25-g *O. aureus* were then placed in the tank and left for one hour. The fish were then removed, killed, and the gill arches, buccopharyngeal cavity and stomach examined for the presence of dye particles.

On examination of the gill arches, copious quantities of even the smallest dye particles were found caught up amongst mucus in the gill rakers, whilst comparatively few particles were found associated with the microbranchiospines (Fig. 4a, b). Where dye was detected in the vicinity of the spines, the particles were also found in mucus-bound clumps which were observed lying on top of the spines, rather than trapped in the inter-microbranchiospine

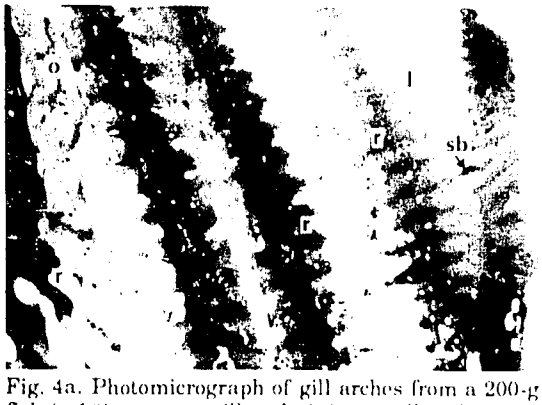

Fig. 4a. Photomicrograph of gill arches from a 200-g fish (x 10). o. outer gill arch, i. inner gill arch, r. gill raker, sb. Sudan black particles.

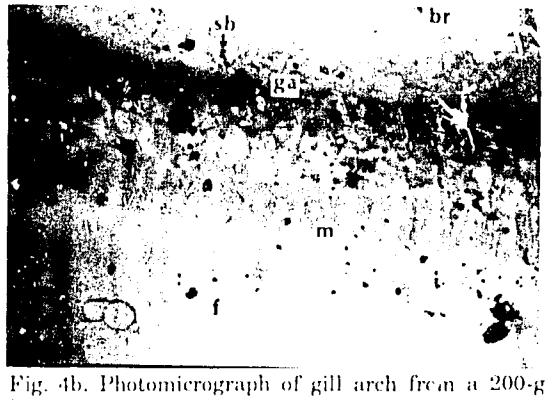

Fig. 4b. Photomicrograph of gill arch from a 200-g fish (x 50). m. microbranchiospine, f. gill filaments, ga. gill arch, br. base of gill raker, sb. Sudan black particles.

spaces by the denticles as had previously been envisaged (Fig. 4b). Large quantities of dye were also observed in the stomach, confirming that the particles had been ingested.

There is thus no necessity to invoke the involvement of microbranchiospines in filter feeding in tilapias in order to explain their abilities to ingest small particles, although in view of their location and the microridges present on the surface epithelium, entrapment of some mucus-bound particles is inevitable. Microbranchiospines are also very small (see above) and it is difficult to envisage structures of their size, no matter how numerous or complex in structure, being the prime method for plankton filtration. Prior to the discovery of microbranchiospines, Greenwood (1953) proposed that mucus was important in the entrapment of algae and the above experiment confirms this. Moreover, a number of tilapia species which have no microbranchiospines and comparatively few gill rakers utilize planktonic algae as a source of food (Table 1), although, as pointed out by Trewavas (1983), these species occur in alkaline,

Table 1. Comparison of feeding and gill raker characteristics of tilapias with and without microbranchiospines. (Sources: Balarin and Hatton 1979; Bower 1982; Trewavas 1983)

Species	Microbranchiospines	Gill raker number	Adult diet (predominant)	Distribution
O. niloticus	Present	14-27	Phytoplankton including cyanobacteria	Wide: Senegal Chad Niger Nile
O. Nyasalapia spp.	Present	17-26	Plankton, algae	Wide: Zaïre, Malawi, Zambezi
O. Neotilapia spp.	Present	22-26	Algae	L. Tanganyika
O. niger	Present	15-19	Epiphytic and epilithic algae	Athi River
O. aureus	Present	18-22	Phytoplankton, zooplankton	Wide: Jordan, Senegal, Chad
O. mossambicus	Present	14-20	Macrophytes, benthic algae, plankton	Wide: Lower Zambezi, Limpopo
O. esculentus	Present	15-21	Phytoplankton	L. Victoria, Kyoga, Nabugabo
O. variabilis	Present	17-23	Algae, benthic sediments	L. Victoria, Kyoga
S. melanotheron	Present	14-19	Algae, detritus, invertebrates	Zaïre to Senegal
O.s. percivali	Reduced	13-16	Filamentous algae	Hot spring, N. Uaso, Nyiro
O.a. grahami	Absent	11-14	Epilithic cyanobacteria	L. Magadi
O. alcalicus	Absent	9-14	Blue-green algae	L. Natron, L. Magadi
O. amphimelas	Absent	12-16	Green algae	L. Manyara, Eyasi, Kitangiri
D. franchettii	Absent	10-12	Algae	L. Afrera

soda lakes, where the algae tend to be long chain species (e.g., *Spirulina*) which occur in clumps. the data in Table 1 indicate that tilapias other than filter-feeding species possess microbranchiospines and it has also been shown that the dimensions of the microbranchiospine "sieve" bear no relationship to feeding habit (Beveridge et al., unpublished data).

Although there is undeniably some correspondence between gill raker modification and the feeding behavior of fishes (Hyatt 1979), Wright et al. (1983) and others have shown that the relationship is not simple since other modifications, such as tubular mouths, epibranchial organs and possibly mucus production may be involved. The situation amongst the tilapias seems to support this view.

The Role of Microbranchiospines in Protection of the Gill Filaments

Fryer and Iles (1972) have also expressed doubts that the principal function of the microbranchiospines is concerned with filter feeding since they occur in tilapias with diverse feeding habits. They suggest that in view of their situation and design they are ideally suited to protect the delicate gill filaments from abrasion. However, the experiment conducted using Sudan black dye particles also suggests that the principal method of removing suspended particulate matter is through entrapment among the gill rakers in a mucus film. It is also difficult to see how such small structures are able to carry out any but the most minimal of protective functions.

The Role of Microbranchiospines in Sensory Detection

Two types of mechanoreceptor, proprioreceptors and nociceptors, have been found to occur in the branchial regions of fish (Nilsson 1984). Although there is a degree of overlap between the two, in general the former is involved in the control of respiration by responding to changes in water pressure in the inhalent current whilst the latter is able to detect slight changes in mechanical pressure caused by suspended particulate material, thus initiating a cough or expulsion reflex. Mechanoreceptors are commonly found both in the gill rakers and gill filaments (Sutterlin and Saunders 1969).

In order to assess whether the microbranchiospines might play some role in mechanoreception, serial longitudinal, transverse and horizontal 5-15 µm sections of buffered formalin-fixed gill arches were cut and stained with one or two stains specific for nervous tissue, Palmgren's silver impregnation method, with and without the toning step, and a modified Weil haematoxylin method for myelin (see Drury and Wallington 1980) (Figs. 5 and 6). With Palmgren's stain, the microbranchiospines appeared brown/black on either a yellow/brown or grey background, depending on whether or not the toning step was used, whilst with the latter method, all tissues other than red blood cells and eosinophilic granular cells appeared light brown in color. No nervous tissue was observed in any of the preparations.

On present evidence it must be concluded that the microbranchiospines are unlikely to serve any mechanoreceptor function, although it is desirable to corroborate the histological evidence with electrophysiological data.

Discussion and Conclusions

There is no evidence from histological preparations that microbranchiospines serve any mechanoreceptor function, and it is suggested that they play only an incidental role in filter feeding and protection of the gill filaments by virtue of their situation on the branchial arches. It is also demonstrated that their complex structure is not essential to the entrap-

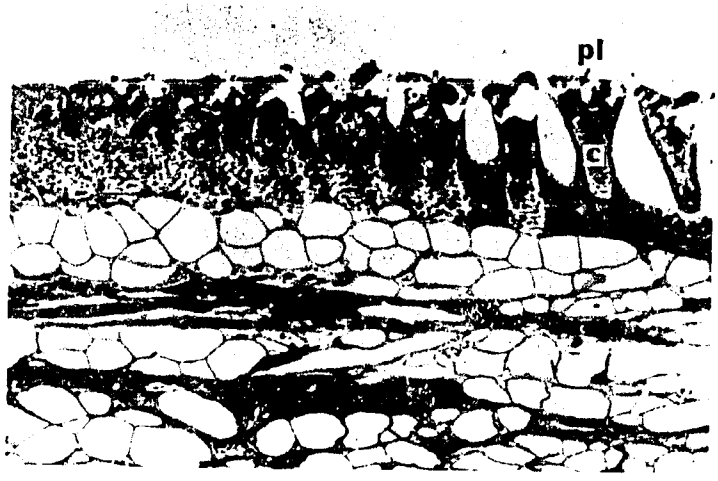

Fig. 5. Longitudinal section through row of microbranchiospines from a 220-g fish. Palmgren's silver impregnation method (x 100). c. connective tissue, pl. plate.

Fig. 6. Longitudinal section through microbranchiospines from a 250-g fish. Modified Weil hematoxylin method (x 200). pl. plate, d. denticle, c. connective tissue.

ment of suspended particles, matter being ensnared in mucus-bound strings on the spine surface rather than between the spines as had previously been supposed. Hughes (1979) and others have suggested that the microridges frequently observed on the epithelial surface of gill structures may assist in the anchoring of mucus and it seems probable that the microbranchiospines entrap algae and other particulate material in the same way. It is also possible that the microbranchiospines act as hydrodynamic "spoilers", their mucus covered surfaces ensuring a laminar flow across the gills. However, it remains difficult to explain their complex, Christmas tree-like structure.

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The Effect of Various Feeds on Seed Production by Taiwanese Red Tilapia*

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Abstract

Three different feeds (tilapia pellets, 24% protein; eel feed, 44% protein and trash fish flesh, 22% protein) were fed to Taiwanese red tilapia broodstock (*Oreochromis mossambicus*/*O. niloticus* hybrids) to investigate nutritional effects on seed production. The broodstock used were 2-3 years of age and were stocked in duplicate concrete ponds (3.4 m^2) each with 10 females and 2 males. The critical weight ranges were 159-445 g for females and 295-448 g for males. Eggs, yolk sac fry and fry were collected every 8-9 days for 32 days.

Eel feed gave highest the total number of seed harvested (18,349) compared to tilapia pellets (9,137) and trash fish (9,707). These results are discussed in relation to feed composition, female weight and pond area. The conclusion is that feeding broodstock a high protein diet pays dividends in high seed production and broodstock growth. The results are compared with those of similar studies on *O. niloticus*.

Introduction

Red tilapias are cultured in several countries of the world including the Philippines, Taiwan, Israel and USA. They are gaining popularity because of their appealing color: much like the popular red sea bream. In Taiwan, red tilapias also have a fast growth rate and command higher market prices than other tilapias.

Most nutritional studies on tilapia have focused on growth from fingerlings to adults in ponds and cages (e.g., Shell

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1967; Guerrero 1980; Hepher 1982). Little is known, however, about the effects of various feeds and nutritional ingredients on the reproductive performance of broodstock. Factors affecting seed production in red tilapias are not understood. This study was conducted to determine the effects of different commercial feeds and trash fish on seed production by Taiwanese red tilapia, an *Oreochromis mossambicus/O. niloticus* hybrid (Liao and Chang 1983).

Materials and Methods

The broodstock used in this study were maintained at the Tungkang Marine Laboratory and were 2-3 years old. Ten females and two males were stocked in each of the six outdoor 3.4-m^2 concrete ponds with continuous aeration: about 3.5 fish/m^2. Three different feeds: tilapia pellets (obtained from the President Feed Company), eel feed (fish meal, mixed with water before feeding) and minced trash fish were used to study their effects on fry production. The composition of these feeds and the weights of broodstock are shown in Table 1. Feed analysis was performed using AOAC (Association of Official Analytical Chemists) methods. Each feeding treatment was duplicated. Water temperature, monitored daily, ranged from 27.3°C to 34°C. Broodstock were fed to satiation twice a day, totalling 1-2% body weight/day for tilapia pellets and eel feed and 3-5% body weight/day for trash fish. Every 8-9 days the pond water was drained to a level of about 10 cm in depth; the broodstock were caught gently with a fine mesh dip net, the females inspected and all the eggs and yolk sac larvae were washed out from the mouths of brooders and collected into a bowl. Eggs and larvae were incubated artificially in aerated plastic jars. The numbers from each female were recorded. Any fry present were also collected. The breeding period lasted for 32 days. All the broodstock were weighed at the end of the experiment.

Results and Discussion

Twenty-four pond harvests were made. Red tilapia given eel feed treatment produced 18 batches of viable total seed which included eggs, yolk sac fry and fry. The tilapias given tilapia pellet feed and trash fish treatments each produced 12 batches. The seed production from broodstock given the three feeds is shown in Table 2. The total seed production on eel feed treatment was 2.0 and 1.9 times higher than those on tilapia pellets and trash fish. Table 1 shows that eel feed contains about twice the crude protein content of tilapia pellets or trash fish. This indicates that high protein feed could be important for high seed production. Similar results were reported for *Oreochromis niloticus* when a diet containing 40% crude protein produced significantly higher number of fry compared to 20% crude protein and

Table 1. Proximate analysis (%) of three feeds given to Taiwanese red tilapia (*Oreochromis mossambicus/O. niloticus* hybrid) broodstock to study effects on seed production. The size ranges of broodstock are shown for each feeding treatment. For details of feeding rates, see text.

Component	Tilapia pellets[1]	Eel feed[2]	Trash fish[3]
Crude protein	24.0	44.0	21.7
Crude lipid	4.5	6.0	1.3
Crude fiber	5.0	1.2	-
Carbohydrate	45.5	19.8	0.3
Ash	12.0	16.0	1.2
Moisture	9.0	13.0	75.5

[1] Broodstock sizes (g): 159-445 (♀); 295-379 (♂)

[2] Broodstock sizes (g): 184-279 (♀); 337-448 (♂)

[3] Broodstock sizes (g): 193-363 (♀); 315-422 (♂)

controls (Santiago et al. 1985). There was considerable variation in seed production among individual ponds of each treatment at each harvest. This may be a result of differences in fecundity (Mires 1982) and spawning frequency of individual females (Lee 1979).

The numbers of the three categories of seed collected are shown in Table 2. The percentage composition for eggs, yolk sac fry and fry, respectively, were: tilapia pellets, 64.3, 25.0, 10.7; eel feed, 44.6, 20.8, 34.6 and trash fish, 68.8, 21.2, 10.0. For all three feeding treatments, eggs constituted the clear majority. Eggs probably suffer higher mortality than yolk sac fry and fry. Hence the relatively high percentage production of yolk sac fry and fry on eel feed is interesting and would probably lead to greater survival to fingerlings. Again, the higher protein content of the eel feed may be the key.

Table 3 shows individual seed production and relates this to body weight. The highest total seed production expressed in terms of seed/female/day was 29, obtained on eel feed. The figures are somewhat higher than most obtained for *O. niloticus* by Hughes and Behrends (1983). This may due to the different species used or the larger size of the broodfish used in our experiment.

The highest seed production expressed in seed/g female (average of initial and final weights)/day was 0.12 for eel feed treatment compared to 0.06 for the other two kinds of feed treatments. These results were similar to those obtained for *O. niloticus* by Hughes and Behrends (1983). The highest seed production expressed in seed/m²/day was again obtained on eel feed: 84.32, about twice that on tilapia pellets and trash fish. However, the number for the eel feed treatment was higher than those obtained for *O. niloticus* by Hughes and Behrends (1983). The difference between the two studies may be due to the different tilapia

Table 2. Total number of Taiwanese red tilapia (*Oreochromis mossambicus*/*O. niloticus*) hybrid seed produced under three feeding treatments.

Harvest	Treatment		
	Tilapia pellets	Eel feed	Trash fish
1	3,106	5,952	709
2	1,932	3,321	2,736
3	837	1,379	2,577
4	3,262	7,697	3,685
Total seed	9,137	18,349	9,707

Table 3. Total seed production (eggs, yolk sac fry and fry) from 159-445 g Taiwanese red tilapia (*Oreochromis mossambicus*/*O. niloticus* hybrid) broodstock at 5:1 (♀ : ♂) sex ratio in concrete ponds at 3.5 fish/m² for 32 days, expressed as seed/female/day; seed/g body weight female weight/day and seed/m² of pond area/day.

	Treatment		
	Tilapia pellets	Eel feed	Trash fish
Seed/female/day	14.28	28.67	15.17
Seed/g female/day[1]	0.06	0.12	0.06
Seed/m² pond/day	41.99	84.32	44.61

[1] Based on the average of initial and final weights of females.

Table 4. Means ± S.D.'s of initial and final body weights of Taiwanese red tilapia (*Oreochromis mossambicus*/*O. niloticus* hybrid) broodstock receiving three different feeds to compare the effects on seed production.

Feed	Mean body weights (± S.D.)			
	Female		Male	
	Initial	Final	Initial	Final
Tilapia pellets	242.3 ± 62.1	260.7 ± 78.1	336.0 ± 34.6	364.8 ± 90.6
Eel feed	231.3 ± 35.9	267.1 ± 53.1	398.0 ± 46.3	435.5 ± 68.3
Trash fish	236.4 ± 49.9	244.8 ± 26.1	364.0 ± 51.0	373.8 ± 32.7

species used in the study, the size of our broodstock (which were larger), the lower stocking density for our broodfish (3.5/m2) and differences in breeding facilities.

Eel feed not only gave higher seed production; it also gave a better broodstock growth as shown in Table 4. For both males and females, eel feed gave higher growth than the other two treatments. Trash fish in particular resulted in very little growth. These data are indicative only and are presented here without statistical significance tests in view of the variation in initial weights.

This study shows that feeding red tilapia broodstock with a high protein diet, such as eel feed, gives high seed production and good growth. This agrees with Santiago et al. (1985) for *O. niloticus*. It is probable that more females fed with eel feed were able to spawn at a higher frequency and the number of eggs spawned also increased as female body weight increased (Lowe-McConnell 1982).

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Comparison Between Natural Feeding Alone and Supplemental Feeding with Pellets Containing Locally Available Ingredients for Cage Culture of *Oreochromis niloticus* in Thale Noi, Thailand

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Abstract

Nile tilapia *(Oreochromis niloticus)* was chosen as a suitable fish for cage culture in Thale Noi, a slightly acidic lagoon in upper Songkhla Lake, Thailand. It was reared in 1-m^3 cages with 35 fish/cage from April to September 1984. There were twelve feeding treatments, varying the composition of feed pellets. The ingredients were dry and fresh aquatic weed *(Ceratophyllum demersum)*, concentrated pig feed, fish meal, cassava starch and chicken pellet mix, with three replications for each treatment. The fish were fed at 5% of body weight/day for 5 months. Survival for all treatments ranged from 90 to 99%. Mean initial weight of the fish ranged from 11.7 to 18.5 g. The best growth was obtained from the fish fed on chicken pellets alone: average percentage weight gain, 638%. Control fish with no feeding exhibited better growth than some fish fed on pellets containing *C. demersum*. Feed conversion ratios were not significantly different and ranged from 3.87 to 4.38. Rearing tilapia in 1-m^3 cages in Thale Noi is therefore possible without supplemental feeding at present.

Introduction

Thale Noi, the uppermost part of Songkhla Lake, is an acidic, freshwater lagoon of approximately 25 km^2. Aquatic weeds flourish there year-round with few species predominating. Some of them, such as *Hydrilla verticillata* and *Ceratophyllum demersum* are used by villagers as pig feed.

This study was undertaken to utilize *C. demersum* in Thale Noi as a feed for herbivorous fish. Nile tilapia *(Oreochromis niloticus)* was chosen because of its high tolerance to adverse environmental conditions, relatively fast growth and suitability for cage culture. Tilapias feed both as herbivores and detritivores; phytoplankton are a major feeding source for adult *O. niloticus* in natural habitats (Bowen 1982).

Materials and Methods

Oreochromis niloticus fingerlings were raised in 1-m^3 cages at 35 fish/cage from April to September 1984. The cages were made of 1-cm mesh galvanized steel wire (with epoxy paint coat) and a wooden frame. There were twelve feeding treatments (Table 1). The nutrient contents of each experimental feed were analyzed by standard methods (AOAC 1975) (Table 2). The fish were fed once a day at 5% of body weight/day adjusted monthly after weighing all fish in each cage. Water surrounding the cages was monitored at monthly intervals at 0900 hours for pH, dissolved oxygen, turbidity and temperature by a Horiba water checker (Horiba U-7, Horiba Ltd., Japan); alkalinity and phytoplankton biomass were analyzed by standard methods (APHA-AWWA-WPCF 1981).

Table 1. Ingredients of experimental feeds for cage culture of *Oreochromis niloticus* fingerlings, expressed as % dry weight.

Feed no.	Dry *Ceratophyllum demersum*	Fresh *Ceratophyllum demersum*	Concentrated pig feed*	Fish meal**	Cassava starch	Chicken pellet***
1 (no feed)	-	-	-	-	-	-
2	-	-	-	-	-	100
3	-	100	-	-	-	-
4	97.5	-	-	-	2.5	-
5	87.5	-	10	-	2.5	-
6	67.5	-	30	-	2.5	-
7	47.5	-	50	-	2.5	-
8	27.5		70	-	2.5	-
9	87.5	-	-	10	2.5	-
10	77.5	-	-	20	2.5	-
11	62.5	-	-	35	2.5	-
12	47.5	-	-	50	2.5	-

* = Hogdonal 252 (commercial pig feed)
** = Local fish meal
*** = P. Charoenpan 005 (commercial chicken pellet)

Tilapia growth and feed utilization data were anlyzed using Duncan's Multiple Range Test (Walpole and Myers 1978).

Results

Table 2 shows that for feeds containing dry *C. demersum*, crude fiber content declined with decreasing content of dry *C. demersum*, but crude protein and crude lipid content increased. Table 3 summarizes tilapia survival, growth, production and feed conversion results. There were no significant differences in survivals among fish fed on feed numbers 1 to 4 (no feed, chicken pellet, fresh aquatic weed and 97.5% dry aquatic weed mixture pellet, respectively), but these were significantly lower than those for fish fed on feed numbers 5 to 12. The fish fed on fresh *C.*

Table 2. Nutrient content of experimental feeds for cage culture of *Oreochromis niloticus* fingerlings. The data are expressed as % dry matter and are means of six replicate analyses. For details of feed composition, see Table 1.

Feed no.	Moisture	Crude protein	Crude lipid	Crude fiber	Ash
1 (No feed)	-	-	-	-	-
2 (Chicken pellet)	11.42	19.87	4.47	4.52	8.01
3 (Fresh *C. demersum*)	-	-	-	-	-
4	13.08	17.28	1.36	10.06	40.94
5	14.17	19.25	2.76	10.57	28.38
6	11.31	24.19	2.81	7.76	27.75
7	10.42	27.91	4.08	9.25	23.11
8	11.92	31.50	3.47	9.07	27.63
9	14.35	20.18	1.76	10.34	34.40
10	19.93	22.08	2.10	7.63	34.52
11	14.59	28.92	2.11	5.76	33.93
12	11.54	37.98	1.65	4.48	34.29
Dry *C. demersum*	15.72	16.23	1.48	8.25	19.66
Fish meal	9.17	52.68	0.63	1.65	35.26
Concentrated pig feed	10.22	37.76	5.00	5.18	18.69

demersum showed the lowest survival rate (90%) while the highest survival rates were obtained from the fish fed on feed numbers 6 and 11 (99%). There were no significant differences in % weight gain among fish with no feeding and others, except for the fish fed on feed numbers 2 and 8. The highest % weight gain was obtained from the fish fed on feed number 2 (chicken pellet, 639%) and the lowest from the fish fed on fresh *C. demersum* (295%). There were no significant differences in food conversion ratios (FCR) among all treatments. The lowest net production was obtained from fish fed on feed number 3 (fresh *C. demersum*), 2.18 kg/m^2.

The water quality parameters measured were as follows: the concentration of dissolved oxygen ranged from 3.78 to 7.88 ppm; turbidity ranged from 19.8 to 76.0 ppm suspended solids; water temperature ranged from 28.32 to 30.0°C; pH ranged from 5.90 to 8.18, water depth ranged from 58.0 to 88.0 cm, alkalinity ranged from 15.5 to 21.9 mg CaCO3/l and phytoplankton biomass ranged from 952 to 4,251 mg/m^3.

Discussion

The water in Thale Noi supports good tilapia growth on natural feeding alone. Aquino and Nielsen (1984) also found that *O. niloticus* grows well in cages on natural foods. However, if cage culture expands, natural feeding may become inadequate and therefore supplemental feeds such as those studied here may be required. If so, it should be remembered that the results here were obtained in the presence of natural feeds. They suggest that chicken pellets, despite a relatively low protein content (19.9%) could be a useful supplemental feed. The relative merits of the other feeds would have to be investigated by more nutritional assays (against a background of reduced natural feeding) and economic analyses of the costs of ingredients against the weight gains produced and tilapia sales values. The results suggest that dry *C. demersum*, in combination with other ingredients such as fish meal and pig feed is well worth further study as a feed component (see Table 3, feeds 8 and 11). Its chief merit is low cost as it is abundant in Thale Noi and easily collected. Economic aspects of its use as a fish feed in Thale Noi are discussed by Tantikitti et al. (this vol.).

The crude protein and crude lipid contents of dry *C. demersum* are lower than the requirements of tilapia during grow-out: 25-35% and 6-10%, respectively (Jauncey and Ross 1982). The common problems encountered with the utilization of aquatic macrophytes as fish feed are low levels of essential nutrients, high level of crude fiber and poor digestibility and

Table 3. Growth, survival, net production and feed conversion of *Oreochromis niloticus* fingerlings receiving different feeds in 1 m^3 cages in Songkhla Lake, Thailand, for 5 months, April-September 1984. The feeding rate was 5% of body weight/day and stocking density 35 fish/cage. The data are means and standard errors from 3 replicate cages/feed treatment. Mean values with a common superscript are not significantly different ($P < 0.05$). For details of feed composition, see Tables 1 and 2.

Feed no.	Initial weight (g/fish)	Final weight (g/fish)	Weight gain (%)	Survival (%)	Net production (kg/m^2)	Food conversion ratio (g feed/g fish)
1	12.71 ± 0.96	72.29 ± 2.11	405 ± 49^{BCD}	93 ± 3^{N}	2.28 ± 0.12^{P}	
2	13.53 ± 1.65	108.72 ± 2.60	639 ± 95^{A}	93 ± 3^{N}	3.43 ± 0.14^{U}	3.93^{E}
3	16.67 ± 1.83	71.20 ± 2.38	295 ± 40^{D}	90 ± 4^{N}	2.18 ± 0.11^{P}	4.35^{L}
4	16.09 ± 2.12	75.89 ± 5.93	320 ± 27^{CD}	94 ± 3^{N}	2.42 ± 0.16^{PQ}	4.16^{E}
5	14.90 ± 0.23	80.45 ± 2.90	391 ± 18^{BCD}	97 ± 6^{M}	2.53 ± 0.15^{QR}	4.07^{E}
6	14.27 ± 0.62	81.57 ± 3.54	419 ± 22^{BCD}	99 ± 1^{M}	2.79 ± 0.15^{ST}	3.99^{E}
7	15.78 ± 1.02	84.00 ± 6.08	402 ± 23^{BCD}	98 ± 2^{M}	2.80 ± 0.16^{ST}	4.38^{E}
8	15.49 ± 0.98	98.71 ± 3.82	506 ± 61^{AB}	97 ± 3^{M}	3.30 ± 0.15^{U}	4.13^{E}
9	11.72 ± 0.31	69.42 ± 4.82	429 ± 28^{BC}	96 ± 3^{M}	2.28 ± 0.21^{P}	4.02^{E}
10	14.20 ± 0.83	77.20 ± 1.55	349 ± 44^{CD}	97 ± 2^{M}	2.54 ± 0.16^{QR}	3.88^{E}
11	18.54 ± 1.19	87.75 ± 1.02	309 ± 14^{CD}	99 ± 1^{M}	3.01 ± 0.07^{T}	3.87^{E}
12	15.47 ± 0.81	82.69 ± 5.30	371 ± 46^{CD}	96 ± 1^{M}	2.72 ± 0.14^{RS}	4.05^{E}

feed conversion (Rifai 1979; Chiayvareesajja 1984).

There are various strategies that can be implemented in tilapia cage culture in Thale Noi to yield better production, such as formulation of better feed, monosex male tilapia culture, and adjusting feeding rate and feeding frequency (Guerrero 1980; Coche 1982).

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Effects of Varying Protein Levels on Spawning Frequency and Growth of *Sarotherodon melanotheron*

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Abstract

Sarotherodon melanotheron breeders were fed with dry pellets containing 20 to 50% total protein. Their frequency of spawning and their growth in length and weight were determined over a 16-week period.

There were no significant differences ($P > 0.05$) among mean spawning frequencies and mean numbers of eggs per spawning with different diets. Although better growth was obtained with higher percentage of dietary protein, there was no significant correlation between a breeder's weight and the number of eggs spawned each time.

There was an increase in mean weight gain of the males as the dietary protein increased up to 50%. The mean weight gain of fish fed with 50% total protein was 77.29% greater than those fed with 20% total protein.

Introduction

The potential of *Sarotherodon melanotheron* for aquaculture in brackishwater has been mentioned (Pauly 1976; Legendre 1986). However, despite its early maturation and multiple spawning, it has relatively low fecundity which limits mass production of fry. To enhance fry production of various tilapias, hormone treatments (Dadzie 1970a), manipulation of temperature and photoperiod to induce spawning (Cridland 1961, 1962), and removal of eggs from the brooding parent (Dadzie 1970b, Lee 1979) have been attempted.

The supply of high quality feed to satisfy the nutrient requirements of breeders is believed to be essential for successive spawning (Santiago et al. 1983). The aim of the present study was to determine the effects of varying dietary total protein levels (20-50%) on the spawning frequency and growth of *S. melanotheron* breeders.

Materials and Methods

The study was carried out at the Layo aquaculture experimental station of the Centre de Recherches Océanographiques which is 40 km West of Abidjan, Côte d'Ivoire, in an oligohaline area along the Ebrié Lagoon. The area is strongly influenced by the proximity of the mouth of Agneby river. The salinity ranges from 0 to 5 ppt (Durand and Skubich 1982, Albaret and Legendre 1983).

Six isocaloric practical diets with varying protein levels were prepared by thoroughly mixing the dry ingredients with gelatinized starch (40 g in 500 ml water) (Table 1). The mixture was then extruded as moist 3 mm pellets using a mincer (Alexanderverk AGM-8876). The moist pellets were dried in an oven at 50°C until the moisture content was 10% or less. Sample of the diets were subjected to proximate analysis; the results are presented in Table 2. Diets were stored at 20°C until required (maximum, 2 weeks).

Six-month-old *S. melanotheron*, previously kept in pens, termed 'enclos' (cage enclosures), were used. Males and females were separated and held in concrete tanks (2 x 2 x 1 m) for 14 days prior to the experiment, fed daily to satiation with a pelleted diet. The teeth of the outermost row of the upper and lower jaws of the males were removed to prevent the males from injuring the females during courtship.

Initial lengths and weights ranged from 18.9 to 20.5 cm and 145 to 160 g for females and from 23.0 to 23.7 cm and 229 to 242 g for males. One female and one male were stocked in 18 wooden aquaria (120 x 38 x 40 cm) each containing 175 l of

Table 1. Composition of experimental diets.

Protein level	20%	25%	30%	35%	40%	50%
Ingredients (%)						
Fish meal (tuna waste)	21.85	27.50	32.60	39.10	43.80	53.56
Cotton seed cake (without gossypol)	11.78	14.65	16.27	18.54	22.52	26.26
Fish oil	5.00	4.49	3.16	2.99	2.10	1.82
Copra cake	26.77	21.24	18.57	13.88	8.09	3.85
Wheat bran	25 60	23.12	20.40	16.49	14.49	5.51
Starch	3	3	3	3	3	3
Mineral premix[1]	4	4	4	4	4	4
Vitamin premix[2]	2	2	2	2	2	2
Calculated						
Lysine	1.81	2.13	2.38	2.67	2.99	3.48
Methionine/Cystine	1.02	1.12	1.22	1.33	1.43	1.60
Estimated						
Digestible energy[3] (Kcal/100)	270	270	270	270	270	270

[1] Mineral premix contains (mg/kg of premix): cobalt, 25 mg; iron, 22.000 mg; iodine, 2.500 mg; manganese, 13.500 mg; copper, 1.500 mg; zinc, 75.000 mg; selenium, 15 mg.

[2] Vitamin premix contains (mg/kg of premix): Vit. A, 2.000.000 UI, Vit. D3, 1.000.000 UI; Vit. E, 25.000 mg; Vit. B1, 5.000 mg; Vit. B2, 6.000 mg; Vit. B6, 5.000 mg, Vit. PP, 40.000 mg; Vit. B12, 4 mg; pantothenic acid, 16.000 mg; Vit. K, 5.000 mg; folic acid, 1.000 mg; choline, 250.000 mg.

[3] Adapted from values for *Oreochromis* : 5,5 Kcal/g protein, 9.1 Kcal.g fat; 4.1 Kcal/g carbohydrates (Jauncey and Ross 1982).

Table 2. Laboratory analysis of experimental diets on a moisture free basis.*

Component	Diet 20%	25%	30%	35%	40%	50%
Protein (N x 6.25) (%)	20.37	25.72	30.43	35.81	41.99	52.34
Lipids (%)	6.80	7.40	12.00	10.10	7.50	13.40
Ash (%)	10.81	11.88	12.12	13.31	13.52	15.15
Cellulose (%)	8.11	7.02	6.32	6.50	4.83	4.33
Non nitrogen extr. (%)	53.91	47.98	39.13	34.28	32.16	14.78
Calcium (%)	1.93	1.71	1.58	1.98	1.49	1.67
Phosphorus (%)	1.55	1.35	1.46	1.69	1.83	1.91

*Done by the Animal Nutrition laboratory, The Agricultural School, Abidjan, Côte d'Ivoire.

tap water and having constant aeration and a recycling system. The aquaria had a glass front for observation of the fish. Aquaria were cleaned everyday by siphoning out feces and/or excess feed. About one half of the water was replaced daily. Average water temperature was 28°C. The experiment lasted for 16 weeks (July 15 to November 16, 1986). Statistical analysis was carried out by the Kruskal-Wallis test.

Six levels of protein with three replicates each were randomly assigned to the aquaria. A fixed feeding regime was adopted: 3% of the body weight per day, divided into two equal feeds at 8:00 AM and 4:00 PM for all fish.

The fish were observed prior to each feeding to determine which female had spawned. The success of spawning was recorded when eggs were seen in the mouth of the male. The eggs were removed from the male's buccal cavity one or two days after spawning (Lee 1979, Santiago et al. 1983), and were counted. Total length and body weight of male and female were measured at two weeks intervals or after removal of eggs for brooding males.

Results and Discussion

Frequency of spawning

Spawning behavior of the breeders in aquaria was similar to that previously described by Lee (1979), Rothbard (1979) and Santiago et al. (1983) for *Oreochromis niloticus*.

All female fish spawned at least once (Table 3). The highest mean spawning

Table 3. Effects of varying dietary protein levels on the spawning frequency of female *Sarotherodon melanotheron* over 16 weeks.

Treatment	Spawning frequency Total	Range	Mean[1]	Number of eggs per spawning Range	Mean[1]
20%	6	1-4	2 (2.1)[2]	239-569	417 (20.9)[2]
25%	9	2-5	3 (2.1)	360-586	337 (40.5)
30%	14	5-8	6 (2.1)	220-678	575 (33.6)
35%	11	3-4	3 (0.7)	304-540	508 (5.6)
40%	7	0-3	3 (2.1)	299-525	477 (4.2)
50%	8	2-6	4 (2.8)	153-508	361 (11.3)

[1]Means are not significantly different (P > 0.05).
[2]Standard deviation.

frequency of the treatment was obtained from females fed with 30% protein, but spawning frequencies in all treatments were not significantly different ($P > 0.05$). The mean number of eggs produced per spawning ranged from 337 to 575. Treatment means were not significantly different. However, small numbers of eggs (20-70) were found in the pseudostomach of some males. The daily cleaning of aquaria probably disturbed some brooders causing them to swallow their eggs. Therefore the recorded totals may be underestimates.

Growth

Weight gains and increases in total length of the females over a 16-week period are shown in Table 4. For females that had just spawned before sampling or were about to spawn, the total weight increment was calculated by adding the body weight gain of a female and the total weight of eggs spawned by the female, as recommended by Lee (1979).

There was an increasing mean total growth of all females as the dietary protein increased up to 50%. Total growth of females fed with 20% protein was 60.18 g which was significantly different from all other treatments. Growth appeared to with increasing dietary protein level, but no significant correlation was found between the weight of a spawner plus spawned eggs and the clutch size or number of eggs per spawning. Lee (1979) had similar findings for one-year-old breeders of *O. niloticus* and *O. aureus* in aquaria but he observed a tendency towards increasing clutch size through successive spawnings of individual females. Fryer and Iles (1972) reported, however, that based on limited data, bigger females of *O. niloticus* produce larger numbers of eggs per clutch. In the present study, not enough females were used to investigate these relationships.

There was a similar apparent increase in mean weight gain of the males as the dietary protein increased up to 50% (Table 5) but again the means were not significantly different apart from those of the 20% and 50% protein levels.

It appears that for *Sarotherodon melanotheron* fed to satiation with high quality protein diets, that increasing dietary protein has no significant effect on spawning frequency (at least when eggs

Table 4. Mean weight gains and increases in total length of female *Sarotherodon melanotheron* fed with different protein levels over 16 weeks.

	Percentage dietary protein					
	20%	25%	30%	35%	40%	50%
Mean increase in length (cm)[1]	2.20 (0.23)[2]	3.70 (0.29)	4.98 (0.52)	4.02 (0.33)	2.90 (0.27)	2.50 (0.24)
Mean weight gain (g)	56.90 (13.64)	63.80 (13.09)	69.98 (10.65)	70.15 (14.11)	77.04 (11.75)	82.60 (14.43)
Mean weight of eggs collected (g)	3.28	2.60	4.71	4.50	3.81	2.75
Mean total growth (g)[1]	60.18*	66.40	74.69	74.65	80.85	85.35

[1] Means are not significantly different except for the asterisked value which is significantly different from all other mean total growth values ($P > 0.05$).

[2] Standard deviation.

Table 5. Mean weight gain and increases in total length of male *Sarotherodon melanotheron* fed with different protein levels over 16 weeks.

	Percentage dietary protein					
	20%	25%	30%	35%	40%	50%
Mean increase in length (cm)[1]	2.70 (0.27)[2]	2.83 (0.28)	3.26 (0.22)	3.40 (0.45)	3.11 (0.24)	4.05 (0.32)
Mean weight gain (g)[1]	39.75 (8.5)	42.09 (8.7)	58.80 (6.4)	60.30 (15.1)	56.50 (9.7)	62.80 (3.1)

[1] Means are not significantly different ($P > 0.05$).
[2] Standard deviation.

are removed from the brooder) and that the growth of breeders is broadly similar within the 20%-50% dietary protein range. These results have important economic implications, because in the absence of previous information on the nutrient requirements of *S. melanotheron* breeders, there is a tendency to offer expensive (over 30% protein) diets.

Acknowledgement

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An Evaluation of Fixed and Demand Feeding Regimes for Cage Culture of *Oreochromis aureus*

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Abstract

Growth, feed conversion, marketable fish production, and labor required for feeding were compared for fixed and demand feeding regimes for cage culture of blue tilapia *(Oreochromis aureus)*. Fish growth was not different ($P > 0.1$). Observed mean feed conversion ratios of fish obtaining feed from demand feeders were lower than for fish fed according to a fixed regime. Differences in marketable production of fish fed according to the two regimes were obscured by differential mortality and interference by tilapia recruits to cages. Labor for demand feeding was 6% to 12% of the requirement for fixed feeding.

Introduction

The digestive physiology of tilapias indicates that multiple feedings might result in good growth and efficient utilization of feed. The efficient digestion and assimilation of total amino acids in detritus requires a relatively long intestine with respect to body length (Bowen 1982). Tilapias have a relatively rapid food passage rate of 2.5 to 3.0 hours at 30°C (Popma 1982).

Tilapias feed continuously throughout the daylight hours, reaching a peak of gastric acid secretion around midday (Caulton 1982). Increasing feeding frequency may allow tilapia digestion to proceed more continuously than less frequent, discrete feedings and thereby improve the efficiency of digestion and assimilation (Balarin and Hatton 1979). However, Kubaryk (1980) found that greater feed intake, not greater feed efficiency, was responsible for increased

weight and protein gains in tilapias. Feeding fish to satiation may not make the most efficient use of feed, even though maximum growth may be achieved (Shell 1966). Kubaryk (1980) also found that energy gain was lower and protein gain was higher for fish fed their ration on a restricted consumption regime up to eight times a day.

Feeding-rate tables based on fish body weight are, at best, approximations that do not consider the interactive complex of water quality and other variables which affect fish feeding response, digestion and assimilation. Demand feeders eliminate the need for a feeding rate table, for feeding rate adjustments, and the labor required for regular fish sampling.

The objectives of this study were to compare growth, feed conversion ratios, and marketable production of *O. aureus* fed according to fixed or demand feeding regimes. Labor for feeding fish according to the two regimes was also compared.

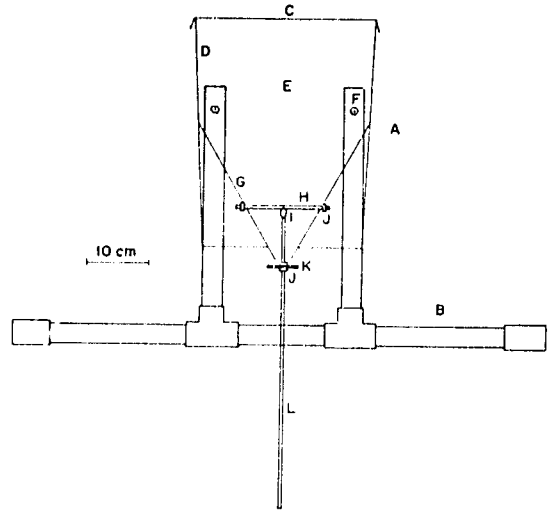

Fig. 1. Cross-sectional view of demand feeder for cage culture of tilapia with side view of support structure superimposed. Key: A - demand feeder; B - PVC support structure; C - cover; D - plastic bucket; E - feed chamber; F - brass nut and bolt; G - plastic funnel; H - horizontal brass rod; I - key ring swivel; J - brass nuts; K - plexiglass plate; L - vertical brass rod.

Materials and Methods

The 1-m^3 cylindrical cages utilized in this study were constructed of semi-rigid, 19-mm mesh, plastic screen tied onto steel hoops. The cages were 1.22 m high x 1.06 m in diameter and floated with approximately 10 cm of freeboard. A feeding ring was suspended from the cage top to a depth of 51 cm.

The demand feeder evaluated in this study was constructed with an 18.9-l plastic bucket into which a polyethylene funnel was inserted (Fig. 1). The feeder was mounted on the cage top and could be activated by a brass rod suspended from the funnel into the water.

Two 0.1-ha and one 1-ha ponds were selected for this study to assess production under the range of pond types found in the Virgin Islands. All three ponds contained populations of *O. aureus* and *O. mossambicus*. There were two experiments.

For the first experiment, four cages were placed in each of the three ponds and stocked at a rate of 400/m^3 mixed-sex *O. aureus* fingerlings. Fish in two cages in each pond were given their daily ration according to a fixed schedule (FF), split into two feedings at 0930 and 1530 hours. Fish in the other two cages in each pond obtained feed from demand feeders (DF) which were checked twice daily during fixed feedings and filled to capacity with 6 kg of feed only when completely empty.

FF fish were fed on a sliding scale from 3% to 1.5% body weight daily (bwd). Monthly feed adjustments for FF fish were based on a sample of fish from each cage. Mid-monthly adjustments were calculated on the basis of the feed conversion ratio from the previous month. Fish were fed a complete, pelleted, floating ration which contained 36% crude protein and vitamin and mineral supplements.

Runoff from heavy rains during the fourth week of the experiment greatly increased the water volume of the two smaller ponds. Subsequent oxygen depletion resulted in high mortality of the caged fish in these ponds. Feeding and

data collection of fish in these ponds was terminated.

After 145 feeding days, caged fish in the 1-ha pond were sorted by size group, weighed and counted. Marketable fish were considered to be those ≥19 cm in length (188 g).

For the second experiment, eighteen 1-m^3 cages were placed in the large pond and were stocked with 200, 300 or 400 sex-reversed male *O. aureus* fingerlings. Each stocking rate was replicated six times. Six 1-m^3 cages were placed in one of the smaller ponds and were each stocked with 300 sex-reversed male *O. aureus* fingerlings.

At each stocking rate and in each pond, fish in three cages were given their daily ration according to a fixed schedule, divided into two feedings; fish in the other three cages obtained feed from demand feeders, which were checked twice each day during fixed feedings and filled to capacity only when completely empty.

FF fish were fed daily according to a feeding rate table. Monthly feed adjustments for FF fish were based on a sample of fish from each cage. Mid-monthly adjustments were calculated on the basis of the feed conversion ratio from the previous month. After two episodes of fish mortality following tropical storms, feeding rates were adjusted based on the number of fish stocked minus the number removed from each cage.

After 197 feeding days, the fish were sorted by size group, weighed and counted.

Results and Discussion

The growth of fish fed according to the two regimes, cultured in the large or the small ponds in the two experiments was not significantly different ($P > 0.1$) (Figs. 2, 3 and 4). Fish growth in all experiments and ponds was nearly linear. In experiment I, DF fish had a higher observed final mean weight (332 g) than FF fish (311 g). FF fish cultured in the large and small pond during experiment II had a higher final mean weight (373 g and

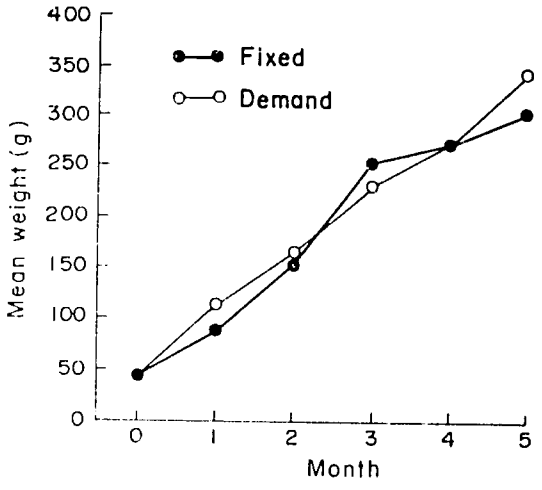

Fig. 2. Monthly mean weight of caged *O. aureus* cultured in a 1-ha pond, experiment I.

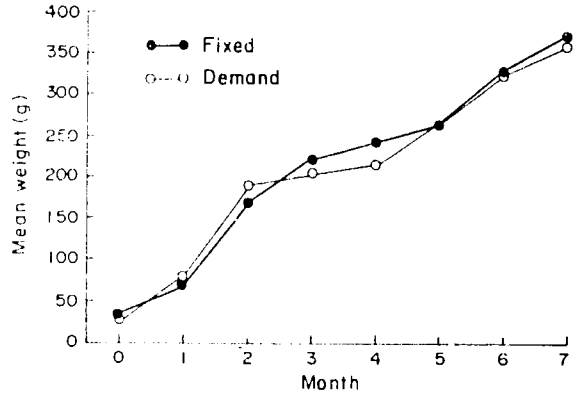

Fig. 3. Monthly mean weight of caged *O. aureus* cultured in a 1-ha pond, experiment II.

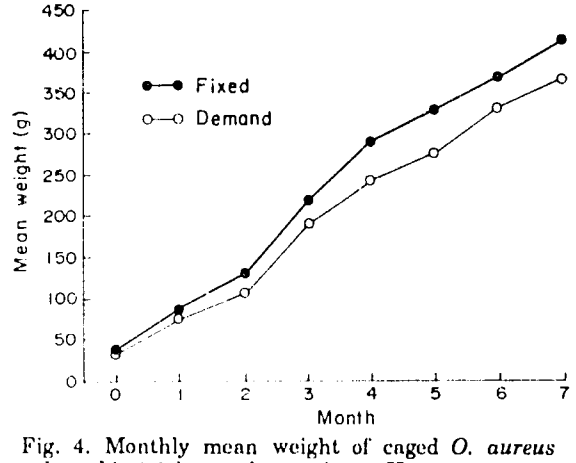

Fig. 4. Monthly mean weight of caged *O. aureus* cultured in 0.1-ha pond, experiment II.

414 g) than DF fish (360 g and 365 g), respectively.

Feed conversion ratios of DF fish (1.55) were lower ($P < 0.1$) than of FF fish (2.30) for experiment I. Feed conversion ratios of DF and FF fish cultured in both ponds during experiment II were not significantly different. In experiment II, observed feed conversion ratios of DF fish (2.17, 2.05) were lower than of FF fish (2.48 and 2.23) cultured in the large and small ponds, respectively.

Fish production in cages was affected by feeding regime, stocking rate, number of marketable fish from the original cage stock, cage recruitment from tilapia populations in ponds, and differential mortality and possible fish escape following runoff events.

Marketable production of DF fish (145.6 kg/m^3) cultured in the 1-ha pond during experiment I was higher ($P < 0.1$) than of FF fish (123.6 kg/m^3). Marketable production was 95% (by weight) of total production from DF cages and 91% from FF cages.

Marketable production of FF fish (80.1 kg/m^3) cultured in the 1-ha pond during experiment II was higher ($P < 0.05$) than of DF fish (59.1 kg/m^3). Marketable production of FF fish (100.7 kg/m^3) cultured in the 0.1-ha pond during experiment II was higher than of DF fish (67.4 kg/m^3).

Interference from open pond fish populations can be a serious limitation of cage culture. Tilapia < 9 g were able to pass through 19-mm mesh. These cage recruits competed for feed and space with the stocked population and were unable to escape after a period of growth. Most of these fish did not reach marketable size during the culture period and therefore represented a reduction in feed utilization efficiency. Cage recruitment accounted for a relatively small proportion (3.8-11.0%) of caged fish production by weight, but could account for more than 1/3 of the mean number of fish harvested. The open-pond tilapia population was composed of previously introduced stocks of *O. mossambicus* and *O. aureus*.

Control of cage recruitment of tilapia by stocking a piscivorous fish species into the open pond or by chemical eradication may be necessary. Use of a smaller mesh size (13 mm) would restrict fish entry into cages, but would also limit water circulation which could affect production.

Demand feeders reduced the labor required for feeding by 88% in experiment I and by 94% in both ponds in experiment II. Meriwether (1986) estimated that demand feeders reduced the labor required for feeding caged *O. aureus* cultured in an Arkansas farm pond by 49%. Demand feeders were refilled on average every 4.3 days in experiment I and 7 days in experiment II. Despite significant savings in labor for feeding, regular observations of feeding response and fish health should be maintained.

In summary, the results indicated that differences in growth, feed conversion, and marketable production of fish fed according to fixed or demand feeding regimes were slight. The use of demand feeders significantly reduced the quantity of labor required for feeding fish. The importance of this factor should be evaluated in terms of the opportunity costs for labor in a commercial culture operation and the local employment situation.

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Phosphorus Nutrition of Juvenile *Oreochromis niloticus*

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Abstract

Using purified diets, the effects of varying dietary phosphorus and dietary calcium on growth and bone mineralization of juvenile (0.4-1.4 g) *Oreochromis niloticus* were investigated. The dietary phosphorus requirement for normal growth and bone mineralization was found to be 0.46% available phosphorus. The apparent availability of phosphorus from casein was demonstrated to be about 70%. The implications for waste production are discussed.

Introduction

Phosphorus is required by all fish for normal growth and bone development, maintenance of acid-base regulation and lipid and carbohydrate metabolism (Ogino and Takeda 1976; Lovell 1978; Cowey and Sargent 1979; Lall 1979). Diets deficient in this element can suppress appetite, normal food conversion and growth and, under extreme circumstances, affect bone formation and lead to death (Lall 1979).

Previous studies have indicated considerable differences in the phosphorus requirements of tilapia *(Cichlidae)* species. Using a source of white fishmeal, supplemented by sodium phosphate, Watanabe et al. (1980) showed that juvenile (6.32 g) *Oreochromis niloticus* required around 0.9% phosphorus in their diet, whilst more recent work by Viola et al. (1986) estimated that requirements for 120-400 g *O. aureus* x *O. niloticus* hybrids were highly variable (0.7-1.0%).

These variations between results are partially attributable to differences between species and fish size but, more importantly, since purified diets were not used in either study, were also a result of the methods employed.

The aim of the present study was to determine with greater precision the requirements of juvenile *O. niloticus* for phosphorus by using purified diets incorporating a wide range of dietary phosphorus levels. The effect of dietary calcium on phosphorus requirements was also investigated.

Materials and Methods

Six-hundred-and-forty juvenile *O. niloticus* (mean weight = 0.4 g) were distributed evenly and at random among thirty-two, 8-l tanks in a warm-water recirculation system. The temperature was maintained at 27°C ± 1°C and oxygen saturation was maintained at or close to 100%.

Sixteen complete diets were formulated from purified ingredients to be isocalorific and isonitrogenous, and the level of phosphorus adjusted to range incrementally from 0.4% to 1.1% of the diet (Table 1). For each level of phosphorus, diets were formulated to contain 0.5% or 1% calcium. Duplicates of the dietary treatments were randomly assigned to the experimental tanks. The trial was conducted over a 40-day period and the fish fed at a rate of 6% body weight per day (dry weight feed/wet weight fish), divided into four equal feeds. The fish were weighed in bulk weekly and the feeding ration was adjusted accordingly. Feeding was carried out on 6 days each week. At the beginning and end of the experiment fish were anesthetized and weighed individually.

In order to calculate absorption efficiency and estimate nonfecal, post absorptive losses due to excretion, whilst maintaining the experimental feeding regime, fish were transferred to static, 8-l tanks one hour after the last feed. Water was aerated and maintained at 27°C. Feces were collected over a 14-hour period overnight in a settling chamber separated from the main base of each tank by 3-mm mesh. The following morning the fish were returned to the experimental recycle system, the feces removed from the settling chamber, dried at 80°C until a constant weight was achieved, and finely ground. Feces collected over five consecutive days were pooled and the apparent absorption of dietary phosphorus determined by the indirect method of Furukawa and Tsukahara (1966) which compares the ratio of chromic oxide indicator to phosphorus in each diet with the resultant feces.

In order to estimate the phosphorus leached from the feces prior to collection and analysis, several experiments were carried out. The duration of fecal leaching was estimated by transferring individual fishes from eight of the diet groups (A1, A3, A5, A8 B1, B3, B5, B8) immediately

Table 1. Composition and proximate analysis of experimental diets. All values are expressed as percentages, except*, which is in Kcal g^{-1}. Proximate analyses were carried out according to methods detailed in ADCP (1980).

Ingredients and composition	Diet A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4	B5	B6	B7	B8
Casein	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Starch	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00
Dextrin	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00
Cornoil	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.0	10.00	10.00	10.00	10.00	10.00	10.00
Vitamin premix[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.0	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[2]	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
$CaSO_4 \cdot 5H_2O$	2.70	2.70	2.70	2.70	2.70	2.70	2.70	2.70	5.40	5.40	5.40	5.40	5.40	5.40	5.40	5.40
Cr_2O_3	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cellulose	10.24	9.78	9.32	8.86	8.40	7.94	7.48	7.12	7.54	7.08	6.62	6.16	5.70	5.24	4.78	4.32
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Crude protein	44.40	42.67	44.81	44.73	44.46	45.29	44.64	42.72	42.34	43.69	42.94	43.22	43.52	42.25	43.88	43.24
Crude lipid	10.13	9.94	9.97	9.82	10.47	9.76	10.04	10.47	10.00	10.04	10.05	10.20	10.16	9.79	9.93	9.89
Ash	7.49	8.18	8.88	9.57	10.27	10.96	11.66	12.35	9.81	10.80	11.91	11.90	12.61	16.33	19.36	13.43
NFE[3]	24.61	25.26	24.23	25.70	20.61	22.80	24.69	22.01	27.85	25.22	24.36	24.66	23.45	18.58	11.43	24.68
Gross energy*	436.60	428.10	430.70	434.80	421.60	430.80	437.70	429.50	437.80	434.71	427.24	431.40	488.80	397.30	378.20	428.70
Phosphorus	0.26	0.28	0.45	0.54	0.67	0.82	0.92	1.04	0.26	0.35	0.46	0.54	0.68	0.81	0.87	1.01

Notes 1 Tacon et al. 1983

2 $MgSO_4 \cdot 7H_2O$ (46.80%); $NaCl$ (22.01%); KCl (18.10%); $FeSO_4 \cdot 7H_2O$ (9.21%); $ZnSO_4 \cdot 7H_2O$ (2.05%); $CuSO_4 \cdot 5H_2O$ (0.30%); $MnSO_4 \cdot H_2O$ (0.90%); $CaIO_3 \cdot 6H_2O$ (0.10%); $CoCl_2 \cdot 6H_2O$ (0.05%); $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.17%)

3 Nitrogen Free Extractives

after feeding to small, perspex tanks and recording the presence/absence of feces every half hour over a 6-hour period. On average, 3.5 hours elapsed between the cessation of feeding and defecation. The leaching rate was calculated by collecting feces from fish from the same eight dietary groups, immediately after egestion, and by introducing 10 mg wet weight into replicate 500-ml vessels held in a water bath at 27°C. Each vessel was aerated and water samples were removed after 10 minutes, 30 minutes, 1 hour and 10.5 hours and analyzed for total dissolved phosphorus.

At the end of the experiment, fish were sacrificed and pooled samples of vertebrae from each treatment were analyzed for phosphorus, calcium and bone ash. Vertebrae were separated from adhering muscle tissue after being subjected to pulsed microwaves for 30 seconds.

All phosphorus analyses were for total phosphorus, following the method detailed (ADCP 1980). Calcium levels were determined by atomic absorption flame photometer (ADCP 1980).

Data on growth and mineral composition were subjected to analyses of variance and Duncan's multiple range test (Duncan 1955) to determine the significance of differences between trials.

Results

Growth data are summarized in Table 2. In all treatments fish increased in weight throughout the experimental period and the highest observed weight gains and specific growth rates (SGRs) corresponded to the diets with highest phosphorus levels.

Using one-way ANOVA, significantly different weight gains ($F = 16.5$; d.f. = 7; $P < 0.01$) and significantly different SGRs ($F = 21.5$; d.f. = 7; $P < 0.01$) were recorded among groups of fish fed the higher calcium diets (i.e., treatment B). However, there were no significant increases in weight gain or SGR between groups of fish receiving diets containing more than 0.46% available phosphorus, with the exception of diet B5 where the fish grew more slowly than in any other group. No significant differences were observed in SGR ($F = 2.0$; d.f. = 7; $P > 0.05$) amongst groups fed on the lower calcium diets (i.e., treatment A). Food conversion ratios (FCRs) appeared to decrease with increasing dietary phosphorus levels in both treatments, although these changes were not significant ($F < 2.1$; d.f. = 7; $P > 0.05$). Using paired sample t-tests of the low and high calcium diets, there were similar results for SGR ($t = 0.08$; d.f. = 15; $P > 0.05$) and FCR ($t = 0.03$; d.f. = 7; $P > 0.05$).

The results of bone mineral analyses are summarized in Table 2. There was no significant difference ($t = 0.1$; d.f. = 15; $P > 0.05$) in the proportion of bone calcium observed in fish fed different dietary calcium levels. However, significantly different levels of bone calcium were observed among groups fed different dietary phosphorus levels ($F = 8.25$; d.f. =

Table 2. Summary of growth data, mineral composition of vertebrae, and apparent absorption efficiency and availability of phosphorus.

	Diet																
	A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4	B5	B6	B7	B8	Control[1]
Initial wt (g)	0.42	0.46	0.37	0.46	0.42	0.42	0.42	0.40	0.41	0.49	0.50	0.46	0.40	0.47	0.46	0.41	
Final wt (g)	1.07	1.19	1.07	1.37	1.23	1.24	1.26	1.25	1.07	1.35	1.31	1.50	0.98	1.48	1.38	1.36	
SGR (% day^{-1})[2,3]	2.39[a]	2.45[a]	2.55[a]	2.72[d]	2.29[a]	2.57[a]	2.75[a]	2.66[a]	2.41[b]	2.51[b]	2.43[b]	2.98[c]	1.70[a]	2.85[c]	2.74[bc]	2.83[bc]	
FCR[3,4]	1.85[a]	1.92[a]	1.69[a]	1.68[a]	1.89[a]	1.57[a]	1.68[a]	1.61[a]	1.98[a]	1.78[a]	1.87[a]	1.33[a]	2.33[a]	1.58[a]	1.61[a]	1.54[a]	
Bone calcium (g 100 g^{-1})[3]	5.28[a]	8.81[ab]	9.42[b]	8.30[a]	9.50[bc]	8.57[b]	7.08[ab]	9.44[b]	6.86[a]	4.91[c]	7.28[a]	11.25[d]	9.73[c]	6.99[a]	8.43[b]	10.17[c]	10.11[c]
Bone phosphorus (g 100 g^{-1})[3]	4.92[a]	6.75[ab]	8.53[b]	6.69[b]	7.49[b]	8.41[b]	9.80[bc]	9.44[b]	5.52[a]	7.48[ab]	7.88[ab]	8.69[b]	9.06[bc]	9.05[bc]	8.87[bc]	9.42[bc]	9.48[bc]
Bone ash (g 100 g^{-1})[3]	25.27[a]	27.43[b]	34.41[c]	28.76	20.83[a]	27.86[bc]	38.91[e]	32.29[d]	23.77[a]	38.97[e]	24.36[a]	35.30[d]	35.54[d]	30.97[c]	30.45[c]	24.72[b]	44.07
Phosphorus leached from feces (g g mg^{-1})	1.29		1.34		1.71			1.81	1.36		1.58		1.71			1.69	
Apparent absorption (%)	68.1	78.1	90.9	83.3	90.1	94.3	93.8	93.5	73.8	79.9	79.3	85.6	90.0	95.8	94.2	97.6	
Available phosphorus (%)	0.11	0.22	0.41	0.14	0.60	0.77	0.91	0.94	0.19	0.28	0.36	0.46	0.62	0.77	0.82	0.99	

Notes: 1 Control data refers to determination of bone composition made prior to experiment
2 Specific Growth Rate (SGR) = (log$_e$ final weight − log$_e$ initial weight) 40 x 100
3 Mean values in the same row not sharing a common superscript letter are significantly different ($P < 0.05$)
4 Food Conversion Ratio = weight of feed offered/live weight gain

15; $P < 0.01$), although the relationship between the two variables was not significant ($t = 0.32$; $P > 0.1$). The amount of phosphorus in the vertebrae increased with increasing levels of phosphorus in the diet and at the higher calcium inclusion level (treatment B) there were significant differences between those groups fed less than 0.46% available phosphorus and those given more than this (see Table 2). Paired comparisons between the high and low calcium inclusion levels showed that calcium levels had no significant effect upon the phosphorus content of the vertebrae ($t = 0.70$; d.f. = 15; $P > 0.05$).

The ash content of the vertebrae varied significantly between dietary phosphorus levels in both treatments ($F_A = 122.2$, $F_B = 383.3$; d.f. = 7; $P < 0.01$), although there was no significant correlation between the two variables in treatment B ($r = 0.09$; $P > 0.05$). Paired comparisons between high and low calcium inclusion levels showed that dietary calcium levels had no significant effect upon ash content ($t < 0.58$; d.f. = 15; $P > 0.05$).

The quantities of phosphorus leached from feces whilst in the collection chamber (10.5 hours) are shown in Table 2. When the amount leached was plotted against dietary phosphorus levels, a significant ($P < 0.01$) relationship was established ($n = 16$; $y = 1.219 + 0.573x$; $r = 0.79$), thus enabling the estimation of phosphorus leached from feces from any of the dietary groups. The proportion of phosphorus in the feces could then be computed as: mg fecal phosphorus + (mg feces x mg phosphorus leached/mg feces)/mg feces. The apparent absorption efficiency was calculated as:

$$100 - 100 \frac{\%\text{ indicator in food}}{\%\text{ indicator in feces}} \times \frac{\%\text{ nutrient in feces}}{\%\text{ nutrient in food}}$$

and the available phosphorus in the diets as apparent absorption efficiency x phosphorus in diet. These data are summarized in Table 2. Absorption efficiency tended to increase with phosphorus level in both treatments.

Discussion

Fish grown on all diets at least doubled in weight over the experimental period, although fish fed diet B5 grew much more slowly than the rest and exhibited a poor FCR. No explanation can be given for this except that the pellets appeared to have poor stability in water.

Approximately 70% of the phosphorus contained in casein was shown to be available to *O. niloticus* and supplementation of the basal diets with primary sodium phosphate resulted in acceleration of the growth response in both trials, although this was more apparent at the higher calcium inclusion levels. In diet treatment B, significant increases in growth with increasing phosphorus were demonstrated up to a level of 0.46% dietary phosphorus with no further acceleration of growth rate up to an inclusion rate of 0.99%. Moreover, the bone mineral data demonstrated that fish fed less than 0.46% available phosphorus had significantly lower bone phosphorus levels. Thus it may be concluded that the dietary requirement for phosphorus of *O. niloticus* fry is around 0.46% available phosphorus which is considerably lower than that estimated in previous studies (Watanabe et al. 1980; Viola et al. 1986). The results also suggest that bone mineral levels and growth are more closely related to dietary calcium levels. The influence of dietary phosphorus on growth has also been demonstrated in carp fry by Hepher and Sandbank (1984).

These results have implications for water quality in intensive culture systems. Phosphorus losses to the environment are influenced both by the availability of phosphorus in the diet and the quantity of available phosphorus since phosphorus which is unavailable to the fish will be evacuated from the gut in the feces, whilst phosphorus surplus to requirements will be excreted via the kidneys and gills (Forster and Goldstein 1969; Nakashima and Leggett 1980). The effects of phosphorus wastes from intensive cage and pen culture operations has been reviewed by Beveridge (1984, 1987) and recent studies of intensive pond systems have

also demonstrated the importance of dietary phosphorus in determining algal densities and water quality (Viola et al. 1986). An understanding of phosphorus metabolism and requirements is therefore of considerable importance in the development of tilapia farming.

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Growth, Ingestion Capacity, Comparative Appetency and Biochemical Composition of *Oreochromis niloticus* and *Tilapia rendalli* fed with *Azolla*

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Abstract

The growth and food utilization of *Oreochromis niloticus* and *Tilapia rendalli* fingerlings fed in aquaria with three different diets (pellets only; 50% pellets:50% *Azolla microphylla;* and *Azolla microphylla* only) were compared. *O. niloticus* grew better than *T. rendalli* on pellets only: mean weights were 25 and 18 g after 10 weeks. *Azolla* incorporation in the diet decreased the growth of both species but *T. rendalli* was less affected than *O. niloticus*. *Azolla* incorporation in the diet also increased the water and ash contents and reduced drastically the lipid content of body tissues for both species, but their crude protein contents were not affected. The food conversion ratios, apparent net protein utilization, protein efficiency ratios and energy retentions were lower with *Azolla*-containing diets for both species, especially for *O. niloticus* fed with *Azolla* alone.

In selective appetency experiments with *T. rendalli*, *A. caroliniana* ADUL-08 was strongly preferred; *A. pinnata* var. *pinnata* ADUL-188 was ranked next and *A. microphylla* ADUL-69, *A. filiculoides* ADUL-67 and *A. pinnata* var. *imbricata* ADUL-07 ranked lower with the last of these three clearly the least appetizing.

Introduction

Azolla spp. are consumed by various fish (Cassani 1981; Antoine et al. 1986) and could improve the availability of nitrogen in semi-intensive agro-piscicultural systems (Vincke and Micha 1985; Barbier et al. 1985; Micha 1985). This could benefit crops, aquatic biota such as phytoplankton and, if the nutritive value of *Azolla* is reasonable, fish (Almazan et al. 1986; Micha, in press).

The present study investigates the value of *Azolla* spp. as foods for *Oreochromis niloticus*, a micro-phagous/planktivorous fish, and *Tilapia rendalli*, a macrophytophagous one. Based on previously published results on the chemical composition of *Azolla* spp. (Van Hove et al. 1987) and the selective appetency of *O. niloticus* for various *Azolla* species (Antoine et al. 1986, 1987; Wery et al. 1987), *A. microphylla* (strain ADUL-69)* was used here for the growth experiments and for studying the biochemical composition of fish fed with different combinations of fish pellets and *Azolla*. For selective appetency studies with *T. rendalli*, and ingestion capacity studies with both species the following *Azolla* spp. and strains were used: *A. caroliniana* ADUL-08; *A. pinnata* var. *pinnata* ADUL-188; *A. pinnata* var. *imbricata* ADUL-07; *A. microphylla* ADUL-69 and *A. filiculoides* ADUL-67.

Materials and Methods

Growth experiment

T. rendalli and *O. niloticus* fry were produced in 200-l aquaria, fed with *Artemia* nauplii for three weeks, then with minced beef heart and Tetra-Min flakes until they reached 1 g. At that stage they were transferred in groups of 40 to 100-l aquaria and fed Trouvit-00 pellets (4% of body weight/day), and minced beef heart supplemented with minerals and vitamins until they reached 2 g. From 2 to 5 g they were fed with pellets (6% of body weight/day) supplemented with minerals and vitamins. The aquarium water was completely renewed every day by a continuous flow system; its temperature was 26°C ± 0.5.

A. microphylla (strain ADUL-69) cultured on a modified, N-free Hoagland solution, was maintained at the linear phase of its population growth curve (Van Hove et al. 1987); fresh samples were collected daily for fish feeding; their dry weight represented 5.06% of their fresh weight.

Twenty fish (5 g ± 0.4 g) of each species were stocked in 100-l (93.5 x 28.8 x 35.5 cm) aquaria. Three diets were tested: 100% Trouvit K30-1 crushed pellets; 50% Trouvit, 50% (on a dry weight basis) fresh minced *Azolla;* and 100% fresh minced *Azolla*.

The feeds were given at 3.6% of the fish weight (adjusted weekly), 6 days per week. *Azolla* was presented in the morning and pellets at four times a day in equal quantities. Duplicate aquaria with twenty fish each were set up for each species and feeding treatment.

In each group of 20 fish, 10 were marked with two colored beads fixed under anesthesia in front of the dorsal fin. For these fish individual growth curves were obtained by weekly weighing. Data were treated by the method of orthogonal polynomials (Winer 1971), using the first degree polynom coefficients (logarithmic growth curves are linear), followed by a three-way ANOVA. Specific growth rates were also calculated for comparison with other published data. All fish were weighed every week whether marked or not.

Freeze-dried *Azolla* and fish samples and fish pellets were analyzed as follows: moisture, weight after 24 hours in a ventilated, 105°C oven; total nitrogen, Kjeldahl; ash, weight after 12 hours at

Azolla strains from the University of Louvain-la-Neuve collection are identified by the acronym ADUL followed by an introduction number.

450°C; crude lipids, cold extraction with carbon tetrachloride for 48 hours (Vervack 1973); crude fibers, according to Kurschner (Vervack 1973); N-free extract (by calculation); gross energy content (by calculation:protein energy = 5.5 kcal/g; lipids = 9.1 kcal/g; carbohydrate energy = 4.1 kcal/g).

Fish analyses were performed on twenty (5 g) fish of each species at the beginning of the experiment and on at least 5 fish per aquarium (10 per species and diet) at the end.

The nutritional values of the feeds were compared using the following coefficients: apparent net protein utilization (ANPU); protein efficiency ratio (PER); energy retention (ER) calculated from the gross energy contents; and food conversion ratio (FCR), calculated as dry matter ingested per fresh fish weight produced.

Appetency experiment

One male and two females of *T. rendalli* (mean weight 150 g ± 10 g) were reared in a 200-l aquarium. Five *Azolla* species/strains (*A. caroliniana* ADUL-08; *A. pinnata* var. *pinnata* ADUL-188; *A. pinnata* var. *imbricata* ADUL-07; *A. microphylla* ADUL-69 and *A. filiculoides* ADUL-67) were cultured on a modified Hoagland solution (Van Hove et al. 1983) maintained at the linear phase of their growth by periodically discarding excess material.

The presentation of the *Azolla* to the fish and estimation of the ingested quantities were as described by Antoine et al. (1986) except that the plastic feeding rings were replaced by 1.5 cm thick polystyrene plates in which five 10 cm diameter holes were cut (Fig. 1); each hole

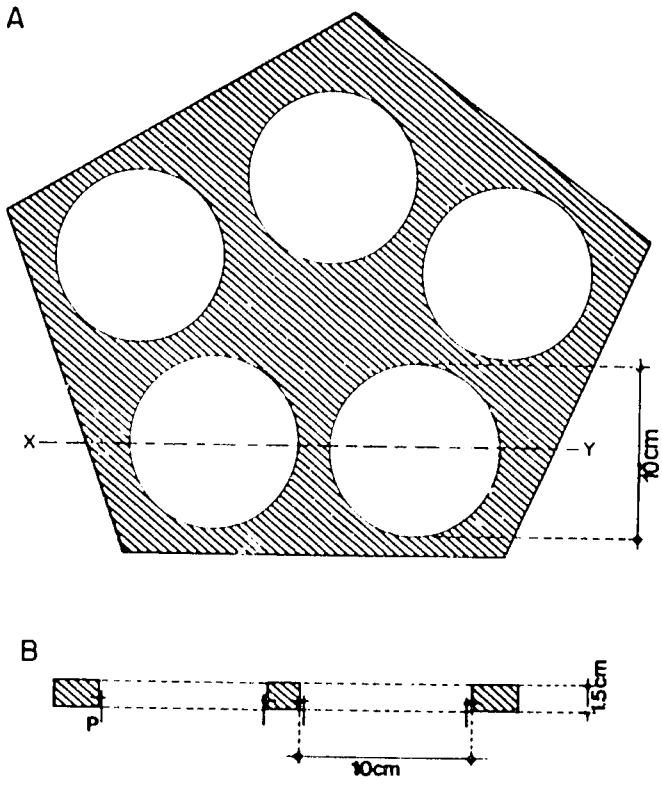

Fig. 1. Polystyrene floating plate used to present different *Azolla* strains as feeds for tilapia. A. Plate seen from above showing five 10-cm apertures. B. Cross-section of XY. P is a thin plastic sleeve that projects 1 cm deep into the water column below the floating plate.

had a 2.5 cm deep plastic sleeve projecting 1 cm below the water surface. Each feeding hole contained 2.5 g fresh *Azolla*. The experiment was repeated on five consecutive days offering two plates per day. A randomized complete block design was used and data analyzed by the non-parametric test of Friedman (Lehmann 1975) followed by the Wilcoxon text (Siegel 1956).

Results and Discussion

Growth experiment

The *O. niloticus* fry produced for the experiment reached average weights of 1 g 55 days after the free swimming stage and 5 g 32 days later; whereas for *T. rendalli* 61 and 42 days were required to bring fry to the same weights. Table 1 presents the chemical composition of the 3 diets. For the same quantity (dry matter) of food, it is clear that the *Azolla*-containing diets have high fiber and ash contents and are low in protein and lipid. They also have a very high water content.

Statistical analysis (Table 2) showed no significant aquarium effect.

Fig. 2 shows the growth curves of *O. niloticus* and *T. rendalli* for each diet. Except for fish fed exclusively with *Azolla*, the curves are exponential. The 100% pellet treatment allowed better *O. niloticus* than *T. rendalli* growth. The two species reached mean weights of 25.0 and 18.3 g after 10 weeks. Replacing 50% pellets by *Azolla* decreased the growth

Table 1. Chemical composition as percentage fresh or (dry) matter of three combinations of trout pellets (Trouvit K30-1) and *Azolla microphylla* (Strain ADUL-69) fed to *Oreochromis niloticus* and *Tilapia rendalli* fingerlings.

Component	Diets I Pellets only	II 50% Pellets: 50% *Azolla*	III *Azolla* only
Crude protein	30.11 (34.34)	2.69 (28.09)	1.10 (21.84)
Crude lipid	6.28 (7.16)	0.48 (5.02)	0.15 (2.88)
Crude fiber	3.45 (3.94)	0.94 (9.79)	0.79 (15.64)
Ash	8.54 (9.74)	1.50 (15.67)	1.09 (21.59)
N-free extract	39.30 (44.82)	3.96 (41.43)	1.93 (38.05)
Moisture	12.31 (0.00)	90.43 (0.00)	94.94 (0.00)

Table 2. Analysis of variance on growth of fingerlings two tilapia species (*Oreochromis niloticus* and *T. rendalli*) fed with three test diets (100% pellets; 50% pellets:50% *Azolla*; 100% *Azolla*). Data were from 10 marked fish in duplicate aquaria for each species and diet.

Effects	Sums of squares	Degrees of freedom	Mean squares	F. obs
Species	54.1	1	54.1	6.2 NS
Diet	1,123.7	1	1,123.7	128.5 **
Species x diet	148.3	1	148.3	17.0 *
Aquarium/species x diet	35.0	4	8.7	1.8 NS
Residual	346.3	72	4.8	
Total	1,707.4	79		

NS = not significant
* = significant (p = 0.05).
** = highly significant (p = 0.01).

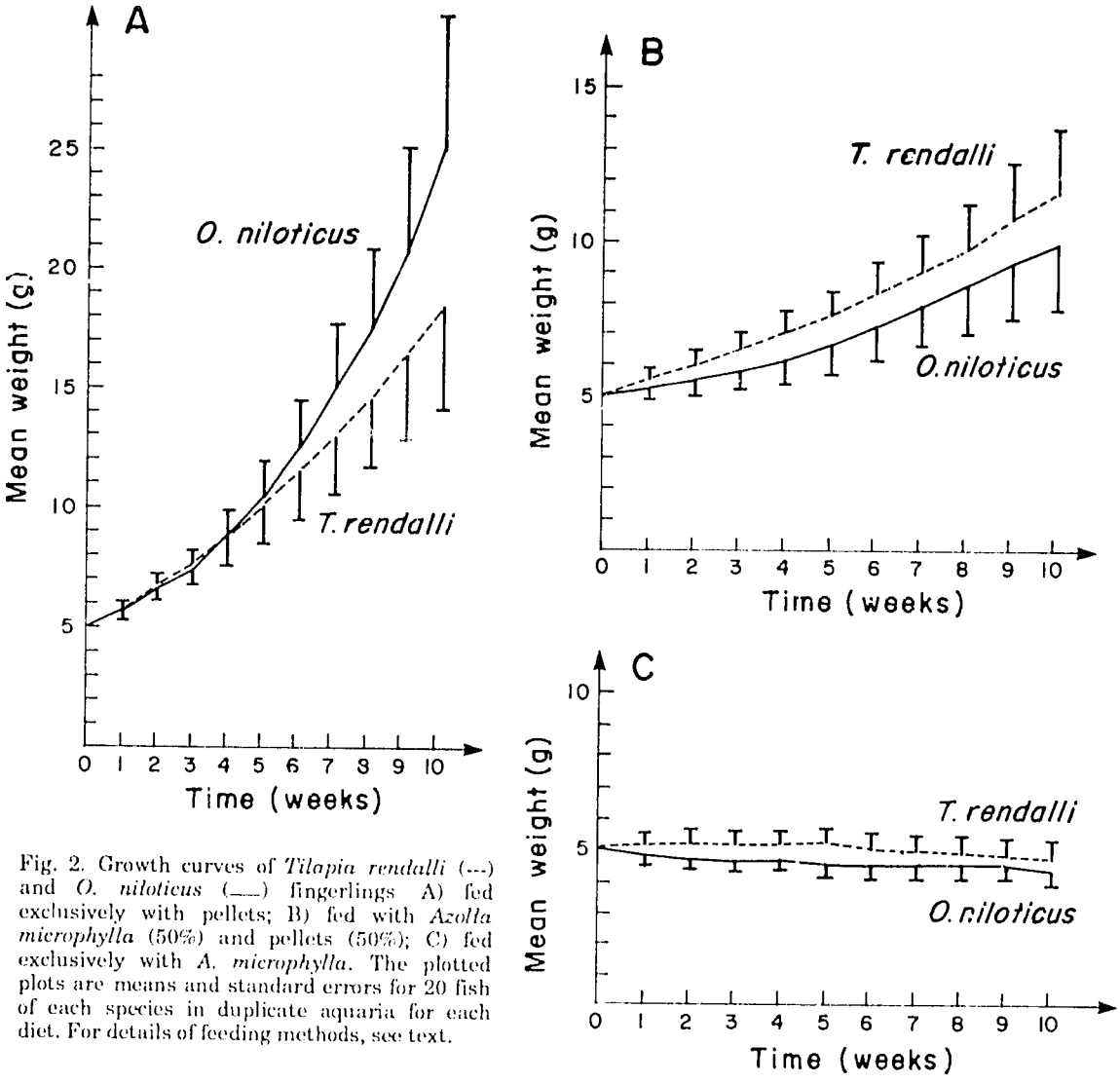

Fig. 2. Growth curves of *Tilapia rendalli* (---) and *O. niloticus* (——) fingerlings A) fed exclusively with pellets; B) fed with *Azolla microphylla* (50%) and pellets (50%); C) fed exclusively with *A. microphylla*. The plotted plots are means and standard errors for 20 fish of each species in duplicate aquaria for each diet. For details of feeding methods, see text.

rate of both species, but *T. rendalli* was less affected (growth reduced to 50%) than *O. niloticus* (growth reduced to 25%); its specific growth rate was even slightly higher (Table 3), but this was not significantly tested with the orthogonal polynomial method ($p > 0.05$). The growth data differences between *O. niloticus* and *T. rendalli* fed on pellets alone and between *T. rendalli* fed on pellets alone and the 50:50 diet are highly significant ($p < 0.01$). The growth curves obtained with the 100% *Azolla* diet were not included in these statistical analyses since non-exponential curves cannot easily be compared with exponential curves. It is noteworthy that 10% of the *O. niloticus* population fed *Azolla* alone died during the experiment, while all the *T. rendalli* survived; the surviving *O. niloticus* lost on average 0.6 g; *T. rendalli* only 0.3 g.

From Table 4, it appears that *Azolla* incorporation in the diet increased fish water content with a maximum of 83.1% for *O. niloticus* only fed with *Azolla*. Lipid content is also drastically reduced to a minimum of 0.6% in *O. niloticus*. Such negative relationships between water and lipid contents have been described by Appler and Jauncey (1983).

Table 3. Growth and food utilization data for *Oreochromis niloticus* and *Tilapia rendalli* fingerlings fed on three test diets (pellets only; 50% pellets:50% *Azolla microphylla* and *Azolla microphylla* only). Data are from 20 fish per species and per diet in duplicate aquaria. For details of feeding method, see text.

Growth/food utilization	*O. niloticus*			*T. rendalli*		
	Pellets only	50% Pellets: 50% *Azolla*	*Azolla* only	Pellets only	50% Pellets: 50% *Azolla*	*Azolla* only
Mean initial weight (g)	5.1	5.0	4.9	5.0	5.0	5.0
Initial standard error (g)	0.4	0.4	0.3	0.2	0.2	0.2
Mean final weight (g)	25.0	9.9	4.3	18.3	11.7	4.7
Final standard error (g)	5.6	2.1	0.4	4.2	2.1	0.6
Growth as % of diet 0	100.0	24.7	< 0	100.0	50.1	< 0
Specific growth rate	2.3	0.9	< 0	1.8	1.1	< 0
Consumed *Azolla* (kg/aquarium)	—	2.3	3.1	—	3.3	4.2
Consumed % of *Azolla* ration	—	78.7	83.7	—	100	98
Food conversion ratio (FCR)	1.2	2.8	—	1.6	2.5	—
Apparent net protein utilization (APNU) (%)	36.1	20.4	—	30.8	23.5	—
Protein efficiency ratio (PER)	2.4	1.3	< 0	1.8	1.4	< 0
Energy retention (ER) (%)	25.2	9.7	< 0	18.9	10.1	< 0

Table 4. Body composition (%) comparison of *Oreochromis niloticus* and *Tilapia rendalli* fingerlings fed with three different diets (pellets only; 50% pellets:50% *Azolla microphylla*; *Azolla microphylla* alone). Data are presented as % fresh weight and (% dry weight).

	Moisture	Crude lipid	Crude proteins	Ash	Total
O. niloticus					
Initial composition	74.4	5.3 (20.7)	15.5 (60.6)	4.6 (17.9)	99.8
Final composition fed pellets only	75.0	5.9 (23.6)	14.9 (59.7)	3.7 (14.9)	99.6
Final composition fed 50% pellets: 50% *Azolla*	75.8	3.6 (14.9)	15.6 (64.6)	4.9 (20.4)	100.0
Final composition fed *Azolla* only	83.1	0.6 (3.8)	10.6 (62.7)	5.5 (32.6)	99.8
T. rendalli					
Initial composition	75.3	5.1 (20.5)	15.5 (62.4)	3.9 (15.6)	99.6
Final composition fed pellets only	73.2	4.9 (18.9)	16.5 (61.8)	5.1 (19.1)	99.8
Final composition fed 50% pellets: 50% *Azolla*	76.0	2.7 (11.3)	15.9 (66.3)	4.6 (19.2)	99.2
Final composition fed *Azolla* only	78.6	0.9 (4.2)	13.7 (64.2)	5.9 (27.6)	99.1

In spite of the low protein content of the *Azolla*-containing diets, the crude protein of the fish content was not affected. Their ash content generally increased with increasing *Azolla* content. No major differences were found between the body composition of the two species.

Table 3 shows that *O. niloticus* never consumed all the available *Azolla* even when they clearly suffered from malnutrition. *T. rendalli* on the other hand normally ingested all the *Azolla* ration (the exception being one group during the first week). The FCR, APNU, PER and ER were better for *O. niloticus* than for *T. rendalli* when both were fed exclusively with pellets. Introduction of *Azolla* (50%) into the diet had in all instances a negative influence on these coefficients, indicating a relatively low nutritive value of *Azolla*, especially for *O. niloticus*.

Appetency experiment

From Table 5, it appears that in nine trials, *T. rendalli* preferentially ate *A. caroliniana*. In all trials, *A. pinnata* var. *imbricata* was the least appreciated. The rankings for the other *Azolla* species/strains were inconsistent. Fig. 3 presents the total consumption of the five species/strains (in %) and the results of the Wilcoxon test.

Further discussion

Azolla incorporation in the diet decreased the specific growth rate and body composition quality of both species; though *T. rendalli* was less affected than *O. niloticus*. In these experiments under controlled conditions, only the direct effect of *Azolla* as feed for fish was tested. In field conditions, especially in semi-intensive agropiscicultural systems, *Azolla* contributes indirectly to the quality of the environment by its decomposition which releases (among other things) nitrogen.

It is unfortunate that the appetency test results were obtained after completion of the growth experiment, in which the strain used (*A. microphylla* ADUL-69) was clearly not the one most preferred by *T. rendalli*. We also know now from unpublished results that *T. rendalli* can ingest much more *Azolla* (> 12% of body weight/day) than the 3.6% used here. Moreover, the crude protein content of the *Azolla* used here (21.84%) was low; values higher than 30% are usual for this strain. Finally the size of the *Azolla* strain used (even though minced) was probably not ideal for feeding fingerlings. Clearly there is scope for much more work on feeding *Azolla* to tilapias, especially *T. rendalli*.

Table 5. The consumption (g/block) of five different *Azolla* species and strains by *Tilapia rendalli* fingerlings in a random block experiment. Bracketed values are the rankings 1-5 for each trial.

Block number	*A. caroliniana* ADUL-08	*A. pinnata var. pinnata* ADUL-188	*A. microphylla* ADUL-69	*A. filiculoides* ADUL-67	*A. pinnata var imbricata* ADUL-07
1	1.58 (1)	1.51 (2)	0.66 (3)	0.52 (4)	0.49 (5)
2	0.96 (3)	1.66 (1)	0.57 (4)	1.43 (2)	0.50 (5)
3	1.67 (1)	1.10 (3)	1.45 (2)	0.30 (4)	0.00 (5)
4	2.13 (1)	0.69 (3)	0.71 (2)	0.36 (4)	0.18 (5)
5	1.47 (1)	0.59 (3)	0.20 (4)	0.62 (2)	0.04 (5)
6	2.02 (1)	0.72 (3)	0.28 (4)	0.86 (2)	0.14 (5)
7	0.93 (1)	0.54 (3)	0.85 (2)	0.41 (4)	0.18 (5)
8	2.38 (1)	0.57 (3)	0.38 (4)	0.70 (2)	0.10 (5)
9	2.45 (1)	1.94 (2)	1.13 (3)	0.53 (4)	0.04 (5)
10	2.45 (1)	1.60 (2)	1.17 (3)	0.64 (4)	0.38 (5)

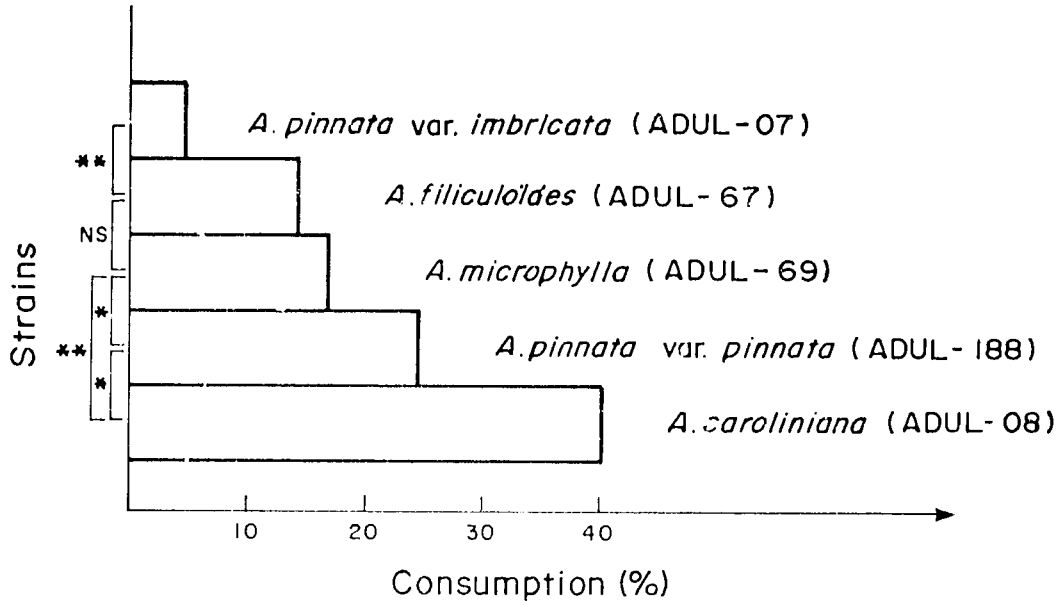

Fig. 3. Consumption of five *Azolla* strains by *Tilapia rendalli* expressed as a percentage of the total quantity of *Azolla* (all strains pooled) consumed during an appetency test. (*: significant; **: highly signficant; NS: not significant).

It seems premature to draw general conclusions concerning the usefulness of introducing *Azolla* into agropiscicultural systems.

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Pito Brewery Waste as an Alternative Protein Source to Fishmeal in Feeds for *Tilapia busumana*

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Abstract

Pito brewery waste, a by-product of brewing by fermentation of sorghum, has a crude protein content of about 32%. Three diets were tested on triplicate groups of ten *Tilapia busumana* fry (mean initial weight, 1.5 g) in glass aquaria. The diets have similar protein content (about 27%) but different protein sources: A, fishmeal alone; B, 5% fishmeal and 5% pito waste and C, pito waste alone. Proximate analysis was performed on all the diets. The following results were obtained in order of diets A, B, C (mean values): specific growth rate, 1.34, 1.46, 1.12; food conversion ratio, 4.10, 3.56, 4.49; protein efficiency ratio, 0.89, 1.05, 0.83. It was concluded that pito waste, despite its inferior growth results is a useful protein source for *T. busumana* feeds, because of its low cost. The feeding costs/kg of fish produced for diets A, B, C were US$ 0.62, 0.27 and 0.03, respectively.

Introduction

Although preliminary marketing survey has indicated potentially high consumer acceptance for *Tilapia busumana* in Ghana, very little information is available on the production of this species, which occurs naturally in Lake Bosumtwi, Ghana. *T. busumana feeds* on periphytic algae (Whyte 1975). It also accepts artificial feeds readily (Oduro-Boateng, unpublished data). Pito waste, a by-product of brewing pito beer from fermented grains of sorghum, has great potential and is high in other nutrients (Eyeson and Ankrah 1975). It is very cheap and easily obtainable from local pito brewers. This study was carried out to evaluate the use of pito waste as a fishmeal substitute in *T. busumana* feeds.

Materials and Methods

Pito waste was analyzed by standard methods (AOAC 1970) for: crude protein, micro-Kjeldahl; total lipids, Soxhlet extraction with petroleum-ether; moisture content, oven drying of 5-g samples to constant weight at 105°C; ash content, ashing 2.0-g samples in a muffle furnace.

Tilapia busumana fry (mean weight, 1.4 g) were collected from Lake Bosumtwi. For nutritional trials, ten fish were placed in each of nine 90 x 60 x 30 cm deep aquaria after seven days acclimatization to captivity. Water temperature and dissolved oxygen were recorded daily for each aquarium and water was replaced twice a week.

Three diets were formulated with different protein sources: A, fishmeal; B, fishmeal (50%) + pito waste (50%) and C, pito waste. The protein level in the diets was maintained at about 27% (that chosen for the pito waste diet, C). Fishmeal was made from sundried anchovies from the local market and wet pito waste was obtained from a pito brewer and sundried. Each ingredient was ground into a fine powder in a hammer mill. Then the various ingredients in the diets were mixed together in their required quantities as shown in Table 1. The feeding rate was 5% of the body weight of fish per day divided into three equal rations of dry diet. Fish were fed for 50 days. The treatments A, B and C were carried out in triplicate aquaria.

Growth performance was assessed as specific growth rate (Brown 1957) and the food conversion and protein efficiency ratios calculated after (Osborn et al. 1919). Statistical comparisons were by the multiple range test of Duncan (1955).

Results

The chemical composition of pito waste presented in Table 2 shows that this feed component has a relatively high crude protein content of 32%. The crude lipid level of 5.7% is also quite high for material of plant origin. Table 3 presents the proximate analyses of the three diets.

The growth performances of *T. busumana* fed the various diets are presented in Table 4. *T. busumana* exhibited best growth when fed on the composite diet (B). Diet C containing only pito waste as a protein source produced the poorest growth.

Food conversion ratios (FCR) recorded for *T. busumana* showed that the composite diet (B) produced the best FCR with the pito waste diet (C) producing the worst. However, the food conversion ratios recorded were poor for all the diets.

Table 1. Composition of test diets A to C used for feeding trials with *Tilapia busumana* fry in glass aquaria. The units are weights of each component in g/100 g.

Ingredients	Diets A	B	C
Fishmeal	37.3	18.7	0
Pito waste	0	46.6	86.0
Cod liver oil	0	2.8	5.0
Dinor oil (palm oil)	5.5	1.0	0
Cooked starch	5.0	5.0	2.0
Uncooked starch	48.2	21.9	3.0
Vitamin/mineral premix	4.0	4.0	4.0

Table 2. Proximate analysis of pito waste - a waste product from the brewing of pito beer by sorghum fermentation.

Component	% Composition (dry weight)
Crude protein	32.0
Crude lipid	5.7
Crude fiber	9.9
Ash	14.9
Moisture	7.9

Table 3. Proximate analysis of test diets used for feeding trials with *Tilapia busumana* in glass aquaria. For details of diets A, B and C, see Table 1.

Component	% composition of diets		
	A	B	C
Crude protein	27.32	27.10	27.52
Crude lipid	10.39	9.82	9.95
Ash	12.07	11.22	8.12
Moisture	10.70	8.54	8.63

Table 4. Growth parameters for *Tilapia busumana* fry fed different diets for 50 days in glass aquaria. The data are means from triplicate aquaria each containing ten fish.

Diet	Mean initial wt (g)	Mean final wt (g)	Mean specific growth rate (%)	Food conversion ratio	Protein efficiency ratio
A	1.60[a]	3.09[a]	1.34[a]	4.10[a]	0.89[a]
B	1.30[a]	2.71[a]	1.46[a]	3.56[b]	1.05[b]
C	1.50[a]	2.66[a]	1.12[b]	4.49[a]	0.83[a]
SEM	0.13	0.16	0.09	0.09	0.10

Data in columns bearing same suffix letter are not significantly different ($P < 0.05$).
SEM - Standard error of the mean.

Discussion

The potential of pito waste as a fish feed component depends on its low cost. A kilogram of pito waste sells on the local market at US$0.01 compared with US$0.4 for fishmeal. Its protein level of 32% (Table 1) is high enough for this feedstuff to be considered as a major protein source even for small tilapias which have a high dietary protein requirement of 30-50% (Jauncey and Ross 1982).

The better specific growth rate, food conversion ratio and protein efficiency ratio (Table 4) for the composite diet (B) than for the fishmeal diet (A) are of interest. It appears that replacing 50% of the fishmeal by pito waste could reduce feeding costs by more than 50%. This suggests that pito waste could be an economical substitute for fishmeal. These results may reflect the herbivorous habit of *T. busumana*. Similar findings were recorded for *T. zillii* (Appler 1985) when fed on a diet having 5% of the fishmeal protein substituted by an algal protein.

Considering the economics of utilizing the different protein sources, feeding diet A costs US$0.62 to produce a kilogram of *T. busumana*, which sells between US$0.8 and US$1.0/kg in the local market. The corresponding feed costs/kg for diets B and C are US$0.27 and 0.03. Thus though all pito waste diet produced inferior growth than the fishmeal diet, it was more economically attractive. These results show that pito waste can prove a useful and cheap source of protein in *T. busumana* feeds.

Acknowledgement

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Growth and Histological Studies on the Liver and Anterior Intestine of Nile Tilapia *(Oreochromis niloticus)* Fed on Mixed Foods: *Daphnia magna, Chlorella vulgaris* and Commercial Carp Pellets

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Introduction

Preliminary work on feeding Nile tilapia *(Oreochromis niloticus)* (Kugler et al. 1987) pointed out that zooplankton *(Daphnia magna)* is a suitable food. Histological observations have confirmed this by demonstrating the good condition of hepatocytes of fish fed on *D. magna*, and that lipid infiltration of liver occurs when commercial carp pellets are given (Tabthipwon et al., this vol.). In nature, fish use a variety of foods to maintain good condition. Moreover, good growth performance is obtained from cultured tilapias fed on mixed diets (De Silva 1985; Kugler et al. 1987).

Fish hepatocytes are a good indicator of dietary quality (Hibiya 1982; Storch and Juario 1983, 1984). When fish are in bad condition from poor nutrition, melano-macrocrophages and lipopigments are formed in hemopoietic tissue (Roberts 1979; Agius and Roberts 1981; Kugler et al. 1987). By coupling observations on liver histology and fish growth, a better

understanding of fish diets can be achieved.

In this study, *O. niloticus* fry were fed with mixed foods: zooplankton *(D. magna)*, phytoplankton *(Chlorella vulgaris)* and carp pellets. Growth measurements were correlated with liver histology (formation of lipofuscins in hepatocytes) and with changes in anterior intestine, which is involved in fat absorption (Noaillac-Depeyre and Gas 1979).

Materials and Methods

Two month-old *O. niloticus* fry were raised on natural feed in a pond and acclimatized to aquarium conditions for 48 hours. Ten fish (1.30 to 1.79 g) were stocked in each of eight 10-l aquaria. Water was well-aerated and maintained at 30°C (± 1°C) monitored graphically on a continuous basis. Aquaria were cleaned and fish feces siphoned out daily. Live *D. magna* was cultured in water fertilized with cow manure. The methods for *C. vulgaris* culture and dilution were as described by Kugler et al. (1987).

The experiment (four duplicated treatments) was divided into two periods of 2 weeks each. Because of availability of local facilities, only some combinations of the three feeds were tested. They were the ones for which we could expect significant changes between periods I and II (Table 1). Control fish were killed at the beginning of the experiment. For the first period, fish were fed on one of the food items: *D. magna*, given three times a day, *ad libitum;* crumbled commercial carp pellets, at 6% body weight of fish/day, split between two feedings; and *C. vulgaris* at 500 x 10^3 cells/ml, supplied at one morning feeding. All feeding was restricted to 6 days a week. For the second period, food items were applied in the same manner (Table 1). The nutrient composition of feeds is given in Table 2. The methods of analysis used were as in AOAC (1965). Fish were weighed to the nearest 0.01 g and their standard length measured to the nearest 0.1 cm every week.

Liver and anterior intestine samples were taken from 3 fish per treatment for histological examination at the beginning, after 2 weeks and at the end of the experiment. Liver samples were fixed in trichloroacetic Bouin's solution and anterior intestine samples in Bouin Holland's solution. For light microscope evaluation the samples were dehydrated in graded ethanol; embedded in paraffin; cut at 7 mm and stained with periodic acid-Schiff (P.A.S.) or Schmorl's solution for liver, and Millot or Mann Dominici's trichrome for anterior intestine. For electron microscopy, tissues were doubly fixed with gluteraldehyde and osmium tetroxide in 0.2 M cacodylate buffer (pH 7.2). They were dehydrated with graded ethanol and embedded in epon. Sections were contrasted with uranyl acetate and lead citrate.

Table 1. Feeding treatments for Nile tilapia *(Oreochromis niloticus)* fry: combinations of zooplankton *(Daphnia magna)*; phytoplankton *(Chlorella vulgaris)* and carp pellets.

Treatment	Period I (2 weeks)	Period II (2 weeks)
1	*C. vulgaris*	*C. vulgaris* & carp pellets
2	Carp pellets	Carp pellets & *C. vulgaris*
3	*D. magna*	*D. magna* & *C. vulgaris*
4	No feed	*D. magna*

Table 2. Nutrient composition of *Daphnia magna*, *Chlorella vulgaris* and carp pellets used as feeds for *Oreochromis niloticus* fry: expressed on a percentage dry weight basis, unless stated otherwise.

Nutrients	*Daphnia magna*	*Chlorella vulgaris*	Carp pellet
Protein	42.5	49.5	35.2
Lipid	6.4	6.3	8.5
N-free extract	23.4	33.4	41.0
Fiber	10.1	3.6	5.7
Ash	14.9	7.3	9.6
Energy[a]	3,862 2	4,666.3	4,393.6
Protein/energy[b]	109.1	106.1	60.2

[a]As kcal/kg feed calculated by using 5.5 kcal/g protein: 9.1 kcal/g lipid: 4.1 kcal/g N-free extract.
[b]As mg protein per kcal of 1 kg feed.

Results

Table 3 summarizes the growth results. For the first period, feeding fish with *D. magna* gave the highest growth, compared with carp pellets and *C. vulgaris*. Deprivation of food and feeding fish with *C. vulgaris* caused weight losses. For the second period, feeding fish with *D. magna* plus *C. vulgaris* gave the highest growth (Table 3). Fish having first lost weight (starved fish and fish fed on only *C. vulgaris*) grew better thereafter on carp pellets and *C. vulgaris* than on *D. magna* (Table 3).

In control fish killed at the start of the experiment, hepatocytes contained abundant glycogen. Abundant acidophilic secretory granules were found in pancreatic acinar cells. The anterior intestine of *O. niloticus* has two layers of smooth muscle and a mucosa forming many deep folds. The mucosa includes enterocytes and irregularly arranged mucus secretory cells.

Table 4 summarizes the results of the histological studies at the light and electron microscope levels.

With Treatment 1, after first feeding with *C. vulgaris*, the hepatocytes resembled those of starved fish, with many lipofuscins; the acinar cells were poor in zymogen granules; and in the anterior intestine, the mucus secretory cells were fewer than normal; microvilli

Table 3. Mean individual weight and specific growth rate (SGR) of Nile tilapia *(Oreochromis niloticus)* fry fed on *Daphnia magna*, *Chlorella vulgaris* and carp pellets in aquaria for two periods of 2 weeks each. The data are means of twenty fish (ten in duplicate aquaria for each treatment). P1 = Period I; P2 = Period II; (1) (2) (3) = mean fish weights at the beginning, after 2 weeks and at the end of the experiment. Data in the same column bearing the same suffix (a-d) are not significantly different (P = 0.05; ANOVA and Duncan's Multiple Range Test).

Treatment	Feeding	Mean weight (g) (1)	(2)	(3)	SGR
1	P1 *C. vulgaris* P2 *C. vulgaris* and carp pellet	1.45a	1.36a	2.00a	1.15a
2	P1 Carp pellet P2 Carp pellet and *C. vulgaris*	1.66a	2.03b	3.22b	2.37b
3	P1 *D. magna* P2 *D. magna* and *C. vulgaris*	1.62a	3.05c	5.26c	4.20c
4	P1 No feed P2 *D. magna*	1.34a	1.08a	1.25d	-0.25d

Table 4. Summary of the histological appearance of pancreatic acinar cells, hepatocytes and anterior intestine of Nile tilapia *(Oreochromis niloticus)* fry fed with four different dietary combinations of *Chlorella vulgaris,* carp pellets and *Daphnia magna:* L = lipids; LF = lipofuscins; LP = lipid particles; MSC = mucus secretory cells; MV = microvilli; n = normal; ZG = zymogen granules; +, +, -, -- represent strong or slight increases and decreases compared to normal tissue. Periods I and II were both 2 weeks long.

Treatment No.	Period I or II/Feeds	Appearance of pancreatic acinar cells	Appearance of hepatocytes	Appearance of anterior intestine
1	I/*C. vulgaris*	ZG (--)	L, LF (++)	MSC, MV (-)
	II/*C. vulgaris* and carp pellets	ZG (n)	L, LF (++)	MSC (n)
2	I/Carp pellets	ZG (n)	L, LF (++)	MSC (n); LP (+)
	II/Carp pellets and *C. vulgaris*	ZG (n)	L, LF (+)	MSC (n); LP (+)
3	I/*D. magna*	n	n	n
	II/*D. magna* and *C. vulgaris*	n	n	n
4	I/No feed	ZG (--)	LF (++)	-
	II/*D. magna*	ZG (--)	LF (++)	-

were shortened and enterocytes resembled those of starved fish. After feeding with a mixture of *C. vulgaris* and carp pellets, the hepatocytes still contained many lipofuscins and were infiltrated by lipids. In the cytoplasm of some hepatocytes, glycogen formation had not yet started. The acinar cells, however, were rich in zymogen granules. The later feeding with carp pellets therefore accelerated lipid deposition and lipofuscin formation in these fish of poor condition. In their anterior intestine, the muscularis was thin but the number of mucus secretory cells did increase.

With Treatment 2, after first feeding on carp pellets, the hepatocytes were loaded with lipid globules and the mucosa of the anterior intestine had deep folds and a thick muscularis. Many mucus secretory cells were present in the epithelial layer and enterocytes stained strongly. Electron microscopy examination revealed abundant particular lipids near the basal lamina. During later feeding with a mixture of carp pellets and *C. vulgaris,* lipid deposition in the hepatocytes persisted but the number of lipofuscins decreased and the hepatocyte cytoplasm became visible. Many zymogen granules were present in pancreatic acinar cells. The anterior intestine revealed enterocytes with dense cytoplasm and numerous mucus secretory cells.

With Treatment 3, after first feeding with *D. magna* for 2 weeks and then with a mixture of *D. magna* and *C. vulgaris,* all tissues were normal.

During Treatment 4, the hepatocytes of fish starved for 2 weeks had lost all their glycogen and many lipofuscins were observed. After later feeding with *D. magna,* glycogen was observed but electron microscopy examination revealed many glycogenosomes showing that this glycogen was being used immediately.

Discussion

Only *D. magna* gave a good growth performance without disturbed metabolism. Mixing *C. vulgaris* with *D. magna* caused no increase of lipofuscins and alteration of hepatocyte structure. *D. magna* is therefore recommended to be used as a standard feed in nutritional tests with *O. niloticus.*

It was demonstrated that lipofuscins are abundant in the hepatocytes of starved *O. niloticus* fry and of fry fed on either carp pellets or *C. vulgaris* alone (see also Tabthipwon et al., this vol.). Lipofuscins are lipochrome substances

(including ceroid pigments and hemofuscins) which are considered as residual bodies resulting from alternative lipid metabolic pathways (Eurell and Haensly 1982). Increased liver lipid deposits may indicate a diet of insufficient vitamin content (Tappel 1972; Smith 1979), too much carbohydrate (Storch and Juario 1983) and high unsaturated fatty acids (Sargent 1976, quoted by Tappel 1972).

Lipid infiltration and abundant lipofuscins in the hepatocytes of *O. niloticus* fry fed on carp pellets was probably due to the high carbohydrate and low vitamin contents of these pellets. By feeding with *C. vulgaris* and carp pellets, growth was improved; this result was similar to that observed by Kugler et al. (1987). *C. vulgaris* probably supplied vitamins. Therefore, *O. niloticus* fry diets of high carbohydrate content should probably be supplemented with appropriate phytoplankton (see Tabthipwon et al., this vol.). However further studies are needed on the reversibility of disturbed lipid metabolism caused by feeding a diet rich in carbohydrate.

The large numbers of hepatocyte lipofuscins and the shortened microvilli of the anterior intestine revealed that starved fish and fish fed on *C. vulgaris* alone (high nutrient content but low digestibility) underwent tissue catabolism. *O. niloticus* fry cannot utilize *C. vulgaris* efficiently. Starved fish and fish fed on *C. vulgaris* showed better responses to mixed diets with carp pellets than to those with *D. magna*. However, such carp pellet feeding may again cause lipofuscin formation and hepatocyte deterioration. *D. magna* is therefore a more suitable food for recovery of condition in starved fry.

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Field Experiments on Growth Enhancement of Tilapia (*Oreochromis niloticus* x *O. aureus* F_1 Hybrids) Using Pellets Containing an Androgen (17α-Ethynyltestosterone)

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Abstract

The effect of a synthetic androgen, 17α-ethynyltestosterone (17 ET) on growth of tilapia (F_1 hybrids of *Oreochromis niloticus* x *O. aureus*) was investigated. The objective was to determine the optimal procedure for androgen application under farm conditions; i.e., the minimal effective duration and dose to achieve commercial results and the effects of season and age population structure of the recipients. 17 ET was tested first in the hatchery by feeding fingerlings for 11 weeks on pellets containing 17 ET at 60 mg/kg or 15 mg/kg food. Fish grew faster than the control at both concentrations but no significant difference was noted between the two treated groups. A field experiment was conducted on fish reared in cages suspended in an earthen pond and fed pellets containing 2.0, 7.5 or 15.0 mg 17 ET/kg food for either 2, 4 or 8 weeks. Growth of fish increased by 25% at a concentration of 2.0 mg/kg food of 17 ET. No significant difference was observed between the control and fish grown on pellets containing 15 mg/kg 17 ET. The growth of the fish at all concentrations was not affected by the duration of treatment. Hence, the lowest concentration (2 mg/kg) and the shortest duration (2 weeks) were chosen for further experiments: two further cage trials (with fish of two different ages and initial sizes) and a growth trial in a commercial polyculture pond. In the cage trials, the growth of 17 ET-treated fish and controls of three graded size combinations (small, large and mixed small + large) were compared in experiments started in spring and summer. In the spring trial, 17 ET-treated small and large fish grew faster than controls.

In the summer trial, the 17 ET-treated large and mixed size fish grew faster than controls, but the small fish did not. The fish in the summer trial grew faster than those of the spring trial. This may be due to the different ages at the onset of the trial (12 months vs. 8 months). In the growth trial carried out in a commercial polyculture pond with common carp, Chinese carps and red tilapias, test fish fed for 2 weeks with 17 ET at 2 mg/kg food reached a mean final weight of 382.0 ± 6.3 g in 6 months as compared with 343.0 ± 7.3 g for controls. All the abovementioned differences were statistically significant (P = 0.01 to 0.05). We suggest that the addition of small amounts of 17 ET to tilapia feed for a limited period may become a practical growth enhancement procedure for tilapia in aquaculture, pending the results of investigations on its elimination during growout and its fate in the culture environment.

Introduction

Acceleration of growth by anabolic steroids is important in livestock husbandry (e.g., Preston 1975). In fish, attempts have been made to enhance growth by androgen-containing food with 17α-methyltestosterone (17 MT) the commonly tested compound (Clemens and Inslee 1968; Yamazaki 1976; Donaldson et al. 1979). However, in *Oreochromis aureus* 17α-ethynyltestosterone (17 ET) seems to be more effective in growth enhancement when applied in lower doses (Guerrero 1975). Previous experiments on growth enhancement in tilapias (Guerrero 1975, 1976; Tayamen and Shelton 1978; Ufodike and Madu 1986) were relatively short term and were carried out on small fry only. The objectives of the present study were to determine the minimal effective dose of 17 ET and duration of treatment as a growth enhancer for F_1 hybrids of *O. niloticus* x *O. aureus*, and the optimal age and size of fish for its application. This paper describes growth trials performed in experimental tanks and cages and in a commercial fish farm, with 17 ET-treated all-male hybrid tilapia.

Materials and Methods

Sources of fish; summary of experimental conditions and feed preparation

The fish used in this study were F_1 hybrid fry of *O. niloticus* x *O. aureus*, produced commercially in the Gan Shmuel Fish Breeding Centre. They were treated with 17 ET to sex inverse the females (Rothbard et al. 1983), nursed to 5-50 g and overwintered for 5-6 months. All fish except those in the hatchery trial (see below), were first overwintered in extremely high densities (100-200 t/ha) in plastic-covered 0.05-ha ponds. The preliminary trial lasting 11 weeks was carried out in the hatchery. Other experiments were performed in cages suspended in a 0.4-ha earthen pond. In a final experiment, fish were tested for growth performance in typical Israeli commercial polyculture.

All fish were fed commercial fish pellets (24% protein; "Ambar" Ltd. Israel). The synthetic androgen, 17α-ethynyltestosterone (17 ET; "Sigma" St. Louis, USA) was dissolved in 95% ethanol and then mixed with the pellets (1 liter ethanol/2 kg pellets). The 17 ET containing wet pellets were dried in the open air. Control pellets used were treated similarly but without the addition of 17 ET. Fresh food was prepared weekly and stored in a cool and dry place.

Hatchery experiment

Two concentrations of 17 ET were tested: 60 mg/kg food (as used routinely for fry sex inversion) and 15 mg/kg food. Groups of 250 fingerlings (average weight - 15 g) were stocked into each of three 2-m^3 fiberglass tanks (Ewos, Sweden) connected to the hatchery recirculating water system. Each group was fed pellets with or without 17 ET (0, 15 or 60 mg/kg food) for 11 weeks at a daily rate of 3% of the biomass. A random sample of 30 fish from each group was weighed individually once a week. The fish were fed continuously, 12

hours a day, during daylight hours, by means of a clock-feeder.

Cage trials

Twelve cages, constructed from paint-coated iron frames and plastic net (5-mm mesh), were suspended in a 0.4-ha earthen pond with average depth of 1.2 m. The cages (Fig. 1) (90 x 90 x 150 cm) were suspended about 40 cm above the pond bottom to prevent the fish from feeding on the accumulated bottom sediments. The food was placed on metal plates (60 cm in diameter) submerged 30-40 cm below the water surface in each cage. Fish were fed once or twice daily (1000 to 1200 hours). A netting frame covered each cage to prevent fish from escaping and to keep out birds.

CAGE TRIAL 1

Determination of the optimal dose and duration of 17 ET treatment. A bifactorial experiment was designed to determine the optimal dose and duration of 17 ET treatment to obtain best fish growth. Groups of 150 fish (average weight 36.8 g ± 1.4) were stocked into 10 cages. Fish were fed with 17 ET at 2.0, 7.5 or 15.0 mg/kg food, each for 2, 4 or 8 weeks. One group served as an untreated control. After the end of feeding with pellets containing 17 ET, the fish were fed regular pellets (24% protein) until the end of the experiment. A random sample of 30 fish was weighed individually every 2 weeks and the daily rations (3% of the biomass) were calculated accordingly. At the end of the experiment, after 21 weeks, all fish were individually weighed. The data were analyzed by a two-way ANOVA and the fish mean weights compared using the Student, Newman, Keuls (SNK) test (Sokal and Rohlf 1969).

CAGE TRIALS 2 AND 3

Determination of the effect of initial size and age on growth acceleration by 17 ET. Overwintered fish were divided into

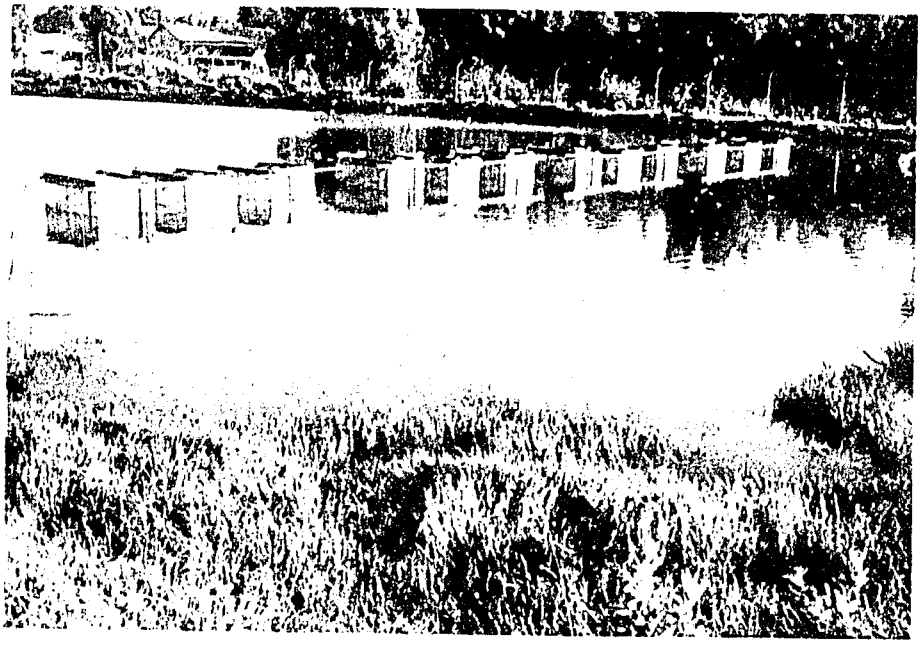

Fig. 1. Cages (90 x 90 x 150 cm) suspended in a 0.4-ha earthen pond; used for growth trials with all-male tilapia (*Oreochromis niloticus* x *O. aureus* F_1 hybrids).

two groups: 1. for a spring growth trial in April (Cage Trial 2) and 2. for a summer growth trial in July (Cage Trial 3). In both trials, fish were divided into three weight classes: small, large and mixed (equal number of small and large fish) (see Table 2). Two hundred fish were stocked in each cage. Each trial consisted of three treated groups (fed pellets containing 17 ET (2 mg/kg) for 2 weeks) and three control groups. Random samples of 30 fish from each cage were weighed individually every 2 weeks. The feeding rates in % body weight/day were: Cage Trial 2 - 0 to 9 weeks, 6%; 10 to 14 weeks, 3%; 15 to 24 weeks, 2%. Cage Trial 3 - 0 to 7 weeks, 3%; 8 to 14 weeks, 2%.

Growth trial in commercial pond polyculture

A group of about 750 fish (37.3 ± 6.0 g, mean ± SEM) was stocked into each of two 3-m^3 tanks. After 1 week's acclimation, fish in one tank were fed pellets containing 17 ET, (2 mg/kg food) for 2 weeks. The second group was fed regular pellets and served as control. After treatment, fish of both groups were tagged with plastic tags and stocked into a 5.5-ha pond together with common carp *(Cyprinus carpio),* Taiwanese red tilapia (*Oreochromis mossambicus/O. niloticus* hybrid line), grey mullet *(Mugil cephalus)* and Chinese carps (silver carp, *Hypophthalmichthys molitrix* and grass carp, *Ctenopharyngodon idella)* to examine their growth under typical Israeli polyculture conditions. The fish were stocked at the end of May and harvested in December 1986. The stocking densities were: common carp, 8,040/ha; red tilapia, 5,000/ha; grey mullet, 510/ha; silver carp, 400/ha; and grass carp, 170/ha.

Results

Hatchery experiment

The mean final weights (± SEM) of the fish fed 15 mg 17 ET/kg, 60 mg 17 ET/kg and the controls were 76.5 ± 3.4 g, 70.5 ± 3.4 g and 62.8 ± 2.2 g, respectively. Both treated groups grew significantly faster than the control, but there was no significant difference between the treated groups (Fig. 2). This indicated that 17 ET does accelerate growth in tilapia and suggested the use of lower 17 ET doses in further studies.

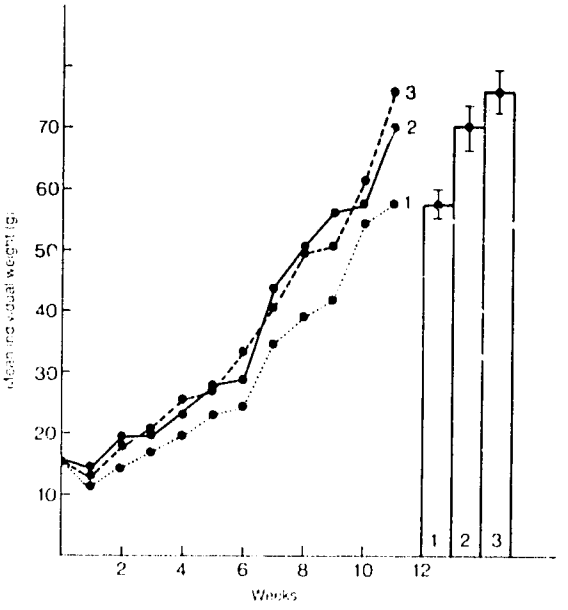

Fig. 2. Growth and final weights (± SEM) of tilapia (*Oreochromis niloticus* x *O. aureus* F_1 hybrids) all-male fingerlings in a hatchery experiment on feeds with or without 17α-ethynyltestosterone (17 ET). Treatment 1 = controls; Treatment 2 = 60 mg 17 ET/g food; Treatment 3 = 15 mg 17 ET/g food. The plotted points are means of random samples of 30 fish from a population of 250/treatment tank.

Cage trial 1

Table 1 and Fig. 3 summarize the results. The mean final weights fell into three subsets (A, B and C) and differed significantly from each other (P = 0.01). The ranking of final weights was inversely correlated with the 17 ET concentration. However, no clear relationship was noted between final weights and the duration of treatment. The highest weight gain was

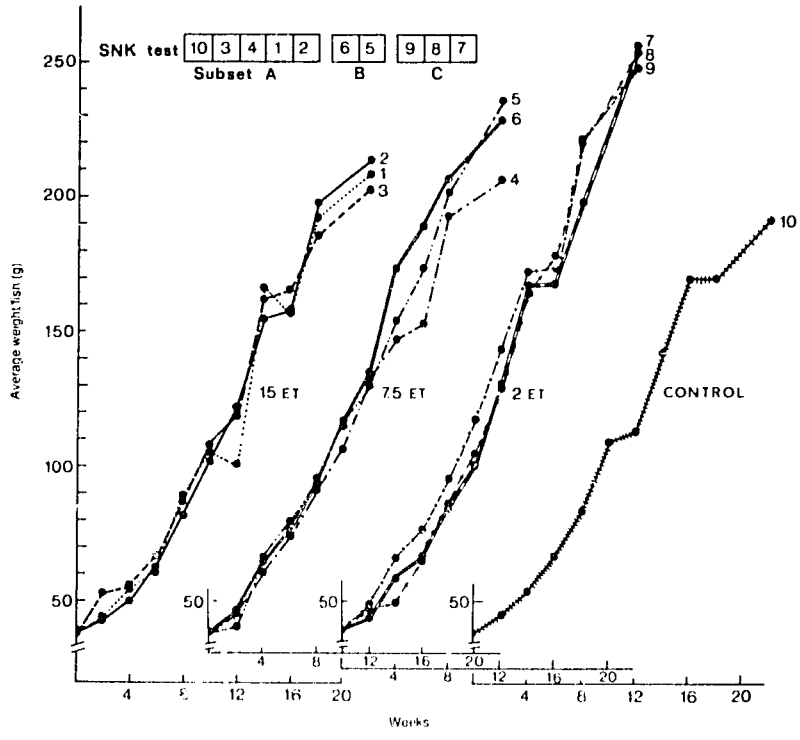

Fig. 3. Mean weights of all-male tilapia (*Oreochromis niloticus* x *O. aureus* F_1 hybrids) fed various concentrations of 17α-ethynyltestosterone (17 ET) at 15, 7.5 and 2 mg/kg food. Numbers at the top of each curve indicate the duration of treatment (1, 4, 7 - two weeks; 2, 5, 8 - four weeks; 3, 6, 9 - eight weeks). Grouping of final mean weights (A, B, C) according to an SNK test is shown at the top. See Table 1 and text (Cage Trials) for further details.

Table 1. Statistical analysis of the mean final weights ± SEM (g) of all-male tilapia F_1 hybrids *(O. niloticus* x *O. aureus)* of mean initial weight 36.8 g (± SEM = 1.4) stocked in growth of 150 in cages suspended in an earthen pond and fed 17α-ethynyltestosterone (17 ET) in food pellets at various concentrations for various durations. The groups A, B, C are significantly different (SNK test, P = 0.01).

Duration of treatment (weeks)	Dosage of 17 ET (mg/kg food) 2.0	7.5	15.0	0	Mean
0				A 192.7 ± 9.3	
2	C 255.6 ± 10.9	A 206.4 ± 12.6	A 209.2 ± 12.5		223.7
4	C 254.7 ± 14.6	B 235.1 ± 12.8	A 213.6 ± 11.4		234.5
8	C 248.6 ± 12.9	B 228.6 ± 11.4	A 202.9 ± 12.3		226.7
Mean	253.0	223.4	208.6		

achieved in the group fed on 2 mg/kg 17 ET.

Cage trials 2 and 3

The results are summarized in Table 2 and Figs. 4 and 5.

Fish treated with 17 ET generally reached final weights higher than their respective controls. However, a significant difference ($P < 0.05$) between treated and untreated fish occurred only in the small and large graded groups in the spring trial, and in the mixed and large graded fish in the summer trial. The daily weight gain in the large graded groups in both trials was considerably higher than that in the small graded groups; that of the ungraded fish had an intermediate value.

Growth trial in commercial pond polyculture

The results are summarized in Fig. 6. About 60% of the fish stocked were recovered, still carrying their tags, when the

Table 2. Effect of initial size and age on growth acceleration of all-male tilapia F1 hybrids *(Oreochromis niloticus* x *O. aureus)* in cages suspended in an earthen pond, by feeding 17α-ethynyltestosterone at 2 mg/kg food. The data are means ± SEM. For details of culture conditions and feeding rates, see text (Cage trials 2 and 3).

Duration of experiment (weeks)	Trait	Small graded		Mixed		Large graded	
		Treated	Control	Treated	Control	Treated	Control
Cage Trial 2 (Spring Trial)							
23 Apr. to 7 Oct. 1985 (24)	Initial mean weight ± SEM (g)	2.26 ± 0.24		4.87 ± 0.66		9.07 ± 1.14	
	Survival (%)	90.5	96.0	95.0	95.5	96.5	92.5
	Final mean weight ± SEM (g)	(a) 175.9±7.6	143.7±7.75	n.s. 186.5±9.6	172.9±10.25	(a) 240.0±11.1	184.2±7.47
	Daily weight gain/fish (g)	0.96	0.84	1.08	1.00	1.34	1.04
Cage Trial 3 (Summer Trial)							
7 July to 15 Oct. 1985 (14)	Initial mean weight ± SEM (g)	39.1 ± 1.8		56.9 ± 3.2		71.3 ± 3.8	
	Survival (%)	97.0	95.5	90.0	92.5	93.5	94.0
	Final mean weight ± SEM (g)	n.s. 155.8±6.8	143.6±5.8	(a) 206.6±8.5	182.9±7.9	(a) 243.5±9.2	214.6±10.5
	Daily weight gain/fish (g)	1.19	1.06	1.52	1.38	1.76	1.46

(a) - significantly different from the control ($P < 0.05$).
n.s. - not significantly different from the control ($P < 0.05$).

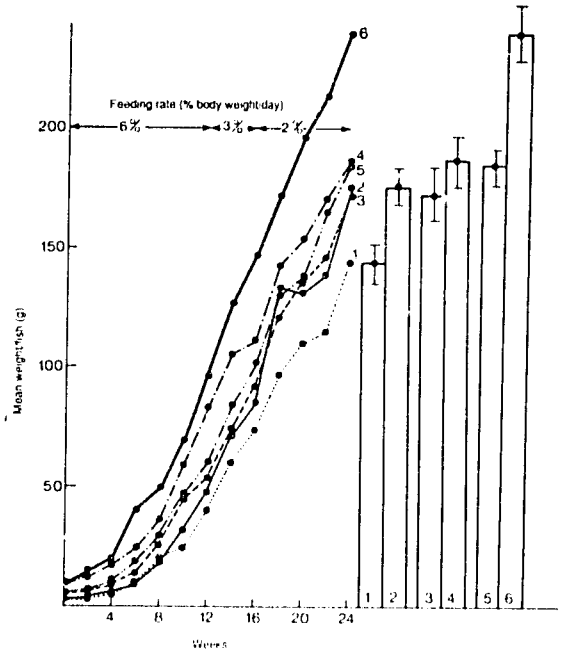

Fig. 4. Growth curves and final mean weights (± SEM) of all-male tilapia (*Oreochromis niloticus* x *O. aureus* F1 hybrids) in three size combinations fed for two weeks with 17α-ethynyltestosterone (17 ET) at 2 mg/kg food and thereafter raised in cages suspended in an earthen pond. The size combinations/groups are: 1, small size control (no 17 ET treatment); 2, small size, 17 ET treatment; 3, mixed size control; 4, mixed size, 17 ET treated; 5, large size control; 6, large size, 17 ET treated. For further details, see text (Cage Trial 2).

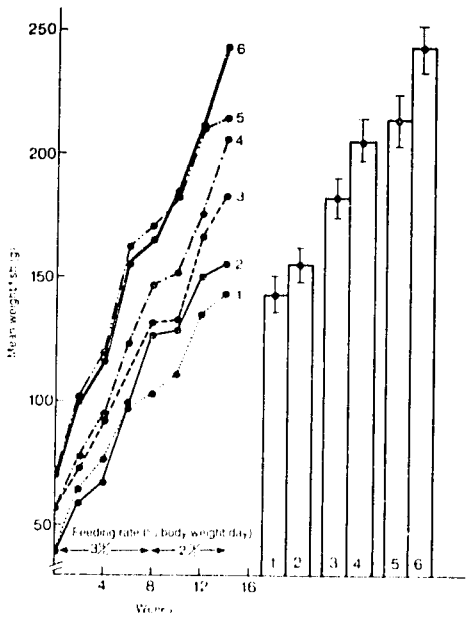

Fig. 5. Growth curves and final mean weights (± SEM) of all-male tilapia (*Oreochromis niloticus* x *O. aureus* F1 hybrids) in three size combinations fed for two weeks with 17α-ethynyltestosterone (17 ET) at 2 mg/kg food and therefore raised in cages suspended in an earthen pond. The size combinations/groups are: 1, small size control (no 17 ET treatment); 2, small size, 17 ET treated; 3, mixed size, control; 4, mixed size, 17 ET treated; 5, large size, control; 6, large size, 17-ET treated. For further details, see text (Cage Trial 3).

pond was drained in December. A further 20% of the fish had lost their tags during the experiment, but could be identified by their color from the commercial stock of red tilapias. The final mean weight of 17 ET-treated fish significantly exceeded that of the controls by more than 11% (382.0 ± 6.2 g vs. 343.0 ± 7.3 g; Fig. 6). Sampling of fish on two occasions during the experiment indicated reduced growth in both groups between September and December, probably due to low water temperature.

Discussion

The objective of the present study was to determine the optimal conditions for growth enhancement in tilapia by a synthetic androgen, under hatchery and grow-out conditions. Previous reports demonstrating androgen growth enhancement in tilapia are based on comparison of all-male hormone-treated fry (for sex inversion of females) and untreated fish of both sexes (Guerrero 1975, 1976; Donaldson et al. 1979; Owusu-Frimpong and Nijjhar 1981). The present study was conducted on all-male hybrid populations which had also been treated with hormone for sex inversion (Rothbard et al. 1983). Our results show that hybrid tilapia fed 17 ET after hormone treatment for sex inversion grew significantly faster than the sex-inversed controls that received no further 17 ET.

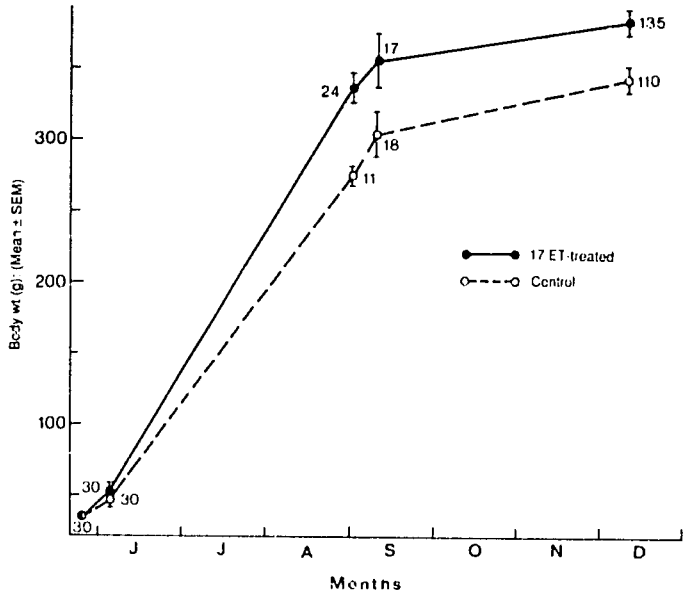

Fig. 6. Growth curves of all-male tilapia (*Oreochromis niloticus* x *O. aureus* F_1 hybrids) reared in a 5.5-ha commercial pond under typical levels polyculture conditions with common carp, Chinese carps and Taiwanese red tilapia after two weeks treatment with 17α-ethynyltestosterone (17 ET) at 2 mg/kg food or control feeding (no 17 ET). Mean weights ± SEM; numbers on the curve indicate sample size. For further details, see text (Growth trial in commercial polyculture).

Two doses of 17 ET (60 and 15 mg/kg food) showed similar growth-enhancing effects in fish reared in tanks (Fig. 2). When tested in cages suspended in a pond, the dose of 15 mg/kg food was ineffective while lower doses of 7.5 and 2.0 mg/kg resulted in growth enhancement (Fig. 3). It is possible that under the improved growth conditions in the pond, as compared with the conditions in the tanks, 17 ET at 15 mg/kg food may have been an overdose while lower concentrations of the androgen were more effective.

Similar results indicating a higher growth response to relatively lower doses of synthetic androgens have been reported for common carp. Feeding with 17α-methyltestosterone (17 MT) at concentrations of 1-5 mg/kg food resulted in 40% weight increase above the control, whereas, at 10 mg 17 MT/kg food, growth was lower (Lone and Matty 1980). It should be emphasized that the carp were treated for 90 days whereas in the present study the duration of treatment was much shorter (14-56 days). There was no advantage in extending the treatment over 2 weeks.

A similar concentration of 17 MT (2.5 mg/kg food) was effective as a growth enhancer also in juvenile coho salmon and proved to be more effective than the naturally occurring steroids, testosterone and estradiol (Yu et al. 1979).

Hormonal treatment of small fry to sex inverse any females nursing to 10-50 g and stocking into overwintering facilities are regular procedures in commercial aquaculture in Israel. Here we investigated the effect of initial size of overwintered fish on growth enhancement in response to 17 ET. In both spring and

summer cage trials, large graded fish responded to the treatment better than the small graded or mixed-size groups. It would appear that some interference occurred in the spring trial where fish of various sizes were stocked together in the same cage. Under these conditions the growth enhancement effect of 17 ET over controls was not apparent.

The results of the commercial polyculture growth trial indicate that 17 ET at a dose of 2 mg/kg food, given for 2 weeks to fish under 50 g can significantly enhance growth of tilapia hybrids under regular conditions of polyculture in Israel.

The metabolic clearance of 17 ET from the fish and its fate in the culture environment are currently under investigation. When the results are available, we shall be able to decide on whether to recommend 17 ET treatment procedures as a management tool for growth enhancement in Israeli tilapia culture.

Acknowledgements

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Response of Nile Tilapia *(Oreochromis niloticus)* Fry to Diets Containing *Azolla* Meal

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Abstract

Sun-dried *Azolla pinnata* was ground and incorporated into experimental diets at various levels (8.50, 17.00, 25.46, 34.00 and 42.45% of the diets) to replace fish meal in a control diet isonitrogenously. All feeds contained 35% crude protein and 250 kcal digestible energy/100 g. They were fed to Nile tilapia *(Oreochromis niloticus)* fry (mean body weights, 14.9 mg in Experiment I and 11.2 mg in Experiment II) at 45% of fish biomass daily for 7 weeks. Results of the two experiments showed that *Azolla* meal is a suitable component of diets for Nile tilapia fry. Growth increased and feed conversion ratios improved as the level of the dietary *Azolla* meal increased. Survival rates were not affected by the levels of *Azolla* in the diets.

Introduction

Nile tilapia *(Oreochromis niloticus)* is an important tropical food fish that readily takes prepared feeds from the fry stage to adult size. Inasmuch as feeds play a major role in the production of fish in certain culture systems, adequate but relatively inexpensive feed supply is needed and the utilization of nonconventional feedstuffs like those already developed for land animals (Devendra 1985) is highly encouraged.

The *Azolla-Anabaena* symbiont has been successfully used as an organic nitrogenous fertilizer in agriculture (Lumpkin and Plucknett 1980) and its consumption by fish has also been

reported (Lahser 1967; Cassani 1981). A number of studies on the nutritional value of *Azolla pinnata* as a nonconventional feed for Nile tilapia have been undertaken (Pullin and Almazan 1983; Almazan et al. 1986; Pantastico et al. 1986). This study was conducted to determine the effect of various levels of dietary *A. pinnata* meal on the survival and growth of Nile tilapia fry.

Materials and Methods

Preparation of Azolla meal

A. pinnata was cultured in outdoor tanks using water from Laguna de Bay (a eutrophic lake adjacent to Metropolitan Manila) and cow dung as fertilizer. *Azolla* was harvested, rinsed with tap water and sun-dried. The dried *Azolla* was ground finely using a food grinder and blender and then passed through a No. 60 standard testing sieve (250 µm mesh size).

Experimental diets

Two sets of six diets each were formulated for two feeding experiments. Diets were designed to contain 35% crude protein and 250 kcal digestible energy/100 g. Table 1 presents the composition of diets for Experiment I. Fish meal (Peruvian) in the control diet was isonitrogenously substituted by *Azolla* meal. Because of the restrictions on protein and energy levels, the highest amount of *Azolla* meal incorporated in the diets was about 42%. Cod liver oil and vegetable oil served mainly as energy supplements. Dextrin was added as a low-density energy source to preclude the use of any non-nutritive filler. The control diet contained fish meal as sole source of protein and dextrin as an energy supplement. Vitamin and mineral premixes were kept constant in all diets.

For Experiment II, levels of fish meal, *Azolla* meal, and vitamin and mineral premixes in the test diets were the same as in the diets for Experiment I. However, the amounts of dextrin were reduced, the levels of oil supplements were increased and kept within a narrow range, and cellulose was used as a filler (Table 2). All diets were pelleted and then crumbled before feeding to the fish.

Proximate analyses of ingredients and diets for Experiment I were made in our laboratory and in a nearby analytical service laboratory using standard methods (Lovell 1975). Duplicate determinations were made per sample. Diets for Experiment II were not analyzed.

Experimental fish and management

There were six dietary treatments with three replicates each. Eighteen aerated glass aquaria (60 x 30 x 30 cm)

Table 1. Ingredient composition (%) of diets for *Oreochromis niloticus* fry used in Experiment I.

Ingredient	Diet Number 1	2	3	4	5	6
Fish meal	55.82	52.45	49.06	45.70	42.30	59.25
Azolla meal	8.50	17.00	25.46	34.00	42.45	-
Dextrin	30.85	24.92	18.75	12.47	6.26	36.42
Cod liver oil	0.25	0.65	1.20	1.75	2.33	-
Vegetable oil	0.25	0.65	1.20	1.75	2.33	-
Vitamin mix*	0.73	0.73	0.73	0.73	0.73	0.73
Mineral mix*	3.60	3.60	3.60	3.60	3.60	3.60
Estimated crude protein (%)	35.0	35.0	35.0	35.0	35.0	35.0
Analyzed crude protein (%)	33.2	34.0	34.4	35.4	35.5	34.2
Estimated digestible energy (kcal/100g diet)	250	250	250	250	250	250

*For complete and practical diets (NRC 1977).

Table 2. Ingredient composition (%) of diets for *Oreochromis niloticus* fry used in Experiment II.

Ingredient	Diet Number 1	2	3	4	5	6
Fish meal	55.82	52.45	49.06	45.70	42.30	59.25
Azolla meal	8.50	17.00	25.46	34.00	42.45	-
Dextrin	15.97	13.14	10.35	7.49	4.71	18.77
Cellulose	10.60	8.22	5.86	3.48	1.13	12.95
Cod liver oil	2.39	2.43	2.47	2.50	2.54	2.35
Vegetable oil	2.39	2.43	2.47	2.50	2.54	2.35
Vitamin mix*	0.73	0.73	0.73	0.73	0.73	0.73
Mineral mix*	3.60	3.60	3.60	3.60	3.60	3.60
Estimated crude protein (%)	35.0	35.0	35.0	35.0	35.0	35.0
Estimated digestible energy (kcal/100g diet)	250	250	250	250	250	250

*For complete and practical diets (NRC 1977).

were used for each experiment. Nile tilapia fry were stocked randomly at 100 per aquarium in 20 l of tap water. Initial mean body weights and total lengths (± S.D.) were: Experiment I, 14.9 (1.7) mg, 11.2 (0.7) mm; Experiment II, 11.2 (1.7) mg, 10.8 (0.9) mm. The fry came from the same broodstock in outdoor concrete tanks. Fry were fed the experimental diets at 45% of their biomass daily for 7 weeks, split between three feedings at 0730, 1130 and 15.) hours. Aquaria were cleaned once or twice a day and up to two-thirds of the water was replaced during cleaning. A complete change of water was made after the weekly sampling of fish.

All fish per aquarium were weighed in bulk and counted at weekly intervals to determine growth rates and to adjust rations. After 7 weeks, the total lengths of ten fry from each aquarium were measured. Experiment I ran from 13 October to 1 December 1983. The water temperature ranged from 24 to 29°C. Experiment II ran from 6 February to 26 March 1984. The water temperature ranged from 23 to 29°C.

Statistical methods

Data on weight gain, increase in total length, feed conversion ratio and survival for each experiment were analyzed by one-way ANOVA. Differences in means were compared by Duncan's New Multiple Range Test ($\alpha = 0.05$).

Results

Experiment I

Growth was similar on all diets after 1 week (Fig. 1). Thereafter, however, there were significant differences ($P < 0.05$) in mean body weights. Weight gain after 7 weeks increased as the level of *Azolla* meal increased up to 34% of the diet (Table 3). With the 42.4% *Azolla* diet, weight gain decreased slightly. However, there were no significant differences ($P > 0.05$) among the weight gains of the tilapia fed diets containing 25.5, 34.0 and 42.4% *Azolla* meal. The mean increases in total length followed the trend of weight gain (Table 3). Weight gain and increase in total length of the tilapia fed the control diet were significantly lower ($P < 0.05$) than those for fish fed diets containing some *Azolla* meal. Survival rates, ranging from 84 to 92%, did not differ significantly among treatments.

Feed conversion ratios, calculated as g of feed given per g weight gain, decreased (improved) significantly ($P < 0.05$) as the dietary *Azolla* meal increased from 8.5 to 25.5%. There were no significant differences in the feed conversion ratios for diets containing 25.5, 34.0 and 42.4% *Azolla* meal. The control diet gave the highest (poorest) feed conversion among all diets.

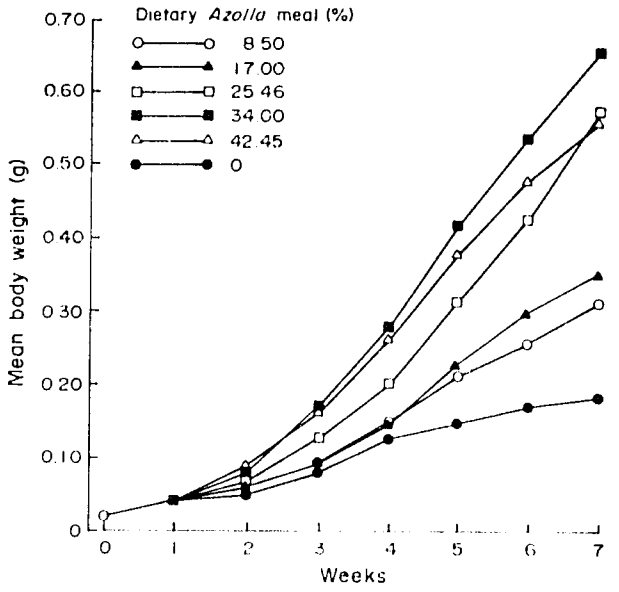

Fig. 1. Mean body weight of Nile tilapia *(Oreochromis niloticus)* fry fed diets containing various amounts of *Azolla* meal. For further details, see Table 1 and text (Experiment I). The plotted points are means of all fry from each of triplicate aquaria for each diet.

Table 3. Growth, survival and feed conversion ratio of Nile tilapia *(Oreochromis niloticus)* fry fed diets containing various levels of *Azolla* meal for 7 weeks. For details of diets and procedures, see Tables 1, 2 and text. Data on columns bearing different suffix letters (a-d) are significantly different: $P < 0.05$.

Diet Number	*Azolla* meal (%)	Weight gain (mg)	Increase in total length (mm)	Feed conversion ratio	Survival (%)
*Experiment I**					
1	8.50	295.8^{b}	15.0^{b}	3.4^{b}	84^{a}
2	17.00	333.3^{b}	16.3^{b}	3.2^{b}	92^{a}
3	25.46	568.7^{a}	19.1^{a}	2.3^{c}	86^{a}
4	34.00	643.4^{a}	19.8^{a}	2.7^{bc}	91^{a}
5	42.45	562.4^{a}	19.5^{a}	2.8^{bc}	87^{a}
6 (control)	0	176.7^{c}	11.9^{c}	4.4^{a}	84^{a}
*Experiment II***					
1	8.50	161.6^{d}	12.5^{b}	4.4^{ab}	81^{a}
2	17.00	182.3^{cd}	14.0^{b}	4.6^{a}	85^{a}
3	25.46	201.9^{c}	14.4^{b}	4.0^{bc}	84^{a}
4	34.00	368.3^{b}	17.7^{a}	2.9^{d}	82^{a}
5	42.45	520.4^{a}	15.6^{a}	2.7^{d}	87^{a}
6 (control)	0	393.5^{ab}	19.6^{a}	3.6^{c}	85^{a}

*Initial body weight = 14.9 ± 1.7 mg; initial total length = 11.2 ± 0.7 mm.
**Initial body weight = 11.2 ± 1.7 mg; initial total length = 10.8 ± 0.9 mm.

Experiment II

The growth curves of the tilapia in Experiment II are shown in Fig. 2. Significant differences ($P < 0.05$) in mean body weights were found as early as 2 weeks. Mean weight gain after 7 weeks increased with the increasing level of dietary *Azolla* meal (Table 3). The highest gain in weight was attained by fish fed the 42.4% *Azolla* diet. The weight gain of fish fed the control diet was comparable to that of fish fed the 42.4% *Azolla* diet or the 34% *Azolla* diet, and was significantly higher than the weight gains of fish in the other three dietary treatments. Similarly, total length after 7 weeks increased as the dietary *Azolla* meal increased from 8.5 to 42.4% (Table 3). However, mean increases in total length of fish fed the control diet, the 34 and 42.4% *Azolla* diets did not differ significantly ($P < 0.05$).

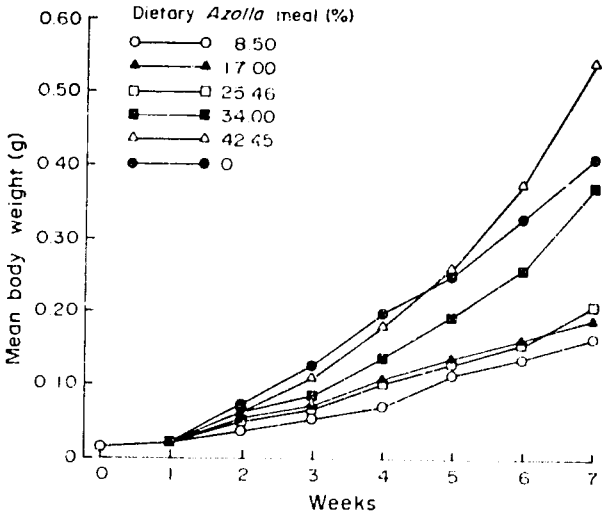

Fig. 2. Mean body weight of Nile tilapia *(Oreochromis niloticus)* fry fed diets containing various amounts of *Azolla* meal. For further details, see Table 2 and text (Experiment II). The plotted points are means of all fry from each of triplicate aquaria for each diet.

Survivals ranged from 81 to 87% and were not significantly different (Table 3). Feed conversion ratios improved as the dietary level of *Azolla* meal increased. The 34 and 42.4% *Azolla* diets gave the best feed conversion followed by the control diet (Table 3).

Discussion

Results have clearly demonstrated the positive growth response of the young Nile tilapia to increasing levels of dietary *Azolla* meal. For diets with higher amounts of *Azolla* meal to produce better growth than those with lower amounts of *Azolla* was rather unexpected. It was initially thought that the growth responses in Experiment I were also directly influenced by the increasing levels of supplemental oils to make the diets isocaloric. The poor growth of the fry fed the control diet was probably due to the absence of oils in the diets.

In Experiment II wherein supplemental oils were present in all diets and maintained within a narrow range, there was a definite improvement on the growth of the tilapia fed the control diet. Although weight gains of fry fed diets containing 8-25% *Azolla* meal were significantly low, the trend of growth responses of the tilapia fed the *Azolla* diets was similar to that in Experiment I.

The Peruvian fish meal used in the study had the following proximate composition: crude protein, 59.1%; ether extract, 5.3%; ash, 21.2%; and nitrogen-free extract, 11.3%. The *Azolla* contained 23.6% crude protein, 1.3% ether extract, 8.6% crude fiber, 15.7% ash and 37.7 nitrogen-free extract. The lipid component of *Azolla* appears low, but the estimated digestible energy based on values of 3.5, 8.1 and 2.5 kcal/g for protein, fat and nitrogen-free extract (NRC 1977), respectively, was 1.87 kcal/g. This gives an energy to protein ratio of 7.9 kcal/g protein which is near the range (8-9 kcal/g) optimum for Nile tilapia (Kubaryk 1980). Moreover, although the fatty acids were not analyzed, it is highly possible that *Azolla*, being a plant feedstuff, contributed some of w6 fatty acids which are required by Nile tilapia (Teshima et al. 1982). This may explain the faster growth response of the tilapia to increasing dietary *Azolla* meal.

Fresh *A. pinnata* was preferred less than *A. filiculoides* by Nile tilapia fingerlings (Antoine et al. 1986). However, fresh *A. pinnata* as supplemental feed was also found effective in enhancing growth of Nile tilapia fingerlings in cages in Laguna Lake (Pantastico et al. 1986). On the other

hand, *A. pinnata* (fresh, dried powder or dried pellet form) as the only feed caused weight loss in Nile tilapia fingerlings and adult males (Almazan et al. 1986). Obviously, *Azolla* is not by itself a complete feed for tilapia. Based on the amino acid analysis (Almazan et al. 1986), *A. pinnata* is low in tryptophan and threonine compared to the requirements for the same amino acids reported for *O. mossambicus* (Jauncey et al. 1983) and *O. niloticus* (Santiago 1985). Other essential amino acids may actually be limiting when corrected for their biological availability. As a dietary component and source of some protein for tilapia, *Azolla* needs supplementation of deficient nutrients particularly when the formulated diet is the only source of nourishment. While fresh *Azolla* may be used as supplemental feed, the dry *Azolla* meal as a dietary component offers the advantage of being more concentrated in nutrient content.

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Effects of Feeding Frequency on Growth, Food Conversion and Survival of Red Tilapia *(Oreochromis mossambicus/O. niloticus)* Hybrid Fry

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Abstract

A study was conducted on the effects of feeding frequency on growth, food conversion and survival of red tilapia *(Oreochromis mossambicus/O. niloticus)* hybrid fry, mean weight (± S.D.), 1.17 (± 0.11) g. They were fed at five feeding frequencies: three times a day (R 1), twice a day (R 2), once a day (R 3), every 2 days (R 4) and *ad libitum* (R 5) for 5 weeks at 10% body weight/day. No feeding was done on the seventh day of every week, when fish were weighed.

There were no significant differences ($P > 0.05$) in mean weight gain among R 1 (4.90 g), R 2 (5.00 g) and R 5 (5.88 g), but these treatments were significantly different from R 3 (3.56 g) and R 4 (3.36 g) ($P < 0.05$). Food conversion ratios were significantly different for R 1 (2.240) and R 2 (2.097) from other treatments ($P < 0.05$). Fry fed *ad libitum* (R 5) showed poor food conversion but good growth. Mean survival was high in all treatments: R 1, 94.8%; R 2, 96.7%; R 3, 92.0%; R 4, 96.0% and R 5, 93.4%. These were not significantly different ($P > 0.05$). The study showed that the optimal feeding frequency was twice a day. These results are discussed in relation to other work on feeding frequency.

Introduction

Little information is available on the effects of feeding frequency on fish growth. Red tilapia, an *Oreochromis mossambicus/O. niloticus* hybrid, was selected as the species for this investigation, being a popular food fish in Malaysia. Tilapia species vary in their feeding habits (Jauncey and Ross 1982). Most cultured

species, including red tilapias, are continuous feeders and feed efficiently in the water column as well as from the pond bottom. Lovell (1980) found that *Oreochromis niloticus* consumed the most food and grew fastest when fed from 4 to 8 times daily but consumed food continuously.

The aim of this study was to determine the effects of feeding frequency on growth, food conversion and survival of red tilapia fry.

Materials and Methods

The study was conducted at the Fish Hatchery Unit, Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia (UPM). Red tilapia fry (an *Oreochromis mossambicus/O. niloticus* hybrid originating from Taiwan) were obtained from Durian Tunggal Melaka fish farm.

A water recirculation system was constructed consisting of fifteen 80-l glass aquaria which drained into a 200-l filter tank containing 0.5-2.0 cm gravel. Water flowed from one aquarium to the next at 1.5 l/min by air lift pumps.

A total of 350 red tilapia fry [mean weight ± S.D., 1.17 (± 0.11) g] were stocked at 25 fish per aquarium. They were acclimatized to aquarium conditions for 2 weeks prior to the experiment. All 350 fish were individually weighed and measured at the start of the experiment and subsequently every 7 days. No feed was given each 7th day.

Shrimp meal (locally made) was used as the dietary protein source. An experiment diet was formulated with a 35% protein content (Table 1). The diet was prepared by thoroughly mixing the dry ingredients and then adding water until a stiff dough resulted. This was then passed through a meat mincer (3-mm diameter die) and the resulting 'spaghetti-like' strings were dried in a convection air dryer at 35°C. The dry feed was broken up into convenient pellet size. Samples of diet were subjected to proximate analysis, the results of which are also presented in Table 1.

Table 1. Composition of an experimental diet for red tilapia *(Oreochromis mossambicus/O. niloticus)* fry (% dry weight).

Component	% dry weight
Shrimp meal	73.7
Palm oil kernel cake	14.3
Soya bean meal	6.7
Fish meal	3.3
Vitamin premix	1.0
Mineral premix	1.0
Crude protein	34.72
Crude lipid	8.41
Crude fiber	8.82
Ash	7.11
Moisture	6.04

Five feeding frequencies were used in this experiment (Table 2). Except for treatment R 5, fish were fed at 10% body weight per day. Each treatment was replicated three times and statistical analyses were carried out by one-way ANOVA and the Duncan's New Multiple Range Test.

Table 2. Feeding frequencies for the red tilapia (*Oreochromis mossambicus/O. niloticus* hybrid) fry used to assess the effects of feeding frequency on growth.

Treatment	Feeding frequency	Times (hr)
R 1	3 times a day	0800, 1600, 2400
R 2	2 times a day	0800, 2000
R 3	once a day	0800
R 4	every 2 days	0800
R 5	*ad libitum*	

Results

The results are summarized in Table 3 and in Fig. 1. The average growth rates at R 3 (once a day) and R 4 (every 2 days) were significantly lower ($P < 0.05$) than those of the other treatments. However, there was no significant difference ($P > 0.05$) among R 1 (three times a day), R 2 (two times a day) and R 5 *(ad libitum)* or was between R 3 and R 4. Feed conversion ratios (Table 3) for R 2 and R 1 were significantly better ($P < 0.05$) than R 3, R 4 and R 5. Percentage survivals for all feeding frequencies were high (Table 3) and not significantly different ($P > 0.05$).

Discussion

The results of this study favor twice a day feeding for optimal growth food conversion. This is in agreement with Grayton and Beamish (1977) who reported that further increasing feeding frequency in rainbow trout resulted in worse food conversion. We found that feeding three times a day and *ad libitum* gave worse food conversion.

Frequent feeding increases food intake and growth only up to a limit, and is governed by appetite which in turn depends on the amount of food in the stomach (Brett 1971). Feeding frequency

Table 3. Initial and final weights, food conversion ratio, specific growth rate and survival of red tilapia (*Oreochromis mossambicus/O. niloticus* hybrid) fry, given 35% protein pelleted feed at different feeding frequencies (R1 - R5). The data are grand means of triplicate aquaria for each R treatment, with 25 fish/aquarium. For details, see Table 2 and text.,

Feeding frequency	R 1	R 2	R 3	R 4	R 5
Initial weight (g)	1.16[a]	1.17[a]	1.17[a]	1.22[a]	1.16[a]
Final weight (g)	4.90[b]	5.00[b]	3.56[b]	3.36[b]	5.88[b]
Average weight gain (g)	3.74[b]	3.83[b]	2.39[a]	2.14[a]	4.72[b]
Specific growth rate (%/day)	3.43[a]	3.46[a]	2.65[b]	2.41[b]	3.86[a]
Food conversion ratio	2.24[a]	2.01[a]	3.12[b]	3.31[b]	3.61[c]
Survival (%)	94.8 ± 1.6[a]	96.7 ± 0.5[a]	92.0 ± 1.7[a]	96.0 ± 1.7[a]	93.4 ± 1.1[a]

Means within the same row followed by different letters are significantly different ($P < 0.05$).

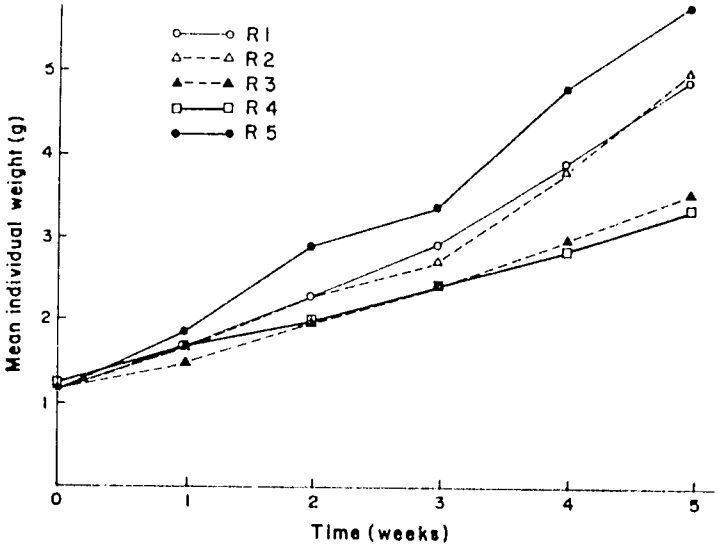

Fig. 1. Growth of red tilapia (*Oreochromis mossambicus/O. niloticus* hybrid) fry fed on a 35% protein pelleted feed at different feeding frequencies (R1 - R5). The plotted points are grand means from triplicate aquaria for each R-treatment, with 25 fish/aquarium. For details, see Table 2 and text.

also varies with species, size and age and with environmental factors and food quality (Grayton and Beamish 1977). The optimum feeding frequency for *Clarias lazera* is continuous feeding (Hogendoorn 1981); for striped bass, four times a day (Powell 1972); for *Ictalurus punctatus*, twice a day (Andrews and Page 1975); for *Channa striata* (Sampath 1981) and *Heteropneustes fossilis* once a day (Marian et al. 1982) and for *Epinephelus tauvina* (Chua and Teng 1978), once in 2 days. Clearly more research is needed on the effects of feeding frequency on growth performance of different tilapia species at different life history stages.

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Influence of *Chlorella vulgaris* and *Microcystis aeruginosa* Mixed with Commercial Carp Pellets on Growth of Nile Tilapia *(Oreochromis niloticus)*

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Abstract

Phytoplankton (the green alga *Chlorella vulgaris* and the cyanobacterium *Microcystis aeruginosa*) were fed to Nile tilapia *(Oreochromis niloticus)* fry (0.40-2.44 g) as additional feeds to commercial carp pellets (35% protein). Various combinations of phytoplankton and carp pellet feeding rates were assayed. The results showed that addition of phytoplankton improved growth on carp pellets but there were confusing effects from fatty degeneration of the liver for fish receiving higher ration of carp pellets (6-10% body weight per day) shown by histological structures on the hepatocytes, and toxicity effects from feeding high levels of *M. aeruginosa*. These effects are discussed in relation to tilapia nutrition.

Introduction

The phytoplankton that usually bloom in fishponds to which feed pellets and fertilizers are applied can be harvested by herbivorous fish, including tilapias (Edwards 1980). For better understanding and efficient utilization of such phytoplankton, we need to know more about its role in fish nutrition, particularly when other kinds of food are present.

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The aim of this paper was to evaluate the possibility of using common phytoplankton such as *Chlorella vulgaris* and *Microcystis aeruginosa* to reduce the use of feed pellets in intensive tilapia culture. The species studied was *Oreochromis niloticus*. An analysis of fish growth and a histological study of the hepatopancreas were performed.

Materials and Methods

Nile tilapia *(Oreochromis niloticus)* fry (0.40-0 55 g) were derived from a stock introduced to France from Bouaké, Côte d'Ivoire, in 1972. The fish were starved and acclimatized for 48 hours at 30°C prior to each experiment. Five fish were stocked in separate 10-l aquaria. Water was aerated and maintained at 30°C (± 1°C) with heaters. Temperature was monitored graphically on a continuous basis. The aquaria were illuminated by two fluorescent lights of 40 watts each (12D/12L photoperiod). Aquaria were cleaned and wastes siphoned out daily.

Monospecific algal cultures were made in sterile media until the quantity of algae reached a volume high enough for mass production in nonsterile conditions. Fluorescent light was provided continuously and temperature varied between 25 and 30°C. Exponentially growing cells were then used for tilapia feeding.

Chlorella vulgaris was cultured in a medium for the Chlorophyta (Dauta 1983). Numbers were counted using a Malassez Cell slide and cultures diluted to the required density. *Microcystis aeruginosa* was cultured with ASM medium (Moriarty et al. 1973). For *M. aeruginosa*, cell counts were very difficult and therefore absorption values were used as standard for dilution (Bianchini et al. 1985) and related to dry weight values (Fig. 1).

Carp pellets were ground and dried. Analysis was later performed for protein (Kjeldahl method); lipids (Folch et al. 1956), ash (samples combusted at 550°C for at least 12 hours in a muffle furnace); carbohydrate (by subtraction from the other nutrients) and water content (samples dried at 105°C for 4 hours). The phytoplankton was centrifuged at 8,000 revolutions/minute and washed twice with distilled water. It was preliminarily dried at 50°C and subsequently analyzed as for carp pellets. Results are presented in Table 1.

Carp pellets were given 6 days/week and the amount was adjusted weekly according to total fish weight. The daily quantity of carp pellet was divided into three and two meals for the first and second experiments, respectively. Phytoplankton was diluted and fed only once in

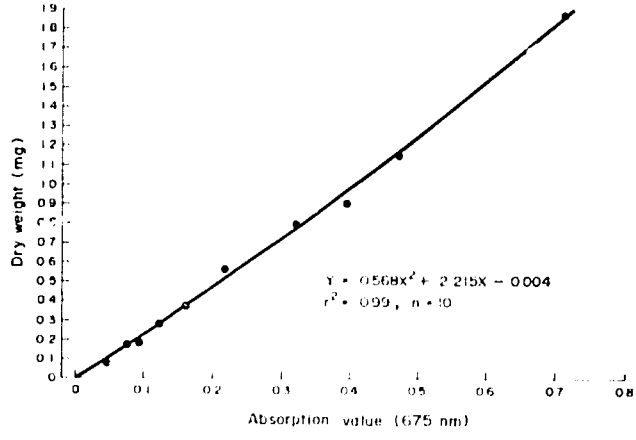

Fig. 1. Relationship between dry weight of *Microcystis aeruginosa* and absorption value at 675 nm of a suspension used to feed Nile tilapia *(Oreochromis niloticus)* fry.

Table 1. Nutrient composition of carp pellets, *Microcystis aeruginosa* and *Chlorella vulgaris*, used as feeds for *Oreochromis niloticus* fry: expressed on a percentage dry weight basis, unless stated otherwise.

Nutrients	Carp pellets	*M. aeruginosa*	*C. vulgaris*
Protein	35.2	50.5	49.5
Lipid	8.5	6.4	6.3
N-free extract	41.0	34.0	33.4
Fiber	5.7	3.7	3.6
Ash	9.6	5.4	7.3
Energy[a]	4,393.6	4,756.7	4,666.3
Protein/energy[b]	60.2	106.2	106.1

[a]Value calculated by using 5.5 kcal/g protein; 9.1 kcal/g lipid; 4.1 kcal/g N-free extract.
[b]As mg protein per kcal of 1 kg feed.

the morning for 6 days/week. Its density was kept as far as possible constant throughout the experiments. All fish were weighed and measured weekly to the nearest 0.01 g and 0.1 cm. Weight changes were used for calculating condition factor and specific growth rates. Due to the unknown quantities of phytoplankton and carp pellets consumed, *apparent* food conversion rate and protein efficiency ratio were calculated.

Two factorial feeding trials were performed, with two replicates, for a duration of 4 weeks each. Different rations of commercial carp pellets, of which the protein content (35%) was suitable for growing tilapia fry, were given by following the recommendation of Jauncey and Ross (1982).

In the first experiment, fish were fed with three rations of carp pellets (0, 10 and 20% fish body weight/day) combined with two levels of *C. vulgaris* (zero and 150×10^3 cells/ml).

In the second experiment, fish of 1.88-2.44 g were fed with two rations of carp pellets (3% and 6% of fish body weight/day) combined with four levels of *Microcystis aeruginosa* (at absorption values of zero, 0.025, 0.050 and 0.075). Data were analyzed for statistical differences using standard procedures such as Student's t test.

Fish liver samples were taken from 6 fish that had fed on carp pellets and carp pellets plus *M. aeruginosa* and were fixed in trichloroacetic Bouin's solution. After dehydration through graded ethanol and embedding in paraffin, sections were cut at 7 µm and stained with Periodic Acid Schiff or Schmorl's reagents.

Results

Fish fed on a 20% ration of carp pellets had a better growth than those on 10% ration (Table 2A). Weight gain increased when *C. vulgaris* was offered additionally. Compared with starved fish, mortality of fish fed only on *C. vulgaris* was higher. In the experiment using *M. aeruginosa*, the growth of fish fed on the 6% ration of carp pellets was higher than on the 3% ration (Table 2B). Statistically, growth rate was enhanced when fish were fed on *M. aeruginosa* and the 3% ration of carp pellets. Furthermore, the highest level of *M. aeruginosa* and 6% ration of carp pellet caused a poor fish growth (Table 2B).

Table 2. Mean individual weights, specific growth rate (SGR), mortality (M), apparent food conversion ratio (FCR), apparent efficiency ratio (PER) and feed conversion ratio, i.e., weight of carp pellets supplied/weight gain of fish (C/F) of Nile tilapia *(Oreochromis niloticus)* fed on carp pellets (0, 10, 20% body weight/day) and phytoplankton *(Chlorella vulgaris* and *Microcystis aeruginosa)*. The data presented are means of duplicate groups of five fish/group.

A. Experiment 1.

Treatment Carp pellets	*Chlorella vulgaris*	Fish weight (g) Beginning	End	SGR	M(%)	FCR	PER	C/F
0	0	0.52	0.45	(-)[1]	6.7	(-)[2]	(-)[2]	-
10%	0	0.48	1.99	5.25a	0	1.42a	1.99a	1.42a
20%	0	0.47	2.30	5.85a	0	2.62a	1.08a	2.62a
0	150 x 10[3]	0.51	0.56	(-)[1]	93	(-)[2]	(-)[2]	-
10%	150 x 10[3]	0.42	2.12a	5.93	0	(-)[2]	(-)[2]	1.13b
20%	150 x 10[3]	0.54	2.85a	6.14	0	(-)[2]	(-)[2]	2.54b

B. Experiment 2.

Treatment Carp pellets (% body weight)	*Microcystis aeruginosa* (absorption values)	Fish weight (g) Beginning	End	SGR	M	FCR	PER	CF
3	0	2.05	3.30	1.50b	0	1.44	1.99	a 1.46 b
3	0.025	2.02	3.32 a	a 1.78	0	1.46	1.74 a	a 1.25 b
3	0.050	2.03	3.67 a	2.15 b	0	1.36	1.92 a	1.04 b
3	0.075	2.05	3.88	2.32	0	1.42	1.81	0.96 b
6	0	2.08	5.51	3.54	0	1.10	2.60 a	1.10
6	0.025	2.07	5.55 a	3.60	0	1.26	2.21	a 1.18
6	0.050	2.19	5.98 a	3.61	0	1.35 a	2.04 a	1.18 a
6	0.075	2.00	5.17	3.45	0	1.47	1.84	1.20

[1] not calculated due to reduction in weight or significant mortality.
[2] not calculated due to the unknown weight of given *C. vulgaris*.
[3] for explanation - see text and Fig. 1.
Individual and grouped data bearing the same letters are not significantly different (P = 0.05).

In the first experiment using *C. vulgaris*, apparent FCR was calculated only for fish fed solely on carp pellets. FCR increased and PER decreased when feeding rates were increased. In the second experiment using *M. aeruginosa*, feeding 6% ration of carp pellets resulted in a decrease in FCR and an increase in PER when the quantity of *M. aeruginosa*, was also considered in calculating these. With increasing concentrations of *M. aeruginosa*, FCR and PER varied for the 3% ration of carp pellets; whereas FCR increased and PER decreased for the 6% ration.

Histological studies showed that hepatocytes from fish fed on the 3% ration of carp pellets and the 0.05 microcystis diet were in good condition with few lipofuscin positive. On the 0.075 microcystis diet, however, lifofuscins became abundant. With the 6% pellet ration and the 0.05 microcystis diet, the liver condition deteriorated: i.e., numerous lipid vacuoles and abundant lipofuscin positive cells appeared. With the 6% ration of carp pellets and high levels of *M. aeruginosa* (0.075) this condition was once more deteriorated.

Discussion

Good growth of Nile tilapia fry was obtained on carp pellets particularly at high rations. FCRs in the range 1.10-2.62 (Table 2) have been reported for other tilapias by Barash (1984) and Winfree and Stickney (1980), and have a curvilinear relationship with feeding rates (Shell 1969). Feeding live phytoplankton like *Chlorella* sp., *Euglena* sp., *Oscillatoria* sp., *Peridinium* sp. and *Scenedesmus* sp. results in poor growth of tilapias (Cridland 1960; Gophen 1980; Pantastico et al. 1985; Moreau et al. 1986). *M. aeruginosa* has a high protein content (Table 1) and appears to be a suitable food for tilapia fry. However, when given in large quantities it may cause problems. Although in this experiment, *M. aeruginosa* was not given alone to fish, poor growth resulted when it was fed at a high concentration. As mentioned by Gorham (1964) blue-green algae, including *Anabaena flos-aquae, Aphanizomenon flos-aquae* and *M. aeruginosa* can be toxic. This could explain the poor growth. Other blue green algae such as live *Chroococcus* sp. (Pantastico et al. 1985) and dry *Spirulina* sp. (Stanley and Jones 1976) can be given alone to tilapia. We therefore have reservations about feeding *M. aeruginosa* alone to Nile tilapia fry. *C. vulgaris* alone is also inadequate. Feeding here with the mixture of carp pellets and phytoplankton (*C. vulgaris* or *M. aeruginosa*) improved fish growth.

The analyses of *C. vulgaris* and *M. aeruginosa* demonstrated their nutritional adequacy for tilapia fry, as recommended by Jauncey and Ross (1982). However, digestibility is also important. The acid gut of Nile tilapia can digest the cyanobacterial (blue-green algal) cell wall better than the green algal cell wall (Moriarty 1973).

Liver histology revealed that feeding Nile tilapia fry with the 3% ration of carp pellets and 0.05 *M. aeruginosa* diet provided the best physiological conditions for the liver, although tilapia can usually utilize high carbohydrate diets efficiently (Anderson et al. 1984). It was shown here that *M. aeruginosa* can be used to feed tilapia fry up to the 0.05 dietary level.

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Nutritive Value of Methionine-Enriched Soybean Plastein for *Oreochromis niloticus* Fry

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Abstract

Feeding trials using *Oreochromis niloticus* fry were conducted to examine the effects of supplemental methionine as crystalline amino acid or enriched soybean plastein on weight gain, feed conversion efficiency (FCE), and protein efficiency ratio (PER). The fry (about 0.4 g) were maintained on diets with the following nitrogen sources (35% crude protein) for 5 weeks: diet 1, soybean protein:gelatin (3 : 1); diet 2, soybean protein:gelatin (3 : 1) + crystalline methionine; diet 3, soybean protein:gelatin (3 : 1) + methionine-enriched soybean plastein; diet 4, casein:gelatin (3 : 1). The lowest and highest weight gains were attained on diets 1 and 4, respectively. Growth was not enhanced by supplementing diet 1 with crystalline methionine. However, supplementing diet 1 with methionine-enriched soybean plastein gave significant improvements: weight gain ($P < 0.05$), FCE ($P < 0.10$), and PER ($P < 0.10$). These results indicate that methionine in soybean plastein is more effectively utilized by the fry *O. niloticus* than crystalline methionine. The present study proves the possibility of using methionine-enriched plastein to improve the nutritive value of methionine-deficient vegetable protein sources.

Introduction

Low nutritive value of dietary proteins for fish is often the result of shortage and imbalance of essential amino acids (EAA) and/or indigestibility and toxic factors of protein sources. It is common practice to attempt to improve the protein quality of fish diets by supplementing with the EAA that are in greatest deficit. However, the effectiveness of supplemental EAA is variable. Generally, some crystalline supplemented EAA are effective in improving the nutritive values of proteins for salmonoid fishes (Halver 1957; Halver et al. 1957; Halver and Shanks 1960) but not for

common carp, *Cyprinus carpio* (Aoe et al. 1970), channel catfish, *Ictalurus punctatus* (Dupree and Halver 1970; Andrews and Page 1974) or the shrimp, *Penaeus japonicus* (Deshimaru and Kuroki 1974, 1975).

Tilapia zillii has been shown to require the same 10 EAA, threonine, valine, methionine, leucine, isoleucine, phenylalanine, lysine, histidine, arginine and tryptophan as other fishes (Mazid et al. 1978). The growth of tilapias has been improved by supplementation of a soybean meal diet with the single amino acids, valine, phenylalanine or leucine (Wu and Jan 1977) and by supplementation with mixtures of lysine-arginine and methionine-tryptophan (Teshima et al. 1986a). However, the growth-enhancing effect of such crystalline amino acids is not marked, which raises the question whether the improved growth response justifies the increased cost. On the other hand, there is evidence that tilapias scarcely utilize for growth amino acids wholly or partially substituted for proteins (Mazid et al. 1978; Teshima et al. 1986a). These results infer that tilapias need diets with well-balanced amino acid profiles, in the form of peptides and proteins, rather than free amino acids in order to ensure goal growth.

Soybean meal is a promising vegetable protein source but is low in methionine and lysine (Table 1). In the present study, methionine was incorporated into a high molecular weight protein-like substance, called plastein, prepared from soybean protein. The methionine-enriched soybean plastein (Met-plastein) was tested for its growth-promoting effect on *O. niloticus*.

Table 1. Amino acid composition (%) of dietary protein sources and whole body protein of *Oreochromis niloticus* (0.3 g fry).

Amino acid	Soybean protein[2]	Met-enriched plastein	Soybean protein-gelatin (3 : 1)	Casein-gelatin (3 : 1)	*O. niloticus*
EAA[1]					
Thr	3.55	2.77	3.17	4.04	4.55
Val	4.63	3.88	4.18	4.49	5.60
Met	1.07	17.78	1.00	2.23	2.35
Ile	4.73	3.58	4.08	3.70	4.87
Leu	6.89	5.93	6.09	6.93	7.81
Phe	5.05	3.88	4.44	4.13	4.87
Lys	5.97	5.42	5.69	6.07	10.17
His	3.33	2.59	2.56	2.48	3.00
Arg	8.43	6.25	8.76	5.42	7.76
Trp	1.01	1.12	1.32	1.41	1.05
Non-EAA					
Asp	11.56	8.37	10.32	7.43	9.79
Ser	4.72	3.79	4.17	5.43	2.89
Glu	20.21	17.18	18.17	26.22	15.89
Pro	5.76	5.85	8.26	4.33	4.70
Gly	3.62	3.05	8.11	5.72	2.85
Ala	3.78	2.93	5.14	4.56	4.06
Cys	1.53	2.57	1.16	0.22	4.84
Tyr	4.15	3.04	3.38	4.44	2.86
Met + Cys	2.60	20.35	2.16	2.45	7.21
Phe + Tyr	9.20	6.92	7.82	8.57	7.73

[1] EAA: essential amino acids.
[2] Guriko-Eiyoshokuhin Co., Ltd.

Materials and Methods

Met-plastein was prepared according to the method of Monti and Jost (1979) as follows: a peptic hydrolysate (10 g, molecular weight = about 1,000) of soybean protein (Repron 90HS; Guriko-Eiyoshokuhin Co., Ltd.) and L-methionine ethylester hydrochloride (5.0 g) were incubated with papain (0.15 g) in 20% acetone solution (43 ml, pH 6.0) containing 0.01M L-cysteine at 37°C for 48 hours. The reaction product was purified by dialysis against water and then freeze-dried to give Met-plastein. The Met-plastein so obtained contained 177.8 mg of amino acid residue per gram of plastein (Table 1).

Amino acid analyses were conducted with soybean protein, Met-plastein, soybean protein-gelatin (3 : 1), casein-gelatin (3 : 1), and whole body protein of *O. niloticus*. Amino acid compositions (%) were determined by using a Shimadzu HPLC LC-3A system after hydrolysis of proteins with 4N methanesulfonic acid containing 0.2% tryptamine as reported by Teshima et al. (1986b).

O. niloticus fry (about 0.3 g) were obtained from a commercial fish farm and maintained on a commercial carp ration (35% crude protein) until used. The fry (about 0.4 g, mixed sex) were randomly assigned to four groups and reared in a recirculating system under the conditions given in Table 2. Feeding trials were conducted in duplicate tanks for each dietary group. Table 3 shows the composition of four test diets which are almost isonitrogenous (35% crude protein) but contain different proteins and amino acid supplements. The basal ration of test diets was the same as described previously (Teshima et al. 1986a). The fish were weighed at the beginning and every week, and the daily ration was adjusted after each weighing.

Weight gain, food conversion efficiency (FCE), and protein efficiency ratio (PER) data were analyzed by ANOVA and significance of differences tested by Student's t-test (Steel and Torrie 1960).

Results and Discussion

Feeding experiments were conducted to examine the effects of supplemental methionine in the forms of free amino acid and plastein on the weight gain, food conversion efficiency (FCE) and protein efficiency ratio (PER) of *O. niloticus* fry. Figs. 1 and 2 and Table 4 show the results of feeding experiments. The fry grew poorly when fed diet 1 containing soybean protein-gelatin (3 : 1) as protein sources (Fig. 1). Soybeans or soybean meal generally have a lower nutritional value than fish meal for tilapias (Wu and Jan 1977; Davis and Stickney 1978; Kesamaru and Miyazono 1978), except when used at low dietary inclusion levels (25% of total proteins supplied; Jackson et al. 1982). The soybean protein used in the present study was low in methionine and lysine (Table 1) as is common for soybean products (Jackson et al. 1982). As shown in Fig. 2, the weight gain, FCE and PER of *O. niloticus* fed diet 2 with a methionine supplement were similar to those of the fish fed diet 1 without a methionine

Table 2. Rearing conditions of *Oreochromis niloticus*.

Condition	Remark
Feeding period	5 weeks (Dec.-Jan.)
Average initial body wt.	0.4 g ± 0.15[1]
Number of fish/tank (30 l)	15
Feeding rate (% of body wt.)	5%
Daily feeding frequency	Twice (0900 and 1600)
Water temperature	27 ± 1°C

[1]Mean ± S.D.

Table 3. Composition (%) of test diets.

Ingredient[1]	Diet no. 1	2	3	4
Soybean protein-gelatin (3 : 1)	45.12	44.73	49.13	—
Crystalline methionine	—	0.51	—	—
Met-enriched plastein[2]	—	—	2.82	—
Casein-gelatin (3 : 1)	—	—	—	40.8
Dextrin	26.0	26.0	26.0	26.0
PLO-Soybean oil (1 : 1)[3]	14.0	14.0	14.0	14.0
Linoleic acid	1.0	1.0	1.0	1.0
Vitamin mixture	2.0	2.0	2.0	2.0
Mineral mixture	4.0	4.0	4.0	4.0
α-Cellulose	7.88	7.76	1.05	12.2
Total	100.0	100.0	100.0	100.0
Crude protein (%)	35.0	35.0	35.0	35.0
+ Agar	3.0	3.0	3.0	3.0

[1] The basal ration of test diets was similar to that described previously, except for nitrogen sources.
[2] Methionine-enriched plastein prepared from soybean protein.
[3] A mixture of pollack liver oil and soybean oil.

Table 4. Results of a 5-week feeding experiment using *Oreochromis niloticus* fry and various test diets (details in Table 3 and text).

Diet no.	Tank no.	Body wt. (g)[1] Initial	Final	Diet consumed (g/fish)	Wt. gain (g/fish)	Wt. gain (%)	FCE[2]	PER[3]
1	1	0.36 ± 0.12	0.73 ± 0.26	0.746	0.37	102.8	49.6	1.42
1	2	0.39 ± 0.11	0.73 ± 0.19	0.815	0.34	87.2	41.7	1.19
2	3	0.39 ± 0.16	0.80 ± 0.31	0.840	0.41	105.1	48.8	1.39
2	4	0.41 ± 0.15	0.77 ± 0.25	0.851	0.36	87.8	42.3	1.21
3	5	0.41 ± 0.15	0.90 ± 0.27	0.906	0.49	119.5	54.1	1.55
3	6	0.41 ± 0.08	0.97 ± 0.24	0.950	0.56	136.6	58.9	1.66
4	7	0.37 ± 0.16	1.03 ± 0.30	0.925	0.66	178.3	71.4	2.02
4	8	0.37 ± 0.20	1.01 ± 0.26	0.910	0.64	173.0	70.3	2.00

[1] Means ± S.D.
[2] Food Conversion Efficiency = gain (g) x 100/feed intake (g).
[3] Protein Efficiency Ratio = gain (g)/protein intake (g).

supplement. Thus, the supplement of a soybean protein-gelatin (3 : 1) with crystallline methionine did not improve its nutritional value significantly. For *O. aureus*, Wu and Jan (1977) have reported that weight gains were not increased by the supplement of crystalline methionine to a soybean meal diet.

On the other hand, the supplementation of the soybean protein-gelatin (3 : 1) diet with Met-plastein was effective in improving the growth of *O. niloticus*. The fry fed diet 3 with Met-plastein gave significantly higher weight gain ($P < 0.05$), FCE ($P < 0.10$), and PER ($P < 0.10$) than those fed diets 1 or 2. This indicates that

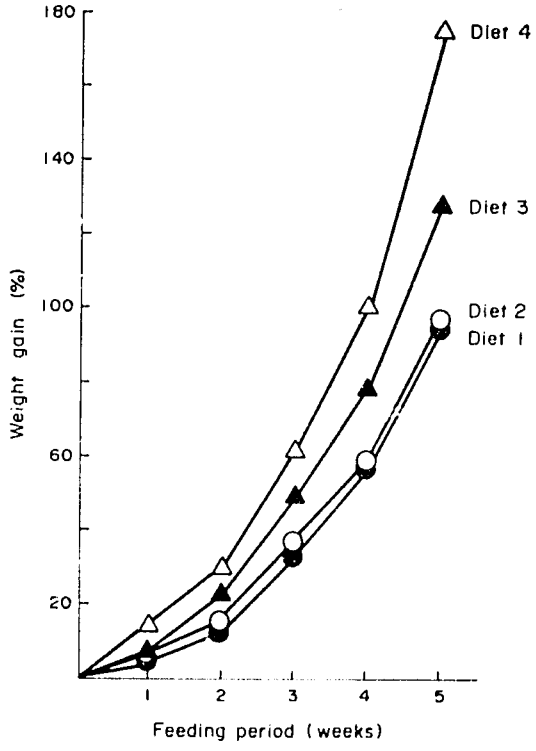

Fig. 1. Growth of *Oreochromis niloticus* fed diets with different nitrogen sources. Mean values were obtained from 2 aquaria.

Diet 1	:	Soybean protein-gelatin (3 : 1)
Diet 2	:	Soybean protein-gelatin (3 : 1) + Cryst. L-methionine
Diet 3	:	Soybean protein-gelatin (3 : 1) + Met-enriched plastein
Diet 4	:	Casein-gelatin (3 :1)

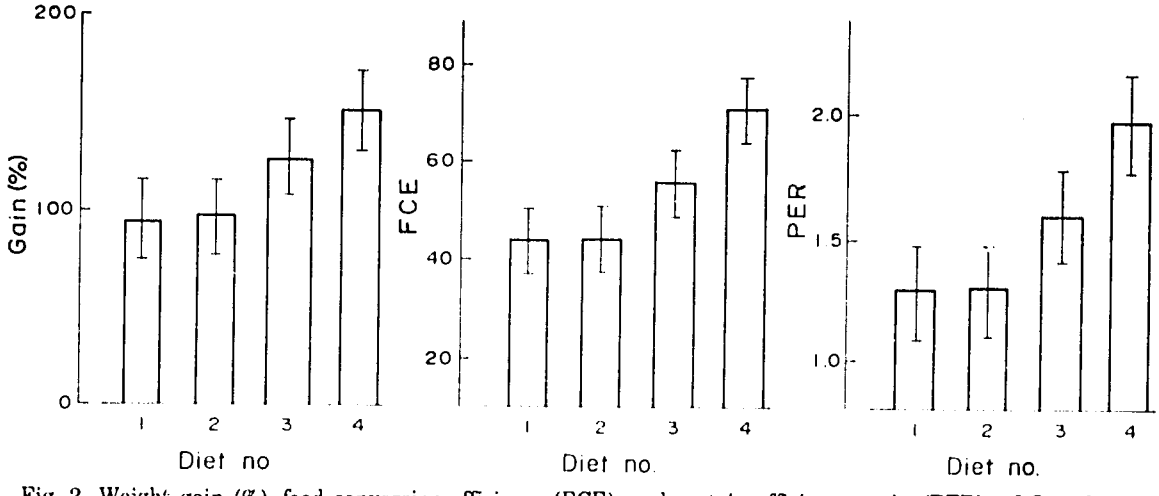

Fig. 2. Weight gain (%), food conversion efficiency (FCE), and protein efficiency ratio (PER) of *Oreochromis niloticus* fed diets with different nitrogen sources. Confidence limits (95%) were ± 20.4, ± 7.9, and ± 0.22 for weight gain (%), FCE, and PER data, respectively. A significant difference ($P < 0.05$) was detected between the following pairs in terms of weight gain (%), FCE, and PER; diets 1:4, 2:4, and 3:4. The pairs of diets 1:3 and 2:3 were significant for weight gain (%) data ($P < 0.05$) and for FCE ($P < 0.10$) and PER ($P < 0.10$).

methionine bound into soybean plasteins is more effectively utilized by *O. niloticus* fry than crystalline methionine. However, the weight gain, FCE, and PER of the fish receiving diet 3 with supplemental Met-plastein were lower than those of the fish receiving diet 4 containing casein-gelatin (3 : 1). Since the EAA contents of diets 3 and 4 did not differ markedly each other (Table 1), some factor other than the shortage of some EAA is responsible for the inferior nutritive value of the former diet.

Tilapia zillii (Mazid et al. 1978) and *O. niloticus* (Teshima et al. 1986a) have been shown to grow poorly when fed an amino acid diet; as also observed in the carp (Aoe et al. 1970) and channel catfish (Dupree and Halver 1970). In these fishes, however, the effects of crystalline amino acid supplements on growth are contradictory even in the same species and vary with the kind of amino acids. For example, the growth of channel catfish has been found not to increase with supplementation of a soybean meal diet with crystalline methionine or cystine (Andrews and Page 1974) and by supplementation of a casein diet with crystalline arginine, cystein, tryptophan, or methionine (Andrews et al. 1977). However, recent studies have demonstrated that the growth of channel catfish was improved by the supplementation of a soybean meal diet with crystalline methionine (Murai et al. 1982) and of a peanut meal diet with lysine (Robinson et al. 1980).

The nutritive value of crystalline amino acids has been improved by coating them with appropriate materials. Murai et al. (1981) have shown that casein-coated amino acids improve the growth of carp more markedly than the same uncoated amino acids, when added to a gelatin diet. Murai et al. (1982) revealed that the growth of carp was improved by the supplementation of a soybean meal diet with methionine coated with aldehyde-treated casein (CHO-casein). The addition of supplemental arginine to a casein diet, irrespective of form, has also been found to enhance the growth of carp (Murai et al. 1981). Also, the growth of channel catfish was improved by the addition of crystalline methionine to a soybean meal diet (Murai et al. 1982). However, the growth-enhancing effect of supplemental methionine for channel catfish was not significantly increased even if this amino acid was coated with casein (Murai et al. 1981) or CHO-casein (Murai et al. 1982).

Further work is required for a better understanding of the metabolism of dietary sources of free and bound amino acids in cultured fishes such as carp, channel catfish, and tilapias. The present study indicates that the nutritional values for tilapias of some vegetable proteins like soybean meal low in methionine may be improved by the supplement of diets with Met-plastein.

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Effects of Dietary Protein Level on Growth and Reproduction in Nile Tilapia *(Oreochromis niloticus)*

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Abstract

The dietary protein requirements of Nile tilapia *(Oreochromis niloticus)* were investigated. Five experimental diets were tested, with protein levels from 20 to 50% at 7.5% increments. In a juvenile growth trial, fish (average weight 24 g) were fed these experimental diets for 6 weeks. Dietary protein requirements for growth were determined in terms of weight gain, food conversion ratio, protein efficiency ratio, apparent net protein utilization and protein digestibility. A diet containing about 27.5-35% protein appeared optimal but there were no significant differences among treatments, except the parameters for the lowest % protein diet (20%) which were significantly inferior (P = 0.05). In the subsequent reproduction trial, fish were fed the same diets. Reproductive performance was determined in terms of onset of spawning, frequency of spawning, egg size (volume and weight), hatchability and fecundity. Fish fed diets containing low and medium levels of protein (20, 27.5 and 35%) had higher fecundity (6.4, 7.0, 6.7 eggs/g, respectively) than those fed diets containing higher (42.5 and 50%) levels of protein (4.7, 4.2 eggs/g, respectively): P = 0.05. Moreover, the former group had smaller eggs and the latter group spawned earlier but less often. Fish fed low and medium levels of dietary protein (20, 27.5 and 35%) spawned later but more frequently. In terms of growth performances during the reproduction trial, the diet containing 35% protein appeared to be optimal compared with the low protein diets (20 and 27.5%) but was not significantly different from the high protein diets (42.5 and 50%).

Introduction

As reproduction requires the formation of gametes which have high protein content (Sa[illegible] . 1973; Springate et al. 1985) it is imperative that an adequate amount of dietary protein be provided to broodstock. Santiago et al. (1985) studied the effects of artificial diets on fry production and growth of *Oreochromis niloticus*

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broodstock and reported that a 40% protein diet consistently gave higher values. However, their experiments, using only two protein levels (20% and 40%) were conducted in outdoor tanks where natural production occurred. It is difficult to quantify the exact nutritional requirements without taking into account the contribution by the natural food organisms.

The objective of the present study was to investigate the dietary protein requirement for growth of juvenile *O. niloticus* and their subsequent reproductive performances, fed varying levels of protein (from 20% to 50%, by 7.5% increments) in a clear water system.

Materials and Methods

Experimental animals

Juvenile *O. niloticus* (average body weight of about 24 g) were collected from a pond fertilized with septage at the Asian Institute of Technology, Bangkok. They were transferred to concrete tanks for 2 weeks for acclimation to tank conditions prior to experimentation, feeding on catfish pellets.

All experiments were conducted in a recirculating water system consisting of fifteen circular concrete tanks (1.2 m diameter; 0.7 m deep; 0.6 m^3 volume) located outdoors under a thatched roof. A header/holding tank supplied water to the tanks by gravity at 0.8 l/min.

Water quality was monitored every 10 days. Temperature, pH and dissolved oxygen varied between 25-28°C; 7.3-8.5 and 5.7-7.5 mg/l, respectively. Nitrite-nitrogen and ammonia-nitrogen increased from 1.6 to 35.8 µg/l and 7.57 to 83.31 µg/l, respectively, at the end of the experiment.

Experimental diets

Five diets were formulated to give dietary protein levels ranging from 20% to 50% with 7.5% increments (Table 1) prepared as described by Wee and Ng (1986). Fish meal, blood meal and soybean meal were used as protein sources. The level of blood meal was kept constant at 5%. The differences in dietary protein levels are mainly due to the differences in the quantity of fish meal and soybean meal the ratio of which, however, remained constant at 3:1. All diets were formulated to contain 10% lipid, 3% of which was provided by corn oil and the other 7% from oil present within the fish meal and addition of *Trichogaster* oil. *Leucaena* leaf meal was mixed into the diets at a constant level of 5%. The calculated gross energy content of all diets was approximately 410 kcal/100 g.

Fish were stocked at 20/tank. At the beginning of the feeding trial, 20 fish were sampled, dried at 80°C for 24 hours and stored for subsequent carcass analysis. At the start and end of the experiment, fish were weighed individually and subsequently batch weighed at 10-day intervals. The fish were fed *ad libitum* three times a day, and the actual amount of feed taken per day recorded. A 50-day growth trial was conducted. On completion, 60% of the stocked fish from each replicate of each treatment were harvested, dried at 80°C for 24 hours and stored for subsequent carcass composition analysis. Prior to final sacrifice of fish, feces accumulated overnight were collected daily from the tanks by siphoning, dried at 80°C for 24 hours and stored for subsequent chemical analysis.

The remaining fish were tagged by clipping a plastic tag through the dorsal musculature at the caudal peduncle, and grown on for the reproduction of experiment for up to 5 months. The stocking density in this experiment was 8-9 fish/tank at a ratio of 6-7:2 females to males. The tank was divided into three parts by placing bricks (40 x 20 x 7 cm) to aid fish breeding activities. The onset of spawning was monitored by daily observation of fish in tanks to ascertain whether they were mouth-brooding eggs. Mouth-brooding females were caught, the total number of eggs from each female counted, and the weight of the female (free of eggs) recorded. One hundred eggs were subse-

Table 1. Composition and nutrient content (g/100 g) of experimental diets formulated to study nutritional effects on growth and reproduction in *Oreochromis niloticus*.

Component	Diet number I	II	III	IV	V
Fish meal	20.8	30.5	40.4	50.2	59.9
Soybean meal	6.9	10.2	13.4	16.7	20.0
Blood meal	5.0	5.0	5.0	5.0	5.0
Corn oil	3.0	3.0	3.0	3.0	3.0
Trichogaster oil	4.4	3.1	1.9	0.5	0.0
Leucaena leaf meal	5.0	5.0	5.0	5.0	5.0
Cassava starch	51.4	39.8	27.7	16.1	3.6
Binder[1]	1.0	1.0	1.0	1.0	1.0
Vitamin premix[2]	1.0	1.0	1.0	1.0	1.0
Mineral premix[3]	1.0	1.0	1.0	1.0	1.0
Chromic oxide	0.5	0.5	0.5	0.5	0.5
			Nutrient content		
Moisture (%)	4.71	4.94	4.98	5.13	5.18
Crude protein (%)	20.24	27.59	35.02	42.64	50.12
Crude lipid (%)	9.81	9.95	10.02	10.04	10.12
Ash (%)	11.03	14.65	18.24	21.23	23.80
Crude fiber (%)	1.57	2.09	2.53	2.88	3.31
NFA (%)[4]	57.53	45.72	34.19	23.12	12.65
Gross energy[5]	437.96	434.67	431.56	430.91	432.42
Metabolizable energy[6]	304.19	318.94	333.66	349.80	366.98
Protein energy (%)[7]	26.34	36.18	46.25	56.40	66.07
P:E ratio[8]	66.54	86.51	104.90	121.90	136.57

[1]Binder: Carboxymethyl cellulose (CMC).

[2]Vitamin premix contains (mg/100 g of diet): thiamine (B1) 2.5 mg, riboflavin (B2) 2.5 mg, pyridoxine (B6) 2.0 mg, pantothenic acid 5.0 mg, inositol 100.0 mg, biotin 0.30 mg, folic acid 0.75 mg, para-aminobenzoic acid 2.5 mg, choline 200.0 mg, nicotinic acid 10.0 mg, cyanocobalamin (B12) 0.005 mg, retinol palmitate (A) 100,000 IU, α tocopherol acetate (E) 20.1 mg, ascorbic acid (C) 50.0 mg phylloquinol (K) 2.0 mg, cholecalciferol (D3) 500,000 IU.

[3]Mineral premix contains (mg/100 g of diet): calcium orthophosphate 727.8 mg, magnesium sulphate 127.5 mg, sodium chloride 60.0 mg, potassium chloride 50.0 mg, iron sulfate 25.0 mg, zinc sulfate 55.0 mg, manganese sulfate 25.4 mg, copper sulfate 0.79 mg, cobalt sulfate 0.48 mg, calcium iodate 0.29 mg, chromic chloride 0.13 mg.

[4]NFE: nitrogen free extracts = 100 - (moisutre + crude protein + crude lipid + crude fiber + ash)

[5]Gross energy: in kcal/100 g, based on 5.7 kcal/g protein; 9.5 kcal/g lipid; 4.0 kcal/g carbohydrate.

[6]Metabolizable energy: in kcal/100 g, based on 5.0 kcal/g protein; 9.0 kcal/g lipid; 2.0 kcal/g carbohydrate.

[7]Protein energy (%): the ratio between energy in protein and gross energy multiplied by 100.

[8]P:E ratio: protein to energy ratio in mg protein/kcal of metabolizable energy.

quently sampled for hatching rate determination in artificial incubators. An additional subsample of 80 eggs was taken for egg size measurement in terms of egg diameter and volume and wet and dry weight of egg.

Moisture, crude protein, crude lipid, crude fiber and ash content were performed on whole fish carcass, feces and diets as described previously (Wee and Ng 1986). For apparent digestibility measurement, chromic oxide content was determined in the feces and diets by the method of Furukawa and Tsukuhara (1966).

Statistical comparisons of the results were made by using analysis of variance (ANOVA). Duncan's multiple range test (Duncan 1955) was used to evaluate the differences between means for individual diets at the 0.05 significance level.

Results and Discussion

On the basis of percentage weight gain, daily weight gain and specific growth rate in both trials (growth and reproduction), there was a progressive improvement in the growth responses with increasing dietary protein levels up to 27.5% and 35% crude protein, respectively. Thereafter further improvements were not significant (Tables 2 and 3). Hence the minimum dietary protein requirement for nonspawning and spawn-

Table 2. Growth performance, feed conversion and protein utilization of *Oreochromis niloticus* fed different experimental diets for 50 days in a growth trial. For details of diets and procedures, see Table 1 and text.

Mean values	Diet number (% protein) I (20.2)	II (27.6)	III (35.0)	IV (42.6)	V (50.1)
Initial weight (g)	24.91a	23.53a	24.46a	23.93a	24.31a
Final weight	46.55a	50.78ab	54.72b	55.83b	55.96b
Percentage weight gain (%)	86.86a	116.83b	123.71b	133.34b	130.15b
Daily weight gain (g/day)	0.43a	0.54b	0.60b	0.64b	0.63b
Specific growth rate (%/day)	1.25a	1.53b	1.61b	1.69b	1.67b
Feed intake[1]	2.49a	2.43a	2.33a	2.27a	2.29a
Food conversion ratio (FCR)	2.06a	1.65b	1.49bc	1.38c	1.48bc
Protein efficiency ratio (PER)	2.40a	2.21a	1.92b	1.70c	1.37d
Apparent net protein utilization (NPU, %)	30.54a	29.89ab	28.86ab	25.90b	21.17c
Daily tissue protein deposition (mg/day)	55.11a	73.75b	91.16c	97.36c	97.90c
Apparent protein digestibility (%)	75.72a	82.44b	87.65c	90.57d	91.60d
Total digestibility (%)	50.42a	58.75a	70.53b	68.66b	66.15b

Note: Figures in the same row having the same suffixes (a, b, c) are not significantly different (P = 0.05).
[1]Feed intake (% body weight/day) calculated on a dry weight basis.

ing *O. niloticus* was estimated to be 27.5% and 35% crude protein, respectively. A similar observation was reported by Chotiyarnwong et al. (1978) where the growth of young Nile tilapia *(Tilapia niloticus)* fed for dietary protein levels ranging from 25% to 40% was not significantly different. In contrast, Jauncey (1982) and Cruz and Laudencia (1977) showed that the growth of juvenile *O. mossambicus* increased with dietary protein levels up to 38% and 40%, respectively, and thereafter decreased with higher levels of protein in diets. Comparing daily weight gain between our two trials, it is clear that mature spawning fish could only gain a small amount of weight per day compared with the preadults. Most nutrients from the diet provided are utilized for egg and sperm production in broodstock. Moreover, mouth brooding reduces feed intake which leads to lesser growth.

In the growth trial, there were significant differences in food conversion ratio (FCR). Better FCRs were obtained with increasing dietary protein levels up to 42.5% and deteriorated slightly with diet V containing 50% protein. In the reproduction trial, although the same trend was observed, the best FCR was with diet III (35% protein) possibly because in tanks with fish fed diets IV and V a size hierarchy was observed. Both the males and females, especially those ready for spawning were excited and very active, fighting and chasing away any fish swimming around them. This was seen especially at feeding time. The more aggressive fish caused other fish to eat less or not to feed; hence, some feed was wasted, leading to poor FCR in this trial. The same observation was reported by Rothbard (1979) and Santiago et al. (1983) which explains how the fish establish a "spawning family" and why some females

Table 3. Growth performance, feed conversion and protein utilization of *Oreochromis niloticus* fed different experimental diets for 86 days in a trial to assess effects of dietary protein level on reproductive performance. For details of diets and procedures, see Table 1 and text.

Mean values	Diet number (% protein) I (20.2)	II (27.6)	III (35.0)	IV (42.6)	V (50.1)
Initial weight (g)	45.26a	51.36ab	53.04b	53.49b	56.28b
Final weight	65.60a	72.05ab	77.67bc	79.46bc	81.58c
Percentage weight gain (%)	45.83a	40.54a	47.14a	48.57a	47.31a
Daily weight gain (g/day)	0.23a	0.23a	0.26b	0.28b	0.28b
Daily weight gain of female (g/day)	0.23a	0.23a	0.27b	0.28b	0.26ab
Daily weight gain of male (g/day)	0.24a	0.24a	0.24a	0.28aab	0.35b
Specific growth rate (%/day)	0.42a	0.40a	0.46ab	0.47b	0.44ab
Feed intake (%)	2.28a	2.23a	2.04b	2.05b	1.92b
Feed conversion ratio (FCR)	5.66a	4.78a	4.20a	4.38a	4.63a
Protein efficiency ratio (PER)	0.89a	0.78al	0.70ab	0.61ab	0.44b
Apparent net protein utilization (NPU, %)	11.43a	10.17a	9.63a	7.21a	5.90a
Apparent protein digestibility (%)	77.70a	85.84b	87.55bc	90.44cd	92.66d
Total digestibility (%)	47.44a	68.83b	69.64b	67.58b	66.62b

Note: Figures in the same row having the same suffixes (a, b, c) are not significantly different (P = 0.05).

delay spawning as a result of retarded growth.

It is commonly observed that protein efficiency ratio (PER) and apparent net protein utilization (NPU) are inversely related to the dietary protein level. In this study, the PER and NPU decreased as dietary protein concentration increased from 20% to 50% in both trials (Tables 2 and 3). Dabrowski (1977) with grass carp *(Ctenopharyngodon idella),* Wee and Tacon (1982) with snakehead *(Channa micropeltes)* and Jauncey (1982) with *O. mossambicus* also reported the same observation, that fish often showed higher utilization and efficiency with dietary protein from lower protein containing diets. Cowey et al. (1972) and Ogino and Saito (1976) considered that the differences in PERs and NPU were due to different levels of energy in diet; diets containing higher levels of carbohydrate would earn higher PER and NPU values than those with lower levels, i.e., less carbohydrate energy.

There was a trend of increasing protein and total dry matter digestibility with increase in dietary protein levels up to 50% protein level. This was probably because of the higher levels of fish meal in the diets. Similar observations have been reported by Rychly and Spannhof (1979) on rainbow trout *(Salmo gairdneri),* Henken et al. (1985) on catfish *(Clarias gariepinus),* Wee (1986) on snakehead *(Channa striata)* and Jobling (1981) on plaice *(Pleuronectes platessa).* It has also been suggested that high carbohydrate content in the diet could adversely affect protein digestibility (Wee and Tacon 1982).

Concerning effects of dietary protein level on reproduction, there were significant differences in the onset of spawning, index of spawning, internal and frequency of spawning, fecundity and egg quality

(Tables 4 and 5). Fish fed higher levels of dietary protein spawned significantly earlier (less than 49 days for diets II, III, IV and V and 58 days for diet I). The same observation was reported for rainbow trout *(S. gairdneri)* (Satia 1973) where broodstock fed high dietary protein levels (44.30% and 55.69% protein) were ripe for spawning 2 weeks before those fed lower protein levels.

In terms of interval of spawning and frequency of spawning, there were no significant differences among diets, although there was a trend of increasing intervals between successive spawnings (from 25 days to 35 days) and decreasing frequency of spawning (from three to two times) with increases in dietary protein. This was probably due to the aggressive nature of some fish, especially males and some females, which were ready for spawning after obtaining enough nutrient and energy from the higher protein diets; such fish were so dominant that a hierarchy was established in the tank, whereby other females were attacked and wounded. This prolonged the time needed to spawn as they required time to recover. Rothbard (1979) as cited by Santiago et al. (1983) reported similar observations with the same species of tilapia, where the females that were not ready to spawn were normally not paired with males, stayed at the upper layer of the water column most of the time and had darker coloration and vertical bands of the body; such fish had reduced opportunity to feed which resulted in later spawning and

Table 4. Reproduction performance of *Oreochromis niloticus* fed different experimental diets. For details of diets and procedures, see Table 1 and text.

Mean values	Diet number (% protein) I (20.2)	II (27.6)	III (35.0)	IV (42.6)	V (50.1)
Onset of spawning (days)[1]	58a	47b	49ab	45b	44b
Per cent of spawning females[2]	58.73a	47.62a	53.17a	46.82a	42.86a
Survival rate of females (%)	94.44a	89.68ab	84.92ab	73.81bc	63.49c
Index of spawning[3]	1.53a	1.48a	1.10a	1.19a	1.11a
Frequency of spawning[4]	2.64a	3.00a	2.00a	2.56a	2.63a
Interval of spawning[5] (days)	25a	27a	28a	33b	35b
Absolute fecundity (eggs/spawning) ± S.D.	327 ± 142ab	388 ± 119a	401 ± 177a	281 ± 116b	270 ± 100b
Relative fecundity (eggs/g female) ± S.D.	6.36 ± 3.20a	7.00 ± 2.37a	6.71 ± 2.77a	4.74 ± 2.10b	4.28 ± 1.61b

Note: Figures in the same row having the same suffixes (a, b, c) are not significantly different (P = 0.05).
[1]Calculated from the beginning of the experiment to the day the female first spawned.
[2]Per cent of spawned females against total females.
[3]Expressed by a ratio between the total spawning times of all females and the total number of females in tank.
[4]The number of spawnings during experiment for each female.
[5]The interval between two successful spawnings in a female.

Table 5. The effect of dietary protein levels on egg quality parameters in *Oreochromis niloticus* (mean value ± S.D.). For details of diets and procedures, see Table 1 and text.

Parameters	Diet number (% protein) I (20.2)	II (27.5)	III (35.0)	IV (42.6)	V (50.1)
Average wet weight[1] of egg (mg)	4.6 ± 0.7a	4.8 ± 0.6ab	4.9 ± 0.6abc	5.1 ± 0.6bc	5.3 ± 0.9c
Average dry weight[1] of eggs (mg)	2.0 ± 0.5a	2.0 ± 0.2a	2.0 ± 0.3a	2.1 ± 0.3a	2.1 ± 0.3a
Average moisture in egg (%)	58.58 ± 3.13a	59.15 ± 1.79a	59.48 ± 1.63a	59.16 ± 1.88a	59.58 ± 1.57a
Average volume[2] of egg (mm^3)	5.01 ± 0.84a	5.38 ± 0.92b	5.43 ± 0.65b	5.77 ± 0.97b	5.72 ± 0.85b
Hatching rate (%)[3]	64.86 ± 13.29a	69.77 ± 12.93a	68.54 ± 13.52a	73.04 ± 13.69a	71.25 ± 12.57a

Note: Figures in the same row having the same suffixes (a, b, c) are not significantly different (P = 0.05).

[1]Egg weight (dry and wet basis): a sample of 50 eggs was weighed and then dried in an oven (at 80°C, for 24 hours) for moisture determination.

[2]Egg volume: 30 eggs were measured using a micrometer for length (L) and width (H), and egg volume was calculated by the formula: $V = \pi/6LH^2$ as used by Rana (1985).

[3]Hatching rate: defined as the ratio between the number of fry and number of eggs obtained from one female, multiplied by a factor of 100. Eggs were incubated in trays (made from hapa material) and fry were counted within 1 day after hatching.

lesser overall growth. This is the main reason for the low survival rate of females, low per cent of spawning females, low index of spawning and long interval of spawning observed from the broodstock fed high dietary protein diets. Santiago et al. (1983), however, reported that the frequency of broodstocks fed higher dietary protein (40% and 50% protein) showed a higher spawning frequency. This contrasts with our results where fish fed higher protein levels showed a lower spawning frequency because of the hierarchial effects.

In the present study, absolute and relative fecundities were significantly higher from the fish fed lower dietary protein than those fed higher levels (Table 5). Dahlgren (1980) also observed a significant difference in terms of fecundity in the guppy *(Poecilia reticulata)* where females fed diets containing 47% protein had a lower fecundity when compared to fish fed a 31% protein diet. The reasons for this are not clear but may reflect differences in egg size. The lower fecundity obtained with fish fed high dietary protein was normally accompanied by heavier and larger-sized eggs. A similar observation was also reported by Mironova (1978) in that *O. mossambicus* females, given a restricted amount of food, spawned considerably more frequently and laid a larger number of small eggs than "replete" females. Santiago et al. (1983) found no significant differences in terms of absolute fecundity of *O. niloticus* fed different levels of protein. In our study although fish fed higher protein levels produced bigger and heavier eggs (wet basis), there were no significant dry weight differences between large eggs from high protein diets or small eggs from low protein diets. Peters (1963) reported that weight differences of tilapia eggs were mainly due to differences in yolk quantity because water content after fertilization amounted to 50-60% of egg weight. According to him, absolute fecundity tends to increase with body weight and is inversely related to the average weight of egg, thus tilapia produce either a large number of small eggs or vice versa - as we observed.

Egg size strongly influences fry health, especially during the early life after hatching. According to Rana (1985), *O. mossambicus* fry hatched from large eggs can live longer without food than from small or medium eggs. Fish fed higher levels of dietary protein in this study (diets IV and V) may thus produce better quality fry. We found no significant differences in hatchability among the diets. However, hatchability could be affected by diet quality because vitamins and iodine are presumably essential for hatching and subsequent growth (Satia 1973).

Another point to consider is broodstock body composition. Landesman et al. (1985) working with the guppy *(Poecilia reticulata)* showed that with increasing protein levels in diets there was an increase in body protein content and a decrease in body fat. In the present study, there was also a significant increase in body protein content with increased dietary protein concentration in the growth trial; however, in the reproduction trial, the differences were not significant (Table 6). Presumably, the higher level of dietary protein intake by fish fed high protein diets contributed to gamete production. Water and ash contents in the fish body were not significantly different among diets. Body lipid was significantly lower in the reproduction trial than the growth trial, presumably due to spawning activities and lipid-rich gamete production.

In conclusion, provision of a high quality diet, in terms of high protein level, results in a significant shift in reproductive strategy by *O. niloticus;* namely, an advance in the onset of spawning, but lengthening in the interval between

Table 6. Gross body composition (wet weight basis) of *Oreochromis niloticus* broodstock in growth and reproduction trials.

Component (%)	Value at start trial	Final values on diets I-V % (protein) I (20.2)	II (27.5)	III (35.0)	IV (42.6)	V (50.1)
Growth Trial						
Moisture	71.4[illegible]	74.11b	74.54b	73.41b	73.49b	73.40b
Crude protein	14.17ab	13.49a	13.84a	14.66bc	14.79bc	14.90c
Crude lipid	8.14a	6.15b	5.21c	5.37c	5.15c	5.05c
Ash	3.90a	3.64a	3.55a	3.63a	3.54a	3.68a
Reproduction Trial						
Moisture		73.36a	75.05ab	75.34ab	76.02b	75.59ab
Initial moisture		74.11a	74.54a	73.41a	73.49a	73.40a
Crude protein		13.34a	13.76a	13.88a	13.40a	14.30a
Initial protein		13.49a	13.84a	14.66bc	14.79bc	14.90c
Crude lipid		5.43a	4.07b	3.57bc	3.30bc	2.92c
Initial lipid		6.15a	5.21b	5.37b	5.15b	5.05b
Ash		4.67a	4.56a	4.90a	4.92a	4.85a
Initial ash		3.64a	3.55a	3.63a	3.54a	3.68a

Note: Figures in the same row having the same suffixes (a, b, c) are not significantly different ($P = 0.05$).

spawning. This results in decreased frequency of spawning, greater mortality in females and a reduction in overall fecundity, which is compensated by bigger and heavier eggs which may have better hatching rates. Hence, a dilemma facing farmers is whether they should go for fewer but better quality eggs by feeding high protein diets (with attendant adverse effects on female survival) or go for a higher number of eggs with poorer hatchability on low protein broodstock feeds. The answer is probably a compromise, the guidelines for which need to be assembled for different hatchery systems.

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SESSION V: PHYSIOLOGY

Effects of Unionized Ammonia on Red Tilapia (*Oreochromis mossambicus/O. niloticus* hybrid) Fry

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Abstract

The effects of unionized ammonia on red tilapia fry (an *Oreochromis mossambicus/O. niloticus* hybrid line, origin Taiwan) (mean standard length, 2.13 ± 0.35 cm) were studied. The 48-hour, 72-hour and 96-hour LC_{50}'s were 6.6, 4.07 and 2.88 mg/l, respectively. The threshold lethal concentration (TLC) was 0.24 mg/l. Prior to death from exposure to unionized ammonia, fry swam erratically and showed hemorrhaging of the gill filaments.

Introduction

Red tilapia, an *Oreochromis mossambicus/O. niloticus* hybrid line obtained from Taiwan (Kuo 1984) has become a popular fish for culture in Malaysia. It has good characters such as fast growth, good food conversion, palatability, tolerance to a wide range of salinities and disease resistance (Galman and Avtalion 1983). As unionized ammonia can affect the growth and survival of cultured fish, this study was undertaken to determine its effects on the survival of red tilapia fry after 48-, 72- and 96-hour exposures.

Materials and Methods

Red tilapia fry, mean standard length (± S.D.), 2.13 (± 0.35 cm) were obtained from the Khoo Peck Wan Aqua Farm, Malacca. Five concentrations of unionized ammonia were tested in the range 0.1 mg/l to 3.5 mg/l, with a control and three replicates at each concentration. The experiments were conducted in eighteen 20-l, 30 x 90 x 30 cm aquaria. Prior to the experiment, the aquaria were treated with 2 ppm methylene blue for 1 week (Mohamed Shariff 1984) to avoid disease infection. Ten fry were stocked in each

aquarium and acclimatized for 3 days prior to the experiment (Sprague 1969). The aquaria were aerated throughout the experiment. Fry were fed with 35% protein pelleted feed at 2% body weight/day. Water temperature, dissolved oxygen, pH and total ammonia were measured every 4 days. The temperature and dissolved oxygen levels were maintained at 24.6 ± 0.4°C and 8.35 ± 0.6 mg/l, respectively. The pH varied from 7.32 to 7.61.

A standard ammonia solution was prepared from ammonium chloride. Total ammonia was analyzed using the blue-indophenol complex method (Solorzano 1969). The percentage of unionized ammonia present in solution was calculated following Emerson et al. (1975).

The numbers of dead fish were observed and recorded every 6 hours. The percentage mortality in each test tank was plotted against the unionized ammonia concentration to determine the LC_{50} for each observation (Vowles and Connell 1980) (Figs. 1 and 2).

Results

The unionized ammonia concentrations in the aquaria and the percentages of the average mortality in these concentrations at different exposures are summarized in Table 1. From these data, graphs were plotted to determine the median lethal concentrations (LC_{50}) at 48, 72 and 96 hours (Figs. 1 and 2). The means for the 48-hour, 72-hour and 96-hour LC_{50}'s were 6.6 mg/l, 4.07 mg/l and 2.58 mg/l unionized ammonia, respectively. The threshold lethal concentration of unionized ammonia for red tilapia fry was 0.24 mg/l (Fig. 2).

Total mortality occurred after 19 days, 9 days and 7 days exposure to 1.854, 2.946 and 3.410 mg/l unionized ammonia, respectively. Fish showed respiratory distress just prior to death and swam erratically at the surface. Hemorrhagic gill filaments were also observed in these fish. However, no histological studies were done.

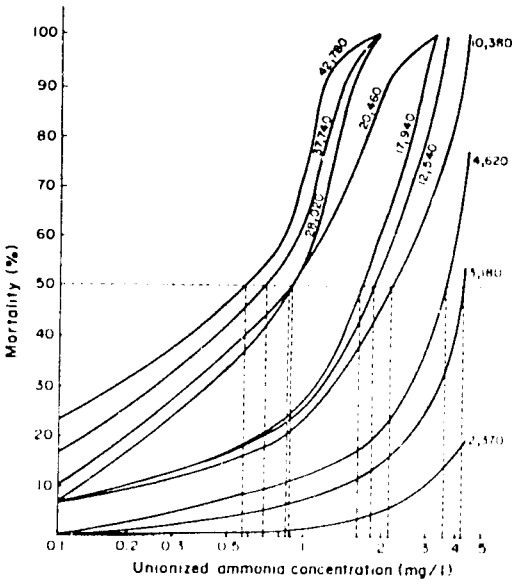

Fig. 1. Mortality curves (%) for red tilapia fry (*Oreochromis mossambicus*/*O. niloticus* hybrid line) in various concentrations of unionized ammonia at given exposure times in minutes.

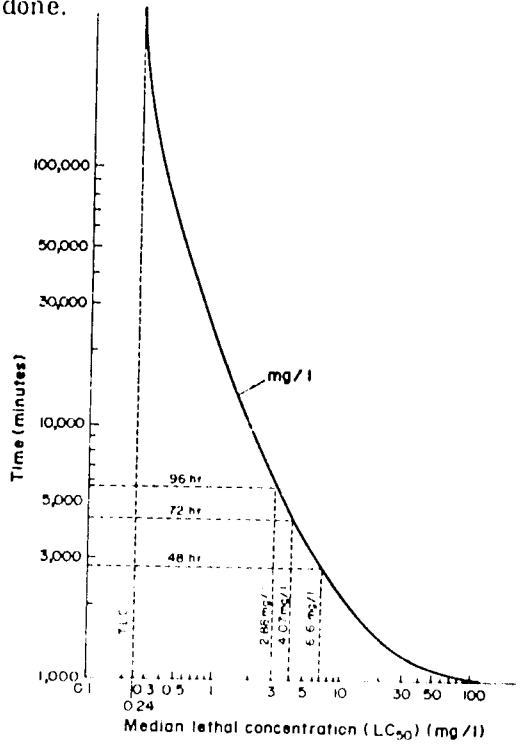

Fig. 2. Median lethal concentration (LC_{50}) of unionized ammonia for 48-, 72- and 96-hour exposure.

Table 1. Percentage of average mortality of red tilapia (*Oreochromis mossambicus*/*O. niloticus* hybrid) fry after given exposure times (minutes) to various concentrations (mg/l) of unionized ammonia. The data are from triplicate aquaria for each unionized ammonia concentration, each having ten fish.

Unionized ammonia concentration (mg/l)	Percentage of average mortality at minutes								
	2,570	3,180	4,620	10,380	12,540	17,580	20,460	28,020	37,740	42,780
0.107 ± 0.024	0	0	0	6.6	6.6	6.6	6.6	10	16.6	23.3
0.951 ± 0.184	6.6	10	13.3	23.3	23.3	23.3	53.3	53.3	63.3	63.3
1.854 ± 0.062	0	10	16.6	46.6	60	63.3	86.6	100	100	100
2.946 ± 0.143	10	23.3	40	76.6	100	100	100	100	100	100
3.416 ± 0.196	13.3	46.3	53.3	100	100	100	100	100	100	100

Discussion

EIFAC (1973) stated that toxic concentrations of unionized ammonia for short-term exposure are between 0.6 and 2 mg/l for most species. Our study showed that 48-hour, 72-hour and 96-hour LC_{50}'s for red tilapia fry [(2.13 ± 0.35 cm (mean ± S.D.) standard length)] were 6.6, 4.07 and 2.88 mg/l, respectively. By comparison, the 24-hour, 48-hour and 72-hour LC_{50} values for *Oreochromis aureus* reported by Redner and Stickney (1979) were 2.46, 2.40 and 2.35 mg/l, respectively.

Ammonia is more toxic when dissolved oxygen concentration is low (Merkens and Downing 1957). However, in our test, the dissolved oxygen in the tanks was maintained at high level in a range of 7.0 to 10.1 mg/l. This could have contributed to the relatively strong resistance of red tilapia fry to unionized ammonia found here. The threshold lethal concentration with no mortality was 0.24 mg/l.

In this study, fish showed respiratory distress and hemorrhaging gill filaments just prior to death. Similar observations were reported by Smith and Piper (1975) and Smart (1978). Redner and Stickney (1979) described lifting of the gill lamella epithelium and dilation of blood spaces in the secondary lamellae of rainbow trout, with capillary congestion and hemorrhaging at the tips of some gill filaments.

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Salinity Tolerances of Red Hybrid Tilapia Fry, Juveniles and Adults

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Abstract

A study was conducted on Lee Stocking Island, Bahamas, to determine the salinity tolerance of red hybrid tilapia (Sipe strain) acclimated in groundwater (1.5-2.0 ppt). For 72 hours following direct transfer, survival of *Oreochromis mossambicus-Oreochromis urolepis hornorum* fry, all-male (sex reversed) juveniles, and adult females in 2-ppt intervals to 100% seawater (37 ppt) was recorded in each of three trials. Fry averaged 6.3 mm standard length (SL), 0.008 g; juveniles 26.5 mm SL, 0.81 g; and adults 133.4 mm SL, 82.63 g. Temperature ranges were 28-29°C in fry and fingerling trials and 30-32°C in the adult trials. Fry and juveniles tolerated direct transfer to 19 ppt without mortality or apparent stress but suffered 100% mortality at salinities above 27 ppt. Adult mortality was observed from 29 ppt, with 100% mortality at 37 ppt. Temporal and behavioral responses of each group to salinity stress were noted over each 72-hour test period.

Introduction

Two of the common red tilapia hybrids are red variant *Oreochromis mossambicus* crossed with either *O. niloticus* in Taiwan and the Philippines (Liao and Chang 1983), or with *O. urolepis hornorum* in Florida. Liao and Chang (1983) found the Taiwanese red hybrid tilapia suitable for brackish- or saltwater cage culture. Its salinity tolerance has been studied by Watanabe et al. (1985).

Research on the culture potential of the Florida red hybrid tilapia in saltwater cages was initiated in 1983 on Lee Stocking Island, Bahamas. Freshwater is not abundant in the Caribbean and the coloration of the hybrid resembles that of desired marine fishes. In preliminary tests at the site, the hybrids survived and grew

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in 100% seawater. This accords with reports of *O. mossambicus* collected at 120 ppt (Whitfield and Blaber 1979) and *O. urolepis hornorum* raised in saltwater ponds (Trewavas 1983). Salinity tolerance of the Florida hybrid has yet to be defined. Accordingly, a study was undertaken to determine the salinity tolerances of fry, juveniles, and adults of the Florida red hybrid tilapia, *O. mossambicus-O. urolepis hornorum*.

Materials and Methods

The study was conducted on Lee Stocking Island, near Great Exuma Island, Bahamas, from July 9-31, 1985. Red tilapia hybrids had been purchased from Florida in 1983. These formed the broodstock from which the experimental animals were obtained. Groundwater (1.5-2.0 ppt) was obtained from shallow wells and seawater (37 ppt) from the adjacent bay.

Prior to the study, fry were removed from the groundwater broodstock tanks when first observed and were given a sex-reversal diet in troughs. They were between one day and one week old and averaged 6.3 mm SL (range 6-7 mm SL), and 0.008 g when used in the study. Juveniles were obtained from outdoor pools containing fish that had completed a successful four-week, sex reversal process. They were maintained in groundwater on a 2% body weight/day diet of trout chow. When used in the study they averaged 26.5 mm SL and 0.8 g (ranges: 17-43 mm SL, 0.3-2.0 g). The response of this group was of most interest as experiments testing growth in seawater cages were to utilize these fish. The adult fish available were females which had been held in a lagoon with variable salinities up to approximately 30 ppt. For the study, 150 fish were reacclimated to groundwater in aerated pools for two weeks. Average length was 133.4 mm SL and average weight was 82.6 g (ranges: 105-160 mm SL, 38.0-157.0 g).

Salinity tolerances of fry and juveniles were tested in aerated, styrofoam boxes filled with approximately 15 liters of the desired salinity. Adults were tested in aerated, fiberglass boxes filled with approximately 80 liters of the test salinity. All salinities were measured with a refractometer (Biomarine, Hawthorne, CA). Accuracy was continuously checked against a distilled water standard. Seawater was diluted with groundwater. Water temperatures ranged from 28-29°C and oxygen levels from 6.6-7.0 ppm in the indoor styrofoam boxes during the fry and juvenile trials. Water temperatures were 30-32°C in the outdoor, shaded fiberglass boxes during the adult trials, and oxygen levels were 3.5-5.5 ppm.

The study tested salinity tolerances at 2-ppt intervals from 19 to 37 ppt of fry and juveniles, and from 25 to 35 ppt of adults. Preliminary testing outlined the critical salinity range for each group. Results within groups and between groups were tested for significant differences at the .05 level by ANOVA and Duncan's New Multiple Range Test.

In each trial, 10 fry, 10 juveniles, and 5 adults per 2-ppt salinity interval were transferred directly to the test containers. Test organisms totalled 300 fry, 300 juveniles, and 85 adults. Time of death and behavioral responses to salinity stress were noted in the initial trial. In later trials, fish that lost equilibrium were removed to groundwater to save animals wherever possible. Fish in each trial were observed for three days, with feed offered the day following transfer. Commencement of vigorous feeding was noted and was assumed to indicate adjustment to the medium.

Results

No mortalities were noted at 19 ppt for fry and juveniles and at 27 ppt for adults (Table 1). Total mortalities ensued at and above 29 ppt (fry), 31 ppt (juve-niles) and 37 ppt (adults). Fry and juvenile tolerances for the most part did not significantly differ, although at most salinities juvenile survival was higher than that of fry. At each salinity, adult

Table 1. 72-hour tolerances of red hybrid *(Oreochromis mossambicus/O. urolepis hornorum)* tilapia fry, juveniles, and adults to various salinities following direct transfer from groundwater (1.5-2.0 ppt). Averages with the same superscript letter (within size group comparisons) or number (between size group comparisons) do not significantly differ at the .05 level.

Trials	Mortality (%)									
Salinity	19 ppt	21 ppt	23 ppt	25 ppt	27 ppt	29 ppt	31 ppt	33 ppt	35 ppt	37 ppt
Fry										
1	0	0	40	70	90	100	100	100	100	100
2	0	20	30	80	100	100	100	100	100	100
3	0	0	10	20	80	100	100	100	100	100
Average	0.0^{a-1}	6.7^{a-1}	26.7^{b-1}	56.7^{c-1}	90.0^{d-1}	100.0^{d-1}	100.0^{d-1}	100.0^{d-1}	100.0^{d-1}	100.0^{d-1}
± S.D	0.0	11.5	15.3	32.1	10.0	0.0	0.0	0.0	0.0	0.0
Juveniles										
1	0	10	0	30	80	90	100	100	100	100
2	0	0	20	60	80	90	100	100	100	100
3	0	0	30	30	80	90	100	100	100	100
Average	0.0^{a-1}	3.3^{a-1}	16.7^{b-1}	40.0^{c-2}	80.0^{d-1}	90.0^{d-1}	100.0^{e-1}	100.0^{e-1}	100.0^{e-1}	100.0^{e-1}
± S.D.	0.0	5.8	15.3	17.3	0.0	0.0	0.0	0.0	0.0	0.0
Adults										
1				0	0	80	40	40	60	100
2				0	0	40	40	40	75	
3					0	0	40	40	75	
Average				0.0^{a-3}	0.0^{a-2}	40.0^{b-2}	40.0^{b-2}	40.0^{b-2}	70.0^{c-2}	
± S.D.				0.0	0.0	40.0	0.0	0.0	8.7	

mortality was significantly less than that of fry and juveniles. At the conclusion of each trial fish were returned directly to groundwater with no stress reactions or mortalities.

Vigorous feeding by fry and juveniles was observed after 18 hours following transfer to 19 and 21 ppt, and after 78 hours in higher salinities. Adults fed normally after 24 hours in 25 ppt and 27 ppt and after 48 hours in higher salinities.

Stress behavior of fry progressed from sluggishness, but with rapid pectoral and gill movement, to frantic swimming to sinking and loss of equilibrium, followed by death. The first death occurred after 60 minutes and the last after 18 hours following transfer from groundwater.

Juvenile stress behavior was similar to that of fry. Rapid pectoral and opercular movements were followed by floating and shimmying, which led to sinking, loss of equilibrium, and death. The first juvenile died 135 minutes and the last 43 hours post-transfer.

Adult fish were less responsive to salinity stress than younger fish. Sluggishness was followed by resting on the bottom and death. Fish were removed when pectoral fin movement stopped, indicating loss of equilibrium. The first fish was removed after 23 hours and the last after 48 hours.

Discussion

The objective of the study was to define the salinity tolerances of fry, juveniles and adult Florida red hybrid tilapia. Two considerations in interpreting

the data are bias from use of all-male juveniles and all-female adults and the effects of possible preconditioning in 1-2 ppt groundwater. Regarding the former, we feel results for juveniles and adults would not have differed significantly if mixed sexes had been tested. The sexes of another euryhaline fish, the desert pupfish *(Cyprinodon n. nevadensis),* did not differ in salinity tolerances (Gerking and Lee 1980); although Love (1978) reported a study by Odense et al. (1966) who found that female cod *(Gadus morhua)* succumb at lower salinities than do males. When transferring a given age group, male tilapia will tend to be larger than females and more salt tolerant, based on the study of Watanabe et al. (1985). The latter question was addressed by holding a group of juveniles in reverse-osmosis desalinized freshwater for 2 weeks prior to rerunning a trial. Results suggested no effect on salt tolerance from groundwater holding.

On the basis of the study fry and juveniles will withstand direct transfer to 19 ppt and adults to 27 ppt. Feeding will resume in one day. Transfer to salinities above these levels will require prior acclimation, for which a safe and convenient salinity is 19 ppt or 50% seawater.

Adults are more salt tolerant than fry and juveniles. The salinities at which all fish die following direct transfer from groundwater are 29 to 31 ppt for fry and juveniles (tested to 20 g) and 37 ppt for adults (tested to 157 g).

Watanabe et al. (1985) found that salinity tolerance after direct transfer from freshwater of the Taiwanese red hybrid tilapia also increased with age beyond day 7 as a function of body size not age. Their results with 10-20 fish, equivalent in weight to fry and juveniles in this study, revealed no mortality at test salinities of 20 ppt and 17.5 ppt, respectively and total mortality at 25 and 32 ppt, respectively.

Apparently, the response of hybrids is very similar to that of *O. mossambicus.* Kader et al. (1981) and Pange (1985) reported no mortality of juveniles following direct transfer from freshwater to 20 ppt, with total mortality at 35 ppt.

The ability of *O. mossambicus* to adjust to high salinities has been inferred from its original distribution in East Africa which includes estuaries and the lower reaches of rivers. Philippart and Ruwet (1982), based on a study of the estuarine distribution of *O. mossambicus* by Whitfield and Blaber (1979), suggested an upper salinity range of 120 ppt with a possible inability to adjust to rapid salinity changes, as *O. mossambicus* were absent from estuaries permanently open to the ocean. They also mentioned other potential causes for the species absence, such as currents, nesting problems, and predation/competition with other marine and estuarine species; which, in light of our results, appear more promising explanations.

The functioning of the remarkable osmoregulatory ability of *O. mossambicus* is not fully understood. Drinking rate, Na-exchange, and NaCl transport are listed as important (Jurss et al. 1984) Foskett et al. (1981) have observed proliferation of chloride cells on the gills upon transfer to 100% seawater. Venkatachari (1974) has noted a slight increase in free amino acids in tissues of *O. mossambicus* exposed to salinities above 8.1 ppt.

The other species in the Florida red hybrid, *O. urolepis hornorum* has also been reported to be highly salt tolerant (Trewavas 1983). It has been grown and has reproduced in marine ponds; however, it has not been reported from the estuaries of the rivers it inhabits (Payne 1983).

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Photoperiod Mediated Variation in Respiratory Rate of *Oreochromis niloticus* and Its Implication for Tilapia Culture

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Abstract

Respiratory rate is an important physiological variable which can considerably influence aquaculture systems. It is known that a wide range of biotic and abiotic factors affect oxygen uptake by fishes. Using computer-operated respirometers and a normal 12D:12L photoperiod, this study showed that the daytime respiration rate of *Oreochromis niloticus* increased by more than 30% over the night-time rate under otherwise constant conditions. Variability in published results for respiration rate in tilapias is discussed and an attempt is made to evaluate these discrepancies in the light of these new data on photoperiod mediated respiratory rate. It is suggested that such detailed studies are necessary in order to gain a complete understanding of oxygen consumption in cultured fishes and for the preparation of comprehensive models of these important processes in aquaculture.

Introduction

Factors affecting oxygen consumption rate are very important in the design and management of intensive and semi-intensive fish culture systems (Meyer and Brune 1982) and can be crucial in the tropics and subtropics where the fish growth and survival in aquatic environment is frequently oxygen-limited (Boyd 1982). Depending on temperature and body weight, fish have a resting (or standard) rate of oxygen consumption which can become elevated due to specific dynamic action (SDA) (Jobling 1983) or swimming activity (Farmer and Beamish 1969). The difference between the resting rate and the maximum aerobic (active) rate of oxygen consumption is known as the scope for activity (Fry 1957) and the

fish's routine rate is variable between these two limits (Priede 1985). The tilapias are very important cultured species in warm waters and a number of authors have measured resting respiratory rates of certain species (Ross and Ross 1983). Estimates of active rate have been made by Farmer and Beamish (1969) and Mishrigi and Kubo (1978a). Routine oxygen consumption has been determined by a number of authors (Ahmed and Magid 1968; Job 1969a, 1969b; Kutty 1972; Magid and Babiker 1975; Mélard and Philippart 1980) although it is, from its definition, a very variable quantity.

In comparing the respiratory data available on tilapias it is clear that there is some discrepancy between authors, with a maximum error of ±30 to 40% of the mean of all values for resting rate. The situation for routine rates is very much worse. These errors may be attributable to stress, the effects of which can be dramatic in the short term and may persist for longer periods (Ross and Ross 1983). Often results have been collected after too brief an acclimation of fish to the apparatus and using respirometers of variable size, design and operation. Overall, the spread of available respiratory data for tilapias means that in the planning of culture facilities a wide range of possible carrying capacities could be calculated depending on the source data used. It is common, therefore, to underestimate carrying capacity by overestimating oxygen consumption rates so as to ensure safe system operation. However, in all situations where fish production and water use are to be maximized, a fuller understanding of the factors affecting oxygen consumption is required so that a reasonably comprehensive model can be used for a given species.

It is known that some species of fish have a varying respiratory rate which has been linked to daily photoperiod (Davis 1962; Holliday et al. 1964; De Silva et al. 1986) and that this may be under hormonal control (Love 1980). Other authors have also shown that respiratory rate may be influenced by longer-term changes in photoperiod (Withey and Saunders 1973) and clearly, this rhythmicity may, in part, contribute to the notable variation in published respiratory data and warrants further investigation.

Continuous medium-term monitoring of respiratory rates in fish, with adequate replication of results by using several respirometers simultaneously can only be carried out in flow-through systems. These can provide the controlled environmental conditions necessary during such experiments (Gnaiger 1983) and, additionally, are easier to arrange for automatic operation. This paper presents new data, derived using self-calibrating, computer-operated respirometers, which show the cyclic nature of resting respiratory rate in the Nile tilapia, *Oreochromis niloticus*, and its relationship to photoperiod. It is suggested that these data are sufficient to account for the variability in published values of respiratory rate of tilapias. The significance of these data in the operation and planning of culture facilities is also discussed.

Materials and Methods

Genetically pure *Oreochromis niloticus* (McAndrew and Majumdar 1982) of 70 to 120 g were acclimatized to 25°C. The fish were anesthetized in benzocaine (Ross and Geddes 1979) and weighed before placing them in the chambers of a six-channel computer-operated respirometer system (Ross and McKinney 1988) at 25°C. The fish were then monitored over a 9-day period without feeding. The photoperiod to which the fish were acclimatized was typical of tropical conditions (0800 to 2000 hours; 12L:12D) and this was replicated in the respirometer system. Five groups of four naive fish were used, producing twenty successful records in about 35 days.

After monitoring a group of fish for 2 days as described above, the illumination regime was changed to continuous light. The fish were then monitored for a further 7 days. Naive fish were used on each occasion and four such groups were used.

Four further groups of naive fish were monitored for 2 days and the illumination was then changed to continuous darkness for a further 7 days.

The results were reprocessed using a five-point running average routine (RSMOOTH) on a mainframe computer (VAX 11/750) running MINITAB (Penn State University), since, in common with other similar long-term respirometry a wide variation in the raw data partially obscured the overall trends.

Results

The smoothed data for all fish subjected to this photoperiod were pooled and averaged in 1-hour blocks. These reprocessed data are shown in Fig. 1. In general, the fish can be seen to be stressed during the first 24 to 48 hours, settling to a cyclic pattern of oxygen consumption over the remaining period in the respirometers. The variation was from 80 mg/kg/hour at night to 120 mg/kg/hour during daylight, with a mean value of about 95 mg/kg/hour. Although the fish were starved throughout this procedure in order to evaluate resting oxygen consumption rate, the respiratory cycles were maintained without apparent decrement until the end of the experiment.

The respiratory activity in continuous light remained cyclic (Fig. 2). The initial stress response was again followed by a cyclic pattern which was maintained over the entire period in the respirometers. The pooled data varied from 80 mg/kg/hour to 110 mg/kg/hour, with a mean value of about 90 mg/kg/hour. The peaks and troughs in the data continued to cor-

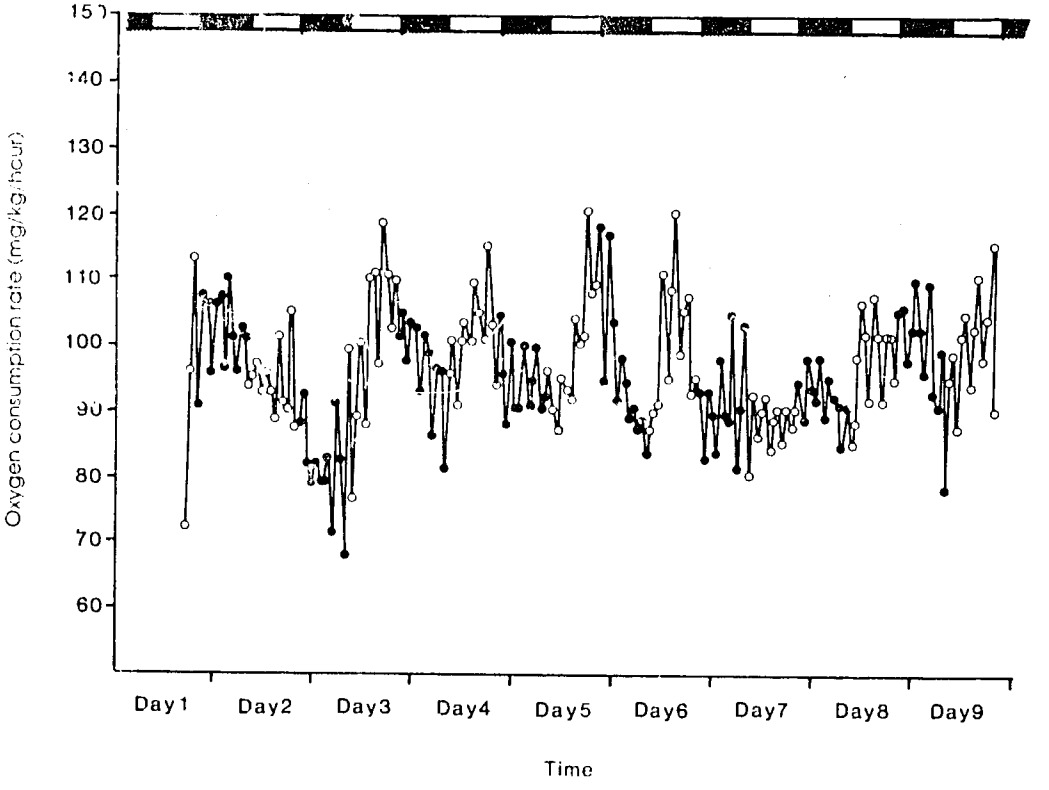

Fig. 1. Computer-smoothed variation in resting respiratory rate for *Oreochromis niloticus* at 25°C and under a normal (12L:12D) photoperiod. The data represent the pooled responses of eight fish.

The bar above the figure shows the actual experimental photoperiod. Open circles are used to show respiration during the light portion of the day, and closed circles indicate respiration during the dark portion of the day, both corresponding to a 12L:12D photoperiod. Grey shading represents the dark phase of the adapted photoperiod, projected next to the curve for comparison.

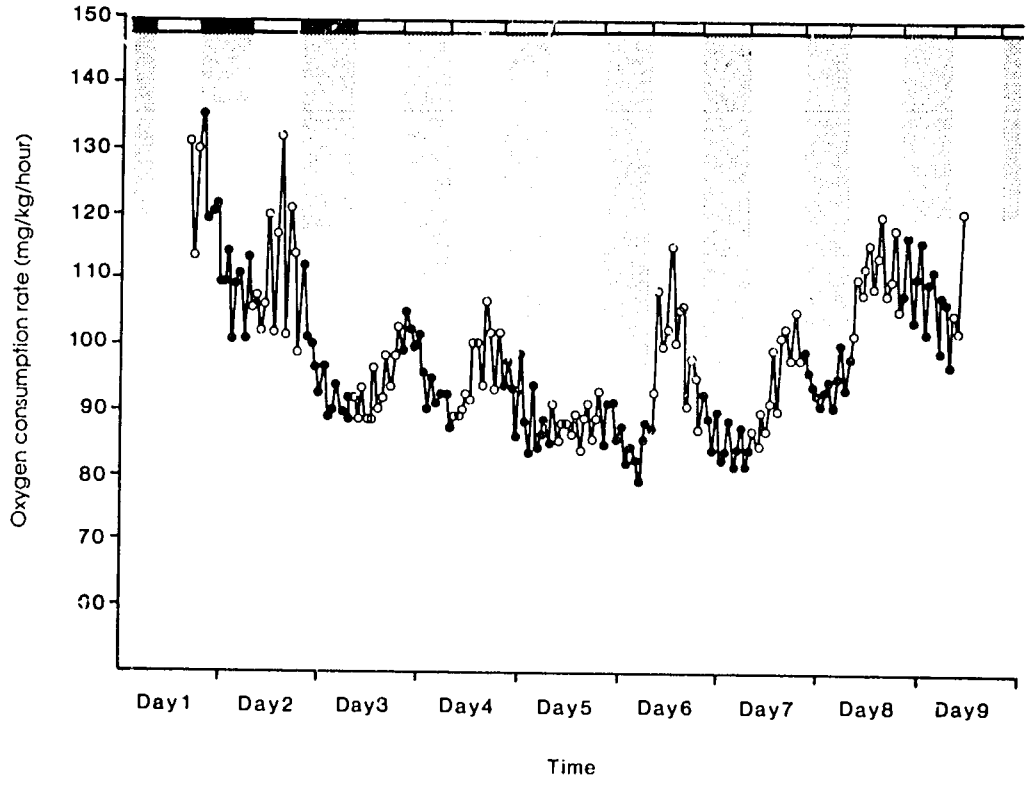

Fig. 2. Computer-smoothed variation in resting respiratory rate for *Oreochromis niloticus* at 25°C and in continuous light (24L:0D). The data represent the pooled responses of twelve fish.

The bar above the figure shows the actual experimental photoperiod. Open circles are used to show respiration during the light portion of the day, and closed circles indicate respiration during the dark portion of the day, both corresponding to a 12L:12D photoperiod. Grey shading represents the dark phase of the adapted photoperiod, projected next to the curve for comparison.

respond closely to the photoperiod to which the fish had been acclimated (12L:12D).

On changing to continuous darkness the fish established a new, steady, pattern in which the residual rhythmicity was much suppressed (Fig. 3). The data varied from 90 mg/kg/hour to 100 mg/kg/hour, with a mean value of about 95 mg/kg/hour. Although there was some correspondence between the variation in respiratory rate and the photoperiod to which the fish were previously acclimated this was not as clear as in the previous experiments.

Discussion

The work described here shows a clear rhythm in resting respiratory rate in *O. niloticus* (Fig. 1). Similar cyclic effects have been shown by Hirata (1973) in *Salmo salar* and Hamada and Maeda (1983) in *Cyprinus carpio*. Crepuscular respiratory patterns, coinciding usually with dawn and dusk peaks in activity have been described by Huang (1975) in *C. carpio* and Nagarajan and Gopal (1983) in *Sarotherodon mossambicus*. There is some

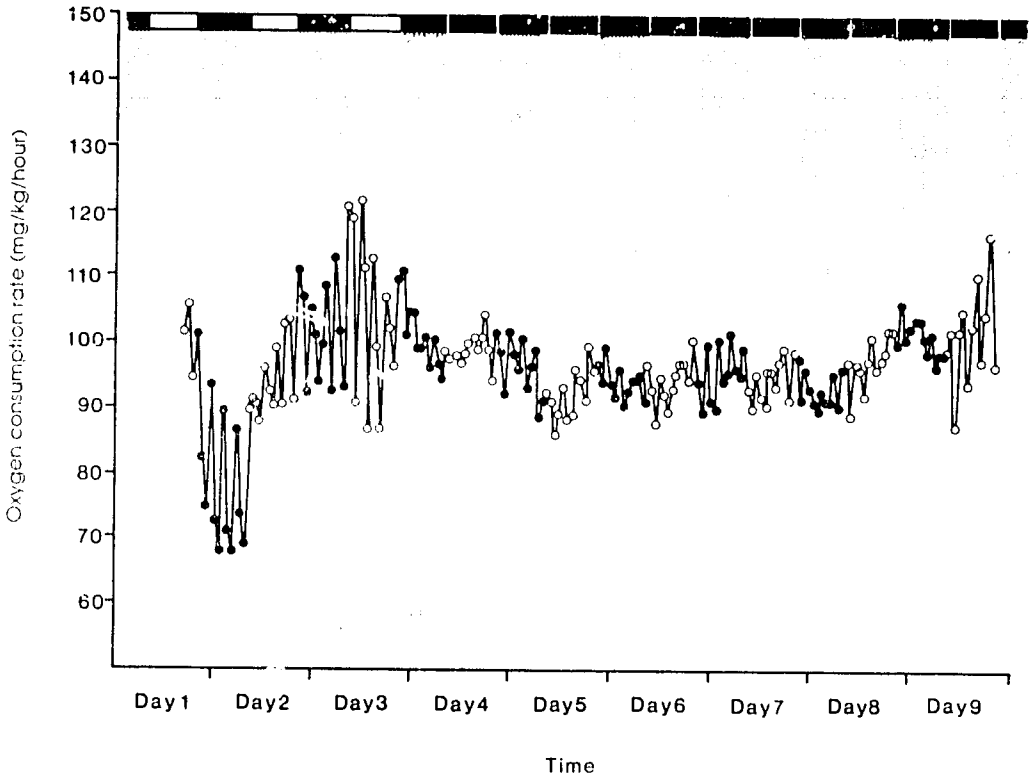

Fig. 3. Computer-smoothed variation in resting respiratory rate for *Oreochromis niloticus* at 25 C and in continuous darkness (0L:24D). The data represent the pooled responses of twelve fish.

The bar above the figure shows the actual experimental photoperiod. Open circles are used to show respiration during the light portion of the day, and closed circles indicate respiration during the dark portion of the day, both corresponding to a 12L:12D photoperiod. Grey shading represents the dark phase of the adapted photoperiod, projected next to the curve for comparison.

variation in the data available for tilapias; for example, De Silva et al. (1986) showed that yolk-sac larvae of *O. niloticus* have a crepuscular respiratory rhythm, whereas 12-mm fry had a single dawn peak in respiration. By contrast, Nagarajan and Gopal (1983) demonstrated crepuscular respiratory rhythms in *O. niloticus* of 5- to 25-g body weight, whereas this study found a single peak in resting rate during daylight in fish of 70 to 120 g. The reasons for these differences is unclear, and may be, at least in part, explained by different experimental techniques (for example, it is not known whether the respirometric techniques employed by De Silva et al. (1986) and Nagarajan and Gopal (1983) were designed to prevent motor activity). However, it is known that many aspects of the physiology of tilapias change as the animals grow, and complex alterations in behavior may be reflected by changes in respiratory strategy as the fish develop.

When illumination became continuous (Fig. 2) the respiratory rhythm remained, without apparent decrement, despite the absence of the presumed zeitgebers after the second day. However, on changing to continuous darkness (Fig. 3), the magnitude of the respiratory rhythm was greatly

reduced and became less clear as time progressed. Thus it appears that the rhythm is mediated at least by light level and probably also by photoperiod and may be exogenous in origin as was found in juvenile Atlantic salmon, *S. salar*, by Hirata (1973).

The data from these experiments have been compared with those of other authors in Table 1, using only values derived for resting respiration rate. The variation in resting respiration rate between authors, for different body weights of fish, ranges from ±30 to 40%. This variation can be substantially accounted for by the daily range seen under a normal 12L:12D photoperiod (Fig. 1, Table 1). It should be borne in mind that the percentage variation shown in our work (±20%) is based on pooled and smoothed data, as described earlier, and consequently the full variability seen in our raw data would easily account for that seen amongst authors.

This cyclic respiratory activity could clearly affect the calculated carrying capacity of a system depending on time of day. It is interesting to compare this performance with data for daily dissolved oxygen (DO) variation in an aquaculture pond. In Fig. 4, respiratory cycles predicted from this paper are compared with the predicted maximum and minimum DO in a subtropical pond using data derived by Meyer and Brune (1982). It is known that tilapias are oxygen-conformers (Ross and Ross 1983) and it can be seen that as DO decreases in the pond the resting respiratory rate of the fish decreases, with a time lag of 2-3 hours. In this example, it should be noted that the minimum DO does not fall below the critical oxygen tension (pC) which would result in reduced, oxygen-dependent respiration. This resting respiratory behavior has obvious adaptive significance in the natural environment but may also have implications for aquaculture in oxygen-limited systems. For example, in view of the additional oxygen requirement for feeding (SDA) it could be advisable to minimize early morning feeding with the bulk of the daily ration given later in the day so as to take full advantage of the available DO at this time.

This work, and that of previous authors, strongly reinforces the necessity to carry out respirometric determinations under precise conditions, which must include a properly arranged photoperiod and consideration of the time of day. Preferably, many measurements must be made so that a mean value can be used. This is particularly important where use will be made of the resulting data, for example, in design of aquaculture facilities where it is necessary to maximize oxygen availability and water use.

Table 1. A comparison of resting respiratory rates for various tilapias at 25°C.

Species	Resting respiratory rate (mg/kg/hour) 50 g	75 g	100 g	Author
Oreochromis niloticus	-	104.4	-	Farmer and Beamish (1969)
O. niloticus	-	171.0	-	Magid and Babiker (1975)
O. niloticus	81.0	75.0	68.0	Mishrigi and Kubo (1978b)
O. niloticus	162.0	140.0	126.0	Ross and Ross (1983)
O. niloticus	-	120.8	-	Verheyen et al. (1985)
O. niloticus	105.0	-	-	Zuim (1979)
O. mossambicus	134.0	116.0	105.0	Caulton (1978)
Tilapia rendalli	187.5	-	-	Caulton (1977)
% Variation	±40%	±39%	±29%	
O. niloticus (light)	-	-	120.0	This paper
O. niloticus (dark)	-	-	80.0	This paper
% Variation	-	-	±20%	

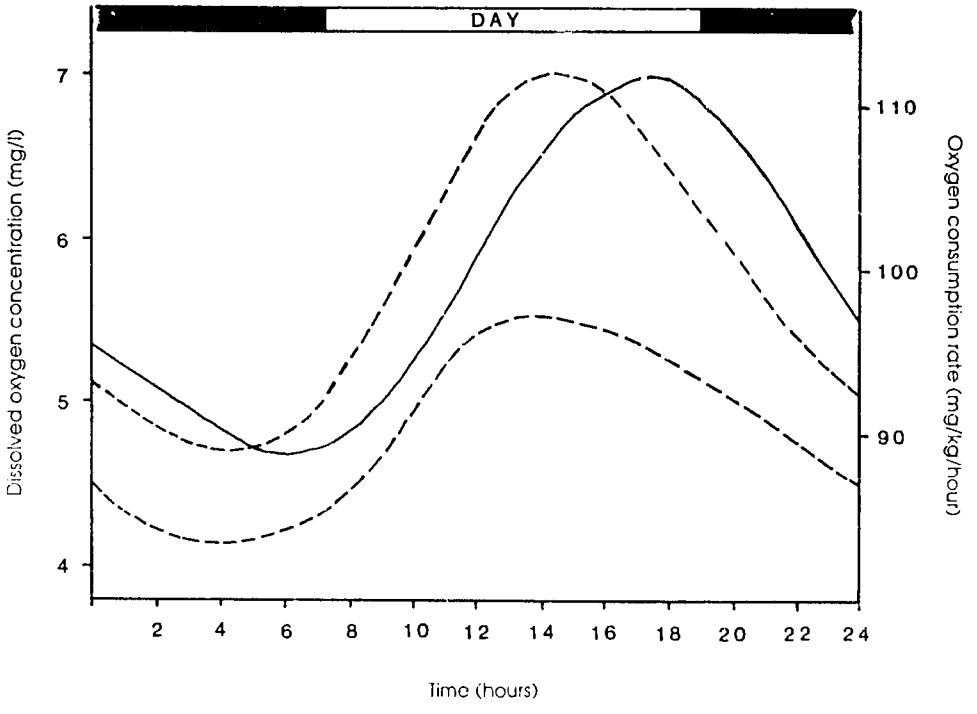

Fig. 4. The relationship between the resting respiration rate (solid line) of *Oreochromis niloticus* under a normal photoperiod (12L:12D) and the maximum and minimum dissolved oxygen concentrations (dotted lines) of a typical tilapia pond (data adapted from Meyer and Brune 1982).

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Food Consumption and Growth Efficiency of Normal and Phenotypic Male *Oreochromis mossambicus*

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VARADARAJ, K. and T.J. PANDIAN. 1988. Food consumption and growth efficiency of normal and phenotypic male *Oreochromis mossambicus*, p. 429-432. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623 p. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Abstract

Attempts were made to understand the role played by the administration of 17α-methyltestosterone (MT) in sex reversal and growth acceleration in *Oreochromis mossambicus*. Bioenergetic data showed that a low dose of 1.5 µg MT/g fish/day administered for a short duration of 11 days significantly changed the food consumption ($P < 0.001$). However, longer treatment with the same dose (1.5 µg MT/g fish/day for 16 days) not only ensured 100% sex reversal but also enhanced feed conversion efficiency. When treated with 1.5 µg MT/g fish/day for 16 days, mixed genetic males (produced by sex reversal) displayed growth rates that surpassed those of genetic males.

Introduction

That the supplementation of diet with synthetic steroids like 17α-methyltestosterone (MT) can induce complete sex reversal in tilapias is an established fact. Sex reversal of female tilapias has been achieved by feeding early fry with food containing 30-60 µg MT/g food for 19-69 days (Clemens and Inslee 1968; Nakamura 1975; Guerrero 1975, 1979; Anon. 1979; Macintosh et al. 1985). Available information on the induction of sex reversal through hormone treatment is also confusing; for instance Nakamura (1975) claimed to have achieved 100% sex reversal but reported that 20% of his fish developed ovarian cavities. Clemens et al. (1968) also claimed that supplementation of diet with 10 or 30 µg MT/g diet ensured 100% sex reversal, but the diet containing 20 mg failed to induce 100% sex reversal. Macintosh et al. (1985) achieved 94% sex reversal (30 µg MT/g for 60 days). However, Pandian and Varadaraj (1987; this vol.) have shown that supplementation of diet with a critical minimum dose of 1.5 µg MT/g fish/day for a minimum period of 11 days resulted in 100% sex reversal of 9-day old *Oreochromis mossambicus* fry.

Supplementation of diets with steroids has been shown to accelerate fish growth (Pandian 1982; Sindhu and Pandian 1984). The steroid docabolin enhances the growth rate of *Channa striata* and is an appetite stimulant (Nirmala and Pandian 1983). Therefore, steroid supplementation of fish diets may accelerate growth by appetite stimulation and/or anabolic properties (Arul 1986) as well as affecting the sex of undifferentiated fry. These effects have been little studied in tilapias, despite the use of a range of treatments. Fryer and Iles (1972) concluded that male growth superiority in cichlids has a genetic basis and is not just a function of the reproductive process. Whether a mixed group of genetic and sex-reversed males would grow as quickly as a wholly genetic male population of tilapia has yet to be investigated. This paper investigates the effect of MT administration on sex reversal and growth in *O. mossambicus*.

Materials and Methods

Nine-day old *O. mossambicus* fry were collected from brooders of similar size on the same day, from indoor tanks. Each treatment was given to duplicate populations of 40 fry each. The minimum daily dose and treatment duration required to ensure 100% sex reversal are 1.5 µg MT/g fish/day and 11 days, commencing on the tenth day following hatching (Pandian and Varadaraj 1987; this vol.). In the first series of experiments, duplicate groups were given 1.5 and 3.0 µg MT/g diet for 11 days, i.e., the minimum dose to ensure sex reversal and a higher dose than that required for sex reversal. Treatment of parallel groups was prolonged to 16 days (Table 1).

During treatment, the fry were reared in aerated 1.5-l aquaria. After treatment all fish from each experimental group were stocked in 45-l aquaria supplied with flowing charcoal-filtered, aerated water at 27 ± 2°C. The fish were fed *ad libitum* on a pelleted diet containing *Spirulina* 30%; fish meal 25%; rice bran 25% and wheat flour 20%. Subsequent to the MT treatment, all the treated groups and an untreated control group, received this pelleted diet at 10% of the body weight/day, adjusted every 5 days. Ten individuals (25% of the surviving individuals of 35 days old) were randomly selected from each group for bioenergetic studies. These were conducted on individual fish in separate aquaria. Feces were collected by siphoning from the aquaria every day. Before taking the initial and final weights, the test fish were starved for 24 hours to ensure complete evacuation of the gut. Control and experimental fish were fed at the rate of 10% of their body weight. When fish had not consumed all the available food, the uneaten food was collected and deducted from the given food.

The following scheme of energy balance was followed (Petrusewicz and MacFadyen 1970):

$$C = F + U + M + P$$

Table 1. Bioenergetics of *Oreochromis mossambicus* fed on diets supplemented with different doses of 17α-methyltestosterone (MT). Each value represents the performance of 10 individuals* (100% survival in the experimental and control groups).

Parameter	Control	Diet treated with the steroid (µg/g fish/day for 11 days) 1.5	3.0	(µg/g fish/day for 16 days) 1.5	3.0
Food consumption**	106 ± 0.1	108 ± 0.2	93 ± 5.0	102 ± 0.1	100 ± 6.0
Food absorption**	93 ± 0.1	96 ± 0.4	88 ± 5.0	92 ± 0.1	91 ± 0.1
Growth**	45 ± 1.4	10 ± 1.4	57 ± 2.3	65 ± 0.5	57 ± 0.6
Metabolic rate**	48 ± 1.2	56 ± 1.0	31 ± 3.2	27 ± 0.2	37 ± 0.2
Absorption efficiency***	88 ± 0.1	89 ± 0.2	90 ± 0.0	90 ± 0.0	90 ± 0.0
Food conversion efficiency***	49 ± 1.4	42 ± 1.1	64 ± 0.7	70 ± 0.6	61 ± 0.6

*Mean ± S.D.
**Mg/g live weight /day.
***In percentage.

where C is the food consumed; (food given - uneaten) F, the feces; U, the urine; M, the energy lost as heat due to metabolism and P, the growth (= food conversion). The weight of the food absorbed (A) was calculated by subtracting F from C, and P by subtracting the initial weight of the fish from the final weight. Rates of feed absorption and conversion were calculated by dividing the respective quantum of wet weight by the products of live fish weight (g) and the duration of the experiment and expressed in terms of mg/g live (wet) weight/day. Efficiency (%) of absorption was calculated by relating A to C, and the conversion efficiency (%) by relating P to A.

A second series of experiments was undertaken to study the growth of mixed (genetic + sex reversed) and wholly genetic populations of males. For this purpose the fry from known parentage were procured and grouped into controls (genetic males and females); unmixed genetic males; unmixed genetic females; mixed genetic and sex-reversed males treated with 1.5 µg MT/g fish daily either for 11 or 16 days and mixed genetic and sex-reversed males treated with 3 µg MT/g fish daily either for 11 days or 16 days. On completion for MT treatment these fry were reared for 72 days, feeding *ad libitum* on a pelleted diet (Table 2).

Results and Discussion

Bioenergetic data obtained for the fry tested under the series 1 are summarized in Table 1. MT treatment at lower dose for a shorter duration (1.5 µg for 11 days) significantly increased food consumption. However, the same or higher dose administered for a longer duration (16 days) reduced the food consumption. Therefore low dose of MT at shorter treatment duration acts as an appetite stimulant.

Control *O. mossambicus* converted the food with 49% efficiency; the efficiency values obtained for the fry treated daily

Table 2. Summary of the growth promotion effect of 17α-methyltestosterone (MT) on *Oreochromis mossambicus* fry (n = 40) reared for 72 days.

Parameters	MT dose (µg/g fish/day)	Duration of treatment, started on the 10th day after hatching	Individual growth in mg (mean ± S.D.)
Controls			
Mixed sex (♂♂ and ♀♀)	—	—	1.23 ± 0.43
Unmixed genetic ♂♂	—	—	1.55 ± 0.23
Unmixed genetic ♀♀	—	—	0.84 ± 0.06
Treated			
Mixed genetic Sex reversed ♂♂	1.5	11 days	a 2.21 ± 0.45
Mixed genetic Sex reversed ♂♂	1.5	16 days	b 2.91 ± 0.40
Mixed genetic Sex reversed ♂♂	3.0	11 days	c 2.45 ± 0.45
Mixed genetic Sex reversed ♂♂	3.0	16 days	d 2.69 ± 0.35

Student 't' test: a Vs b $P < 0.0005$; b Vs d $P < 0.005$; c Vs d $P < 0.05$; a Vs c $P < 0.025$.

with 1.5 µg MT/g for 16 days showed the highest (70%). Likewise the fry treated daily with 3 µg MT/g fish/day either for 11 or 16 days also displayed high (>60%) efficiency. Thus the hormone, when administered for longer duration, induced anabolic enhancement of growth efficiency. Therefore, prolonging the daily treatment with 1.5 µg MT/g fish/day for 16 days not only ensured 100% sex reversal of genetic females but also increased the growth efficiency by 1.5 times.

Data obtained on the growth of fry during the 72-day rearing period are summarized in Table 2. When reared separately or along with the females, unmixed genetic males grew slowly and attained a body weight of about 1.2-1.6 g. However, mixed genetic and sex-reversed males grew faster and attained 2.2-2.9 g. When comparing the MT treated fish with control (Table 2), the mixed genetic and sex-reversed males grew faster than genetic males both during and after treatment period. It may be concluded that the treatment with 1.5 µg MT/g fish/day for a period of 16 days not only ensured 100% sex reversal but also accelerated the growth rate and enhanced conversion efficiency. Guerrero (1975), who observed the faster growth of androgen-treated *O. aureus*, could not determine whether the phenomenon was due to increased feeding and/or due to enhanced growth efficiency. Our study shows that it is not only the dose but the duration of the treatment that evoked significant difference in food consumption and growth efficiency.

Acknowledgement

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Effects of Acid Water on Survival and Growth Rate of Nile Tilapia *(Oreochromis niloticus)*

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Abstract

The effects of acid water on survival, behavior and growth of Nile tilapia *(Oreochromis niloticus)* were studied. All fingerlings (mean total length 3.1 cm; mean weight 0.4 g) and adults (mean total length 14.5 cm; mean weight 45.4 g) died at pH 2.0 and 3.0 within 1 to 3 days. Survival of fingerlings (mean total length 4.0 cm; mean body weight 1.0 g), exposed to pH levels of 4.0, 5.0 and control (7.0 ± 1) for 60 days was 57.8, 82.2 and 84.5%, respectively, whereas survival of adults (mean total length 14.2 cm; mean body weight, 46.3 g), exposed to the same pH levels for 70 days was 86.6, 100.0 and 100.0%, respectively. All fish showed behavioral manifestation of physiological stress almost immediately at pH 2.0 and 3.0: rapid swimming and opercular movements; surfacing and gulping of air and inability to control body position. The mean specific growth rates of fry were 0.70, 1.08 and 0.92%/day at pH 4.0, 5.0 and 7.0 (control), respectively; these were not significantly different. The corresponding mean specific growth rates of adults were 0.51, 0.52 and 0.61%/day and were also not significantly different.

Introduction

The pH of water exerts major effects on water quality and aquatic life (Odum 1959). Most natural water bodies have a pH close to neutrality, but some lakes and reservoirs have acid pH's as low as 2.9 (Beamish 1976). In Thailand, acid water in alluvial soil areas has been reported in Narathiwat, Pattani, Nakhon Sri Thammarat, Surat Thani, Chon Buri and Chachoengsao Provinces (Teinsongrassamee 1978; Suthipradith 1982; Tansakul, unpublished data). The pH of such waters may be below 4.0 (Suthipradith 1982).

This study was made to determine the suitability of acid water for the survival and growth of Nile tilapia *(Oreochromis niloticus)*, generally a fish most tolerant of poor water quality. If tilapia could be successfully reared in low pH water it would provide a cheap protein resource in rural, low income areas in Thailand affected by acid soils and water.

Materials and Methods

Experiment 1. Survival of Nile tilapia fingerlings and adults in acid water

Nile tilapia were obtained from the Pattani Governmental Fisheries Station and acclimatized in the laboratory for 2 weeks before the experiment. Dechlorinated tap water was used, aerated for 24 hours before the beginning of an experiment. It was adjusted twice daily (0900 and 1300 hours) to pH levels of 2.0, 3.0, 5.0 and 9.0 with 0.1M H_2SO_4 and 0.1 N NaOH [without buffers; to eliminate their residual effects (Dunson et al. 1977)]. The control pH 7.0 was not adjusted and varied by ± 0.1 throughout the experiments.

Thirty Nile tilapia fingerlings of mean total length (± S.E.) 3.13 (± 0.02) cm and mean weight (± S.E.) 0.4 (± 0.02) g were held in triplicate 20-l fiberglass tanks and 15 adult fish of mean total length (± S.E.) 14.50 (± 0.14) cm and mean body weight (± S.E.) 45.38 (± 0.92) g were held in triplicate 50-l fiberglass tanks. Fish feces, sediments and one-fifth of the water were removed every day, replacing the water with clean water. The water was changed completely every 10 days.

All fish were fed with chicken grower pellets (containing not less than 19% protein) at 5% of their body weight/day, twice a day (1000 and 1600 hours) The fish were weighed every 10 days for ration adjustment.

Upon exposure to selected pH levels, survivors and dead fish were counted at 1, 2, 3, 12 and 24 hours and at 2 and 3 days and every 3 days thereafter for a total of 30 days. Dead fish were identified by lack of any reaction when touched with a glass rod and lack of movement of gill opercula. They were removed from the water tank when identified. Data were analyzed by ANOVA and least significant difference (Snedecor and Cochran 1967).

Experiment 2. Survival and growth of Nile tilapia fingerlings and adults at pH levels of 4.0, 5.0 and control (7.0 ± 0.1)

Fifteen fingerlings in tanks with 15 l of water per tank and 10 adults in tanks with 20 l of water per tank were reared at pH levels 4.0, 5.0 and control (7.0 ± 0.1) with three replicates at each pH. Treat-

Table 1. Means (and S.D.'s) of initial total lengths and weights and final weights of Nile tilapia *(Oreochromis niloticus)* fingerlings and adults, exposed to different pH's for 60-70 days. For details of tank conditions and feeding, see text.

Fingerlings	Initial total length (cm)		Initial weight (g)		Final weight (g)		Specific growth rate (%/day)
pH 4.0	4.06	(0.08)	1.00	(0.06)	1.52	(0.12)	0.70
pH 5.0	3.97	(0.07)	0.93	(0.06)	1.78	(0.11)	1.68
control pH 7.0 (± 0.1)	4.00	(0.07)	0.96	(0.05)	1.67	(0.11)	0.92
pH 4.0	14.12	(0.14)	44.96	(1.13)	63.77	(2.21)	0.51
pH 5.0	14.16	(0.13)	46.40	(1.29)	66.77	(2.28)	0.52
control pH 7.0 (± 0.1)	14.19	(0.14)	47.53	(1.58)	68.67	(2.56)	0.52

ment groups of both juvenile and mature categories were size matched (Table 1). The pH's were adjusted with 0.1M H_2SO_4 and 0.1N NaOH twice a day (at 0900 and 1300 hours). Changing of water and all other conditions were the same as in Experiment 1.

Survival of fingerlings was followed for 60 days and of adults for 70 days. Total lengths and weights were taken every 10 days. Mean specific growth rate (%/day) was calculated as described by Brown (1957). Data were analyzed by Duncan's New Multiple Range Test (Chantarakana 1980).

Experiment 3. Behavior of Nile tilapia in low pH water

The method was modified from those of Falk and Dunson (1977) and Somsiri (1980), using 15-l cylindrical glass containers. Water pH levels were adjusted to 2.0, 3.0, 5.0 and control (7.0 ± 0.1) as in Experiment 1. Five fingerlings (total length 2 to 5 cm) were placed in each tank. There were two replicates at each pH level. Fish behavior was observed for 12 hours.

Results

Experiment 1. Survival of Nile tilapia fingerlings and adults in acid water

The results are shown in Fig. 1. There was total mortality of fingerlings in pH 2.0 and 3.0 in one hour and three days, respectively. Survival rates of 84.4%, 86.7% and 93.3% were observed in pH 5.0, 9.0 and control (7.0 ± 0.1) water, respectively. The survival in pH 2.0 and 3.0 was significantly lower than in the control but there were no significant differences among survival rates in pH 5.0, 9.0 and control ($P > 0.05$).

There was total adult mortality after 12 hours and one day at pH 2.0 and 3.0, respectively; and survival rates of 88.9%, 97.8% and 100% in pH 5.0, 9.0 and control (7.0), respectively (Fig. 2). Survival rates at pH 2.0 and 3.0 were significantly lower than controls ($P < 0.05$) but there were no significant differences among those in pH 5.0, 9.0 and controls ($P > 0.05$).

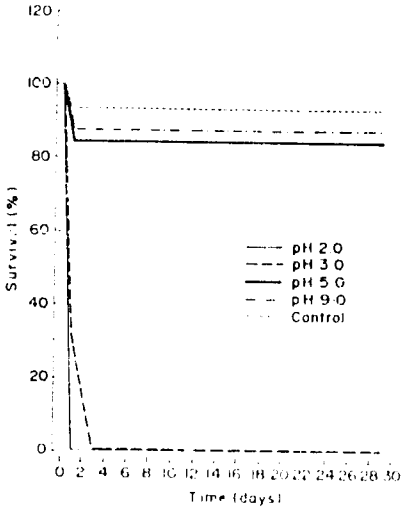

Fig. 1. Survival (%) of Nile tilapia *(Oreochromis niloticus)* fingerlings kept in water of different pH: 2.0, 3.0, 5.0 control (7.0 ± 1) and 9.0 for 30 days. The curves refer to initial populations of 90 fingerlings (triplicate tanks each with 30 fish) for each pH.

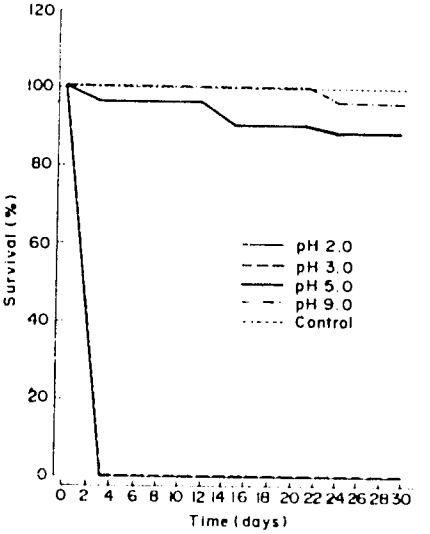

Fig. 2 Survival (%) of Nile tilapia *(Oreochromis niloticus)* adults in water of different pH: 2.0, 3.0, 5.0, control (7.0 ± 0.1) and 9.0 for 30 days. The curves refer to initial populations of 90 fish (triplicate tanks each with 30 fish) for each pH.

Experiment 2. Survival and growth of Nile tilapia fingerlings and adults at pH levels of 4.0, 5.0 and control (7.0 ± 0.1)

In pH of 4.0, 5.0 and control, fingerlings had survival rates of 57.8%, 82.2% and 84.5% and adults 86.6%, 100.0% and 100.0%, respectively (Figs. 3 and 4). For both fish categories, survival in pH 4.0 was lower than in pH 5.0 or controls, but in neither category was this statistically significant ($P > 0.05$). Growth data are summarized in Table 1.

Experiment 3. Behavior of Nile tilapia in low pH water

When fish were released into the glass containers containing water at pH 2.0, 3.0, 5.0 and control, they initially showed excited behavior and moved around; but there were no observable differences in swimming behavior among treatments.

In pH 2.0, some fish had moved to the water surface within 10 minutes and were gulping air with the body held at about 45° to the water surface. Their opercular movements were faster than those of controls and they showed rapid body movements. All fish in pH 2.0 had moved to the surface by 15 minutes, and some had begun to sink to the bottom, where they remained, making jerking movements in an effort to swim. By 20 minutes, some of these fish had ceased to show opercular movements and were presumed dead. All fish died within 30 minutes. Dead fish showed thick mucus on the body surface and over the gills, open mouths and protruding eyes.

In pH 3.0, fish still swam similarly to controls after 20 minutes, but showed faster opercular movements. By 30 minutes, they moved more slowly than the controls and showed a slower reaction than controls when touched with a glass rod. At 60 and 90 minutes some fish stayed at the bottom, where some fish showed jerking movements. After 2-5

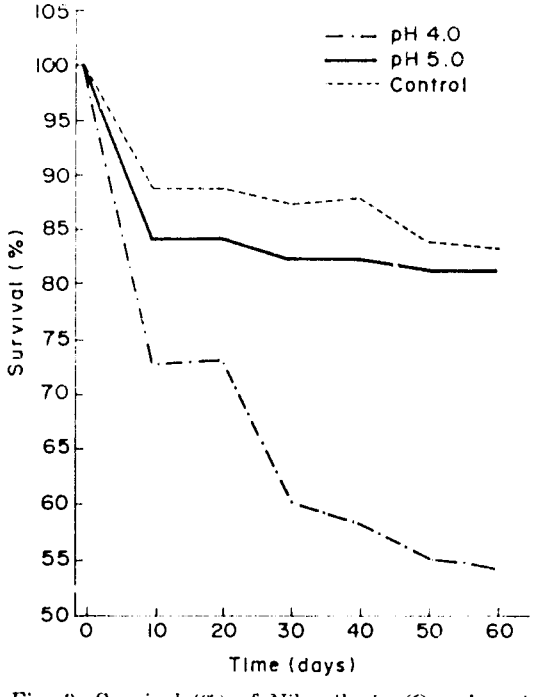

Fig. 3. Survival (%) of Nile tilapia *(Oreochromis niloticus)* fingerlings in water of different pH: 4.0, 5.0 and control (7.0 ± 1.0) for 60 days. The curve refer to initial populations of 45 fish (triplicate tanks each with 15 fish) for each pH.

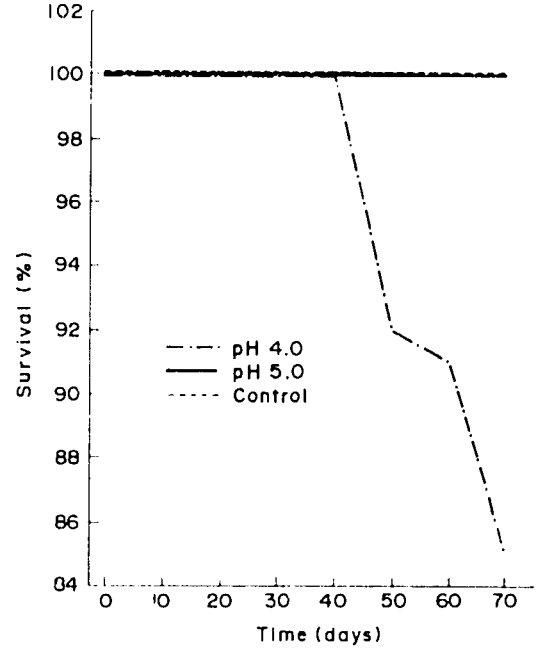

Fig. 4. Survival (%) of Nile tilapia *(Oreochromis niloticus)* adults in water of different pH: 4.0, 5.0 and control (7.0 ± 1.0) for 70 days. The curves refer to initial populations of 45 fish (triplicate tanks each with 15 fish) for each pH.

hours, all these fish had lost the ability to control body position and showed very slow opercular movements. All died within 7 hours and showed thick mucus on the body and over the gills as in pH 2.0.

In pH 5.0, fish showed no differences in behavior compared with controls and zero mortality over a 12-hour period.

Discussion

The study indicates that neither fingerling nor adult *O. niloticus* can tolerate pH levels of 2.0 or 3.0. Their 60-day survival was, however, greater than 80% in pH 5.0 and control, and there were no differences in growth between these two pH levels. The ability of *O. niloticus* to adapt to very low pH seems to be limited with a threshold at about pH 4.0. This is comparable with data presented by Balarin and Hatton (1979) that *O. niloticus* survive in the range pH 4 to 11 but die within 2-6 hours in pH outside this range.

The behavior of fish placed in pH 2.0 or 3.0 water and the appearance of dead fish suggest that the major cause of death may be respiratory failure. Schofield (1976) and Haines (1981) found that acid water destroyed gill tissue, caused redness and swelling of the gills, and increased mucus secretion.

Nile tilapia fingerlings here showed only 42.2% mortality in pH 4.0 even after 60 days, and adults were even more tolerant. However, environmental factors also influence the tolerance of fish to low pH; for example, poor water quality (Zischke et al. 1983); temperature (Dunson et al. 1977) and iron (Balarin and Hatton 1979). Therefore tank experiments such as were performed here are not representative of field conditions and performance. However, this study suggests that the recommendation of Beamish (1976) that the suitable pH of water for fish culture in general should be 5.0 or higher, is applicable to the culture of *O. niloticus*.

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SESSION VI: BIOLOGY AND ECOLOGY

A Morphometric Criterion for Sex Discrimination in Tilapia*

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Abstract

Morphometric analysis is used to differentiate between sexes in tilapia of various age groups. Discriminate analysis, performed on size-corrected values of truss lengths and measures of facial features (mandible, premaxilla, preopercula), were conducted on individuals, over a 5-month period, to study the process of morphometric differentiation during maturation. The model (using upper lip width (premw), and measures of body depth at eye level (A2) and at the insertion of the dorsal fin (A4)) is successful in discriminating the sexes before there is obvious size differentiation. Applications include (a) correcting for sex-related effects in growth and nutrition experiments, and (b) avoiding a male-biased ratio when selecting for rapid growth in genetics programs.

Introduction

There is often a need to distinguish the sexes of tilapia at an early age objectively without relying on the uncertain skills and experience of a human technician. In our laboratory, a continuous measure of sexual differentiation (rather than the male/female dichotomy), is needed for genetics experiments and studies on the ontogeny of dominance hierarchies and their effects on growth. The new "CIRC" technique (Doyle et al. 1987; Talbot et al., this vol.) makes it possible to evaluate the growth rates of fish rapidly in samples from experimental, aquaculture and natural systems. We require a measure of sexual differentiation to control for differences in the distribution of sexes and maturity

*Contribution No. 12 from the IDRC Network of Aquaculture Genetics Projects.

status in these samples (and in experiments dealing with mutation and disease also). Finally, the mechanism of sex determination is still not completely understood in tilapia and somatic or even polygenic involvement appears possible. In some circumstances it is useful to have a continuous measure of sexual differentiation that better reflects the underlying genetic reality.

Materials and Methods

Two morphometric techniques were used to develop a sex discriminating function for tilapia: standard morphometrics and truss morphometrics. Standard morphometrics is a term used to describe the conventional caliper measurements of the skeletal structure of the fish, and truss morphometrics, a technique described by Strauss and Bookstein (1982), measures distances between homologous landmarks on the body outline. The technique of truss cells is more efficient than the conventional measure of body size, as described by Hubbs and Lagler (1947), because (1) it covers the entire body, and (2) the short truss lengths make better use of localized information about body shape. It has been successful in distinguishing between stocks of various marine fish species such as Chinook salmon (Winans 1984) pollock and haddock (McGlade and Boulding 1985) and in the study of growth patterns of the above species. Doubtless it could be used for similar purposes in tilapia as well, for example to quantify differences in hybridizing mixtures of *Oreochromis niloticus* and *O. mossambicus*.

Standard morphometrics

Experience in handling *O. mossambicus* has indicated that fully mature males can be distinguished from females at the same age by their larger size and jaw structure. The pre-opercular bone also seems to be more angular in males than in females although this is not usually used as a discriminating feature when classifying animals by sex. Fig. 1a illustrates the facial parts measured in the present study: length and width (at lip) of the mandible and premaxilla bones; the length of the premaxillary pedicel; and the width, height and angularity of the preopercular bone. The angularity was measured as the distance from the angle of a right angle placed against the posterior and ventral edges of the opercular bone to the apex of the bone. These measurements were made with calipers and a right-angle cut out of plastic. Measurement of these facial features requires delicate probing with the calipers to distinguish between fleshy and bony parts on the live fish.

Truss morphometrics

Ten distinctive and homologous landmarks on the outline of the fish were chosen to describe its form (Fig. 1b). Each fish was positioned with its right side against a sheet of transparent vellum paper, onto which the points were drawn and later entered into the computer using an X-Y coordinate digitizing pad. The Euclidean distances between coordinates, forming a network of 4 truss cells, were calculated, using Pythagoras' theorem. The truss positions are illustrated in Fig. 1b.

Experimental method

In mid-August 1986, the experimental fish, hatched between 3 and 18 February 1986, were transferred from a crowded hatchery-type situation to four spacious tanks. The fish were from the third generation of the Dalhousie stock established in 1983 (Doyle et al. 1987). This stock originated with 100 fish purchased from a local supplier as "Florida red" strain of tilapia -- a hybrid of *O. mossambicus* and *O. urolepis hornorum*. Of these, 102 individuals were identified by spine clipping, and

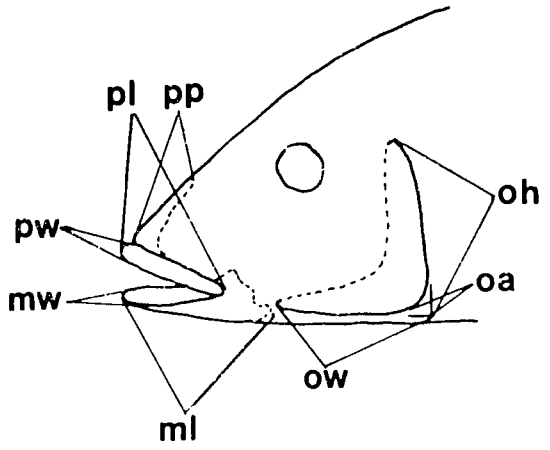

Fig. 1a. Standard Morphometrics - Head of a tilapia indicating measurements taken. Solid lines trace outline of the fish; broken lines trace the underlying bone structure. ml., mandible length; mw., mandible width; oa., angularity of the pre-opercle; oh., pre-opercle height; ow., pre-opercle width; pl., premaxilla length; pp., premaxillary pedicel; pw., premaxilla width.

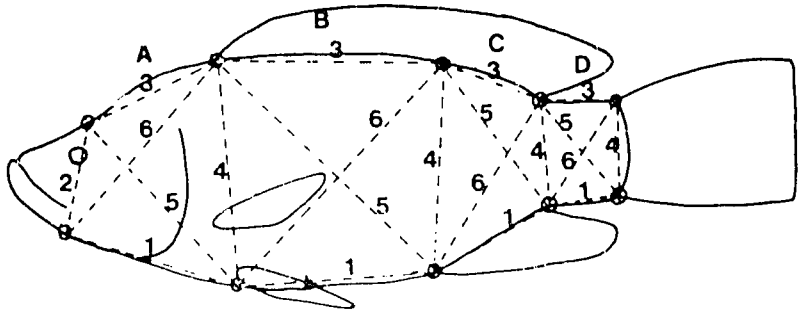

Fig. 1b. Truss Morphometrics - Locations of the 10 landmarks are illustrated as open circles while the broken lines indicate truss lengths. Each truss length is referred to by its corresponding cell (A, B, C or D) and position number (1 to 6). Landmarks refer to: 1) The posteriormost point of the maxilla; 2) The posteriormost point of the eye; 3) The origin of the pelvic fin; 4) The origin of the dorsal fin; 5) The origin of the anal fin; 6) The point between the spinous and soft portions of the dorsal fin; 7) The insertion of the anal fin; 8) The insertion of the dorsal fin; 9) The anterior attachment of the ventral membrane from caudal fin; 10) The anterior attachment of the dorsal membrane from caudal fin; Points 1 and 2 are made on the outline of the fish at a point on a line perpenticular to the horizontal axis of the fish.

morphometric measurements were made every three weeks from 28 August 1986 to 3 January 1987 (from age 6 to 10 months). They were then dissected and sexed by inspection of their gonads. The measurements and truss lengths were entered into the computer, transformed to natural logs, and used to establish a standard measure of body size (calculated as the log mean of all truss lengths). Each observation was then corrected for standard size, as outlined by Strauss and Bookstein (1982): (1) measured variables were regressed on the size of the fish, and (2) each linear observation was divided by its estimate.

The final set of morphometric measurements was analyzed using discriminant analysis (Wilkinson 1986) and, using the resulting canonical coefficients, scores for the previous months were calculated.

The results of univariate F tests between sexes in the tenth month were used as criteria to reduce the data to a smaller set of variables: those with an F of at least 7.0 were included in a reduced model. It was then further reduced using the canonical coefficients (standardized by the standard deviations): variables with coefficient of less than 0.3 (absolute value) were omitted. This reduced the final set to truss lengths A2, A4 and premw (premaxilla width at lip). It is fortunate that the discriminant function loads on these three variables, as they are relatively easy to measure.

Results

The full data set (all 21 truss lengths and 8 standard measurements) discriminated between the sexes in 96% of the fish, while the reduced set (truss lengths A2 and A4, and the premaxilla width at lip (premw)) discriminated sex in up to 91% of the fish. The results presented here arise from the reduced set. The canonical function for sex determination in our experiment is as follows:

$$\text{Score} = -0.85 \cdot (\text{A2}) + 0.45 \cdot (\text{A4}) + 0.73 \cdot (\text{premw})$$

We would expect the coefficients to be somewhat different for different *Oreochromis* species. Canonical scores of less than -0.1 indicate the male sex and those larger than -0.1 the female sex, with some ambiguity in scores close to -0.1. Discriminant analysis performed separately on each set of triweekly measurements resulted in similar canonical scores to those derived from the formula above.

The histograms of the scores from the initial (August 1986) and final (January 1987) set of measurements are drawn in Fig. 2 and the degree of discrimination in each set of consecutive measurements is listed in Table 1.

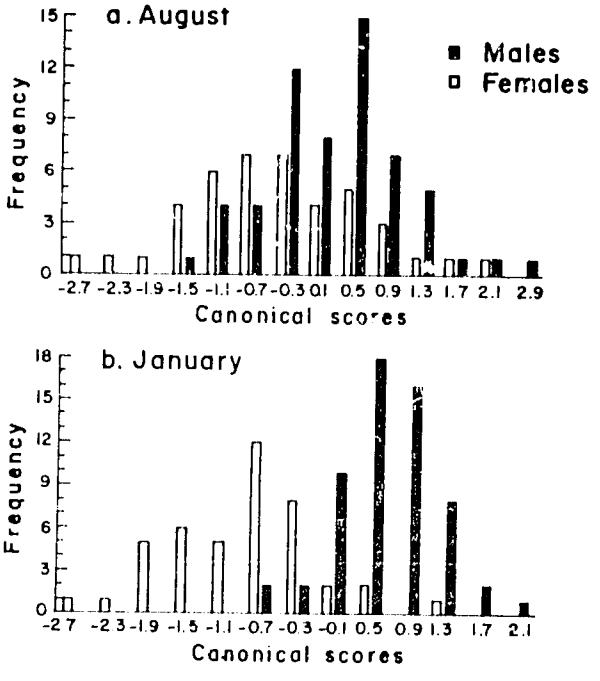

Fig. 2. Histograms of the canonical scores of the August (a) and January (b) morphometric measurements. The scores were calculated by applying the January canonical coefficients to the standardized, size corrected data. Scores of < -0.1 indicate males, scores of > -0.1 indicate females.

Table 1. Discriminant analysis results of each of the consecutive measurements between August and January. Number (N) and percent (%) of males classified correctly out of 43 males. Number (N) and percent (%) of females classified correctly out of 59 females.

Time of measurement	Age (in months)	Males N	Males %	Females N	Females %	Total %
August 28	6.5	27[1]	64.3	38	64.4	64.4
September 19	7.2	32	74.4	45[2]	77.6	76.2
October 10	7.8	39	90.7	51[2]	89.9	89.1
November 1	8.5	35	81.4	53	89.8	86.3
November 22	9	38	88.4	51[2]	87.9	88.1
December 15	9.8	34	79.1	55[2]	94.8	88.1
January 3	10.5	38	88.4	55	93.2	91.2

[1] Out of a total of 42 males.
[2] Out of a total of 58 females.

The morphometric analysis confirms that the lip width (premw) of the male is indeed enlarged, in the sense that it has a different allometric relationship to body size than females and immatures. A plot of the log-transformed premw and body size (means of truss lengths) of the series of measurements pooled together illustrates the allometry of lip width which is used to distinguish visually between fully mature males and females (Fig. 3).

The growth rate of the fish is plotted against discriminant score in Fig. 4. It can be seen that there is a continuous inverse relationship between degree of "femaleness" and growth rate. Relatively undifferentiated animals have intermediate growth rates.

A discriminant analysis was attempted on the three variables corrected for standard length of the fish (Hubbs and Lagler 1947), rather than means of truss length. This resulted in only 76% discrimination in January. Thus standard length does not include enough information to be a good measure of the size of fish.

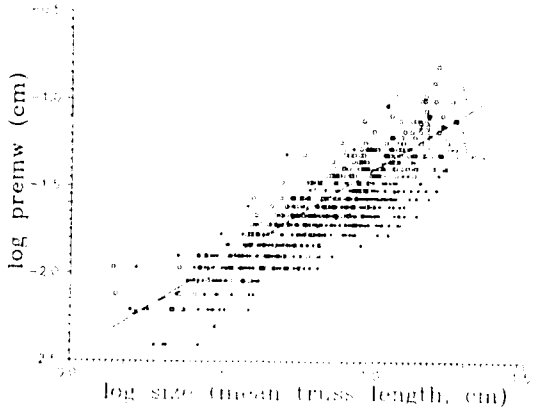

Fig. 3. Bivariate plot of lip width (premw) and body size (mean truss length). The slope of the allometric relationship distinguishes between the sexes. Female sex is indicated by stars and a dashed line (regression $Y = -2.4 + 0.821\ X$, $N = 409$, $r = 0.829$, $F = 891.0$, $P << 0.001$) and males by circles and a solid line ($Y = -2.466 + 1.073\ X$, $N = 301$, $r = 0.873$, $F = 960.8$, $P << 0.001$). Intercepts are not significantly different (ANCOVA, $F = 3.02$, P = NS) but slopes are ($F = 33.65$, $P < 0.001$).

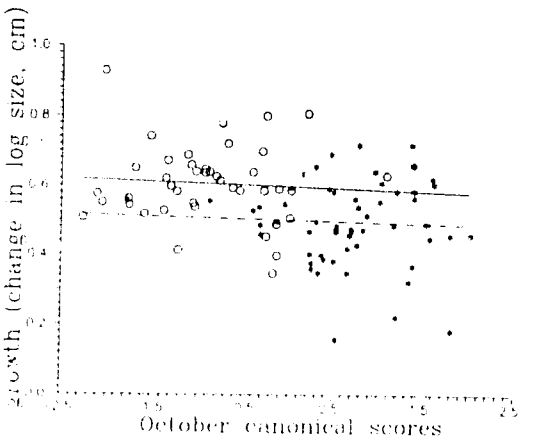

Fig. 4. Bivariate plot of femaleness and growth (calculated as the difference between August and January log mean truss lengths). Sex is indicated as in Fig. 3 (Regression, females: $Y = 0.501 - 0.006\ X$, $n = 58$, $F = 0.079$, P = NS; males: $Y = 0.598 - 0.009\ X$, $n = 42$, $F = 0.133$, P = NS). Intercepts are significantly different (ANCOVA, $F = 7.5$, $P = 0.007$). There is no interdependence of the degree of femaleness and growth rate within the sexes.

Discussion

The data presented here suggest that sex-dependent, differential growth rate and sexual differentiation have a continuous, rather than a dichotomous relationship to one another (Fig. 4). Sex-related differences in growth are evident at early stages of sexual differentiation. If a correction for sex is not made in genetics experiments or selection programs, selection for rapid growth may result in an undesirable skewing of the sex ratio. Detailed studies of the genetics of early growth rates in mammalian systems always use sex-corrected data (Riska et al. 1984). The morphometric criterion described here provides a relatively simple covariate to correct for variation in sex ratio and maturity status in genetics programs (and nutritional or physiological programs too).

People working with *O. mossambicus* often distinguish the sexes by their general body and jaw part sizes. This study quantifies the shape differences and

makes it possible to separate the sexes at an earlier stage when size differences are not observed. Male tilapia have a faster growth rate of the lip width (premw) and of the body depth at the eye level (A2) in relation to size of the fish (see Fig. 3). As has been noted by other authors (Pruginin and Shell 1962; Chervinski 1983), tilapia males grow larger than females. Pruginin and Shell (1962) exploited the differential growth rates of the sexes to discriminate between males and females of *O. niloticus* using a mechanical grader. Although this resulted in a 94% discrimination, their technique operates only with a specific size and age distribution, which, in practice, is very difficult to achieve due to the large environmental variation in the growth rate of tilapia. Size discrimination is weak in the early life stages and is of little use in freely reproducing populations containing a mixture of ages. Although a t-test comparison of the sizes (calculated as the log mean of all truss lengths) of males and females indicates that the differences are significant, this was visually obvious in relatively few fish, even in January.

Observations on the genital morphology of *O. mossambicus* males and females are not applicable to younger fish, as pointed out by Chervinski (1983). The two openings that the female has on the genital papilla were not noticed in our tilapia at 10 months of age; and the enlargement of the upper jaw in males is only clearly visible in older animals.

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Oreochromis mossambicus Is Not Universally A Nuisance Species: The Sri Lankan Experience

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Abstract

During the last decade or so there has been an increasing apprehension that *Oreochromis mossambicus* is literally hindering the development of the fisheries, particularly in Asia, where it was widely introduced in the 1950s. Numerous views have been expressed, taking cases in isolation, of its undesirable effects on fisheries development in the region. Suggestions have been made to remove it from water bodies. The success of this operation is yet to be documented. Here the positive effects of *O. mossambicus* in the inland fishery of Sri Lanka are reviewed, together with aspects of its biology, with a view to establishing that it is not necessarily a nuisance species in every country.

Introduction

A few species of African cichlids have played an important role in the development of fisheries, particularly in the Southeast Asian region over the last 50 years (Ling 1977; Smith and Pullin 1984). Of these cichlids *Oreochromis mossambicus* (Peters) was the first to be introduced into the Southeast Asian region. Ling (1977) noted that *O. mossambicus*, within a short period of 35 years, has come to be the most important pond-cultured species in Southeast Asia. However, over the last two decades *O. mossambicus* has gradually begun to lose its importance and has been declared a nuisance species almost universally (Welcomme 1984; Smith and Pullin 1984).

Through the last decade the role of *O. mossambicus* in intensive culture or semi-intensive culture has declined in the region, most notably in the Philippines and in Taiwan (Smith and Pullin 1984). Nevertheless, this species has a major role to play in other fisheries sectors, at least in some Asian countries. In this paper an attempt is made to highlight the importance of *O. mossambicus* as a protein source for poor people with special reference to Sri Lanka.

The total reservoir area in Southeast Asia is estimated at 3 x 10^6 ha and is predicted to increase five-fold by the year 2000 (Fernando 1977). These waters provide a potentially vast protein source. The paucity of lacustrine species in the indigenous fauna of the region and its possible consequences on fish production has been aptly documented (Fernando and Holcik 1982). As such there is a need to utilize lacustrine species from elsewhere or adopt a stocking and recapture strategy of suitable riverine species indigenous to the region.

Materials and Methods

In this paper, published data on fish yields in reservoirs in some Southeast Asian countries are utilized. In addition, data on the mean landing size and von Bertalanffy growth parameters (L_∞ and K) of *O. mossambicus* populations from randomly selected reservoir populations in Sri Lanka were computed using the ELEFAN I program (Pauly and David 1981). The index of growth performance ϕ', recommended by Moreau et al. (1986) was computed for *O. mossambicus* populations in Sri Lankan reservoirs and compared with mean values of ϕ' from other populations of *O. mossambicus* as well as with those of *O. niloticus* populations from elsewhere. This growth performance index is defined as follows:

$$\phi' = \log_{10} K + 2 \log_{10} L_\infty,$$

where K and L_∞ are the von Bertalanffy growth functions, the growth constant and asymptotic length, respectively.

Results

The yields of reservoir fisheries in the region vary greatly (Table 1). Also, the yield estimates from the same country quoted by different authors differ significantly. It is evident from Table 1 that the mean yield from Sri Lankan perennial reservoirs is the highest in the region (Fernando 1980; Oglesby 1985; Petr 1985). In Sri Lanka the perennial reservoir fishery is a capture fishery, dependent primarily on natural recruitment of *O. mossambicus* populations (De Silva 1983) introduced in 1952 (Fernando and Indrasena 1969). On average, *O. mossambicus* accounts for nearly 85% of the total estimated yield of 27,000 to 39,000 t and in the individual reservoirs, the contribution of this species ranges from 55 to 99% of the fish production (De Silva 1985a).

In some situations, *O. mossambicus* do not reach a desirable market size due to stunting, even though at times *O.*

Table 1. Fish yields from reservoirs in the Southeast Asian region (P.j.—*Puntius javanicus*; B.s.—*Barbus sarana*; L.d.—*Labeo dussumieri*; O.m.—*O. mossambicus*; O.n.—*O. niloticus*; C.c.—*Cyprinus carpio*; *—Sepik river).

Country	Annual yield (kg/ha) Mean	Range	Major species	Authority
Indonesia	99	22 — 353	P.j., O.m., O.n.	Baluyut (1983)
India (Tamil Nadu only)	83	2.2 — 270	Various; O.m.	Sreenivasan (1984)
Papua New Guinea*	(2,000 t/year)		O.m.	FAO (1986)
Malaysia	37	37 — 37		Oglesby (1985)
Sri Lanka	253	84 — 650	O.m.; also L.d.; B.s.	De Silva (1987b)
Thailand	54	8.3 — 135.6	Various including O.m., O.n.	Baluyut (1983)

mossambicus yields could be high in certain impoundments. De Silva and Fernando (1980) pointed out that there is no evidence that stunting of *O. mossambicus* has occurred in the Parakrama Samudra Reservoir, for which data are available for a period of nearly 30 years. Further evidence to this effect is presented in Fig. 1 from six reservoirs. The mean landing size was variable in all reservoirs and there was no apparent trend in the size of the landings over the years. It has been suggested that variability in the mean landing size is related to the water level of the reservoir, at least in the case of one reservoir (Amarasinghe 1987). The mean landing size of this species in the different reservoirs ranged from 19.3 to 23.9 cm in total length and 131 to 2,533 g in weight (Table 2). In almost all reservoirs the mean landing size reaches a desirable market size. The minimum permissible mesh size of 2.5 inches (6.5 cm) for gill nets and the prohibition of the use of any other gear has perhaps had a positive effect on the landing size as well as on the conservation of the stocks (De Silva 1983, 1985a).

The von Bertalanffy growth parameters and the growth index ϕ' (Moreau et al. 1986) of eleven *O. mossambicus* reservoir populations are given in Table 3. ϕ' ranged between 2.29

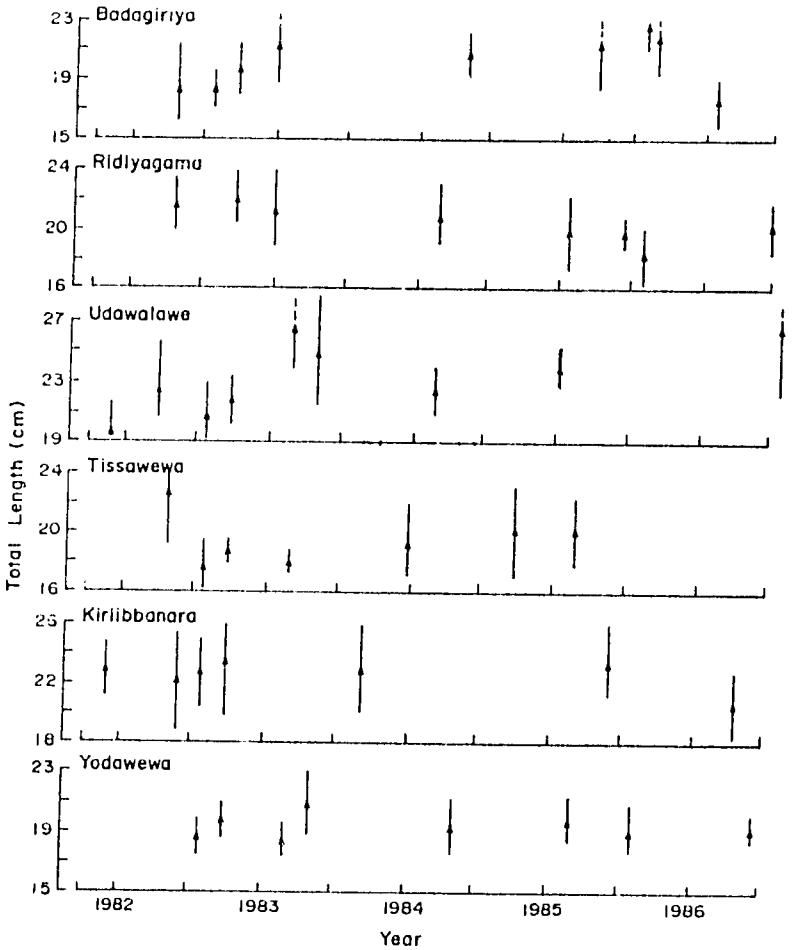

Fig. 1. The mean landing size (TL in cm) of *Oreochromis mossambicus* in six reservoirs over the period 1982 to 1986. The vertical lines indicate the range.

Table 2. The grand mean landing size and the range in length and weight of *Oreochromis mossambicus* of individual reservoir fisheries (data updated from De Silva 1985b; L_{max}/W_{max} - maximum recorded length/weight; X - grand mean; SD - from different landing dates between 1980 to 1986).

Reservoir	Total length (cm)				Weight (g)			
	L_{min}	L_{max}	X	S.D.	W_{min}	W_{max}	X	S.D.
Badagiriya	15.5	27.0	20.2	1.84	77	348	158	35
Chandrikawewa	16.5	31.0	20.0	2.00	87	544	163	58
Giritale	18.0	31.1	23.9	1.72	95	568	244	57
Kaudulla	16.5	28.5	20.4	1.36	65	462	164	38
Kiribbanara	16.5	35.5	22.6	0.97	92	796	243	43
Lunugamwehera	17.0	34.5	23.4	0.70	118	540	253	24
Minneriya	19.0	35.0	23.5	2.37	113	610	220	64
Parakrama Samudra	16.0	28.0	21.1	1.02	74	394	158	23
Pimburettewa	17.5	36.0	23.4	1.14	111	523	250	38
Ridiyagama	16.5	29.5	20.7	1.14	72	385	182	34
Tissawewa	15.5	30.0	19.5	1.82	65	435	144	43
Udawalawe	17.0	38.0	22.6	2.35	91	760	252	93
Urusitawewa	16.5	26.5	21.3	1.60	85	651	210	48
Weerawila	16.0	28.0	20.8	1.30	74	394	131	18
Yodawewa	15.5	25.0	19.3	0.83	72	265	131	19

Table 3. The surface area of eleven reservoirs and von Bertalanffy growth parameters (L_∞ and K) and the growth index ϕ', computed using standard length, of *Oreochromis mossambicus* populations (*data from Amarasinghe 1987).

Reservoir	Area (ha)	SL_∞ (cm)	K ($year^{-1}$)	ϕ'
Badagiriya	482	21.4	0.59	2.43
Giritale	310	25.8	0.43	2.46
Kaudulla	2,537	23.4	0.70	2.59
Kirribbanara	366	23.7	0.64	2.56
Lunugamwehera	3,023	26.2	0.40	2.44
Minneriya	2,560	24.5	0.40	2.38
Pimburettewa*	834	31.4	0.34	2.53
Ridiyagama	888	24.6	0.32	2.29
Tissawewa	234	25.7	0.63	2.62
Udawalawe	3,274	26.6	0.70	2.70
Yodawewa	765	22.4	0.40	2.31

and 2.70 with a mean of 2.48 (± 0.13). A comparison between ϕ' of *O. mossambicus* populations and *O. niloticus*, the species which is considered to be an 'aquatic chicken' (Smith and Pullin 1984), from elsewhere is made in Table 4. The data indicate that the mean growth of *O. mossambicus* of the Sri Lankan reservoirs compares with that of the African populations and *O. niloticus*. Mean ϕ' of *O. mossambicus* populations of Sri Lanka and Africa did not differ significantly (Student's *t* test; $p > 0.05$) from the mean ϕ' of *O. niloticus* from African waters. ϕ' of some *O. niloticus* populations, however, was considerably higher than those observed for *O. mossambicus* which is perhaps indicative of the higher growth potential of the former species and therefore, its suitability for culture.

Table 4. The mean $\bar{\phi}'$, ± sd and the range of *Oreochromis mossambicus* and *O. niloticus* populations (1—Sri Lankan reservoir populations; 2 and 3—African populations from Moreau et al. 1986).

Species	n	$\bar{\phi}'$	± sd	Range
O. mossambicus[1]	11	2.482	0.129	2.29 — 2.70
O. mossambicus[2]	19	2.465	0.165	2.05 — 2.65
O. niloticus[3]	16	2.651	0.222	2.36 — 3.11

Discussion

O. mossambicus is now found in virtually every kind of water body - ponds, ditches, canals, etc. (Ling 1977). It was introduced into or has invaded small bodies of water such as the temple ponds of India (Sreenivasan 1970) where, after a few generations, it began to get stunted. Such factors have perhaps resulted in it being considered a nuisance species.

Where the species has been introduced into sufficiently large water bodies, and where an adequate food supply is available, as in the case of Sri Lankan reservoirs it is a very desirable and a favorable species. There is evidence from Papua New Guinea also that *O. mossambicus* supports a very profitable fishery in the Sepik river and its flood plain lakes (FAO 1986). Hodgkiss and Man (1977) also reported that *O. mossambicus* was not stunted in the Plover Cove Reservoir in Hongkong.

Many reasons have been suggested for the success of *O. mossambicus* in the perennial reservoirs of Sri Lanka. Fernando and Indrasena (1969) suggested that the paucity of lacustrine species in the indigenous fauna and the high predatory pressure on young tilapia were responsible for its success. Subsequently De Silva and Fernando (1980) also hypothesized that its ability to effectively digest blue-green algae, a dominant component of the planktonic vegetation in Sri Lankan reservoirs, would also contribute to its success. More recent and detailed studies on the biology of *O. mossambicus* populations seem to indicate that its ability to switch its food habits in relation to availability of suitable food (Maitipe and De Silva 1985) and to effectively digest zooplankton, phytoplankton and detrital material (De Silva et al. 1984; De Silva 1985b) are contributory factors for its success. The high fishing pressure prevalent in the Sri Lankan reservoirs (De Silva 1985a) and the predatory pressure possibly prevent overpopulation and consequent stunting of the populations. The life pattern in fish, whether altricial or precocial, has relevance to aquaculture and the tendency for a species to get stunted (Balon 1981). The life pattern of *O. mossambicus* could be of either pattern, depending on the environment (Arthington and Milton 1986), and perhaps is an indication of its adaptability.

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Repeatability of Relative Size-Specific Growth in Tilapia*

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DOYLE, R.W. and A.J. TALBOT. 1988. Repeatability of relative size-specific growth in tilapia, p. 451-456. *In* R.S.V. Pullin, T. Bhukasawan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623 p. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Abstract

The growth trajectories of individual tilapia (*Oreochromis mossambicus, O. urolepis hornorum* hybrids) were followed by measuring their lengths at weekly intervals. The repeatability of specific growth SG, where SG = ln [length(2)/length(1)], was determined in two ways: (1) by week-to-week correlation of growth increments, which is the conventional procedure, and (2) by correlation of SG residuals after regression on body length. Use of the residual, termed "relative size-specific growth" (RSSG), improves the repeatability of growth which is otherwise very low. The correlation of RSSG measurements generally exceeded 0.5, after a brief period of exponential growth. This means that fish that grow relatively fast for their size continue to do so as their size increases.

The application to aquaculture genetics lies in using RSSG to select for rapid growth. It is difficult to obtain a tilapia population in which all animals are exactly the same age, as is required when selecting for growth rate using conventional procedures. RSSG is a useful alternative to size-at-age as a criterion for selection. RSSG does not require equal ages--in fact it can be used for mass selection in a freely-reproducing population.

Introduction

A crucial question for aquaculture geneticists is whether the growth of fish is sufficiently heritable to be worth selecting. The "repeatability" of a trait (correlation between values of a trait expressed two or more times in the same animal) sets an upper limit to its heritability. Unfortunately, in tilapia, the serial correlation of growth rates is often low or even negative. As explained in the Discussion section, this is largely an artifact. The repeatability of growth *as it is usually measured* gives us little information about heritability in a fish that, like tilapia, reaches maturity before it is large enough to be harvested.

*Contribution No. 11 from the IDRC Network of Aquaculture Genetics Projects.

The present experiment is concerned with the repeatability of growth in tilapia when growth is measured in relation to body size (length) rather than age. It is one of a series of studies on genetic stock improvement in aquaculture situations where the ages of animals are mixed or not accurately known (Doyle and Talbot 1986a, 1986b; McNaughton 1986; Doyle et al. 1987; Talbot et al., this vol.; Kamonrat and Doyle, this vol.).

Materials and Methods

The fish used in the study belong to a "Florida red" strain of tilapia (a hybridization of *Oreochromis mossambicus* and *O. urolepis hornorum*) kept at Dalhousie University and now in its third generation (Doyle et al. 1987). Fifty fish from a single brood were isolated into 0.6 l containers 3 days after hatching. They were grown at 26°C on brine shrimp nauplii (first 4 weeks) and commercial aquarium food after week 3. After the 10th week they were transferred into individual 4.5 l containers. Standard lengths and weights were measured weekly. Fish were grown as isolated individuals to eliminate competitive effects (Jobling 1985; Doyle and Talbot 1986a) that would affect the serial correlation of growth rate in the present experiment.

The calculation of relative size specific growth (RSSG) is a two-stage procedure. The first calculation provides a conventional growth estimate; the one we use is specific growth (SG), defined as

$$SG = \ln [L_2/L_1]/(\text{duration of growth period}),$$

where L_1 and L_2 are the lengths at the beginning and end of the growth period, respectively. Since all growth periods had the same duration (1 week) in our experiment, the divisor is omitted from subsequent calculations.

Computation of specific growth relative to other fish of the same size (RSSG) consists of pooling all the SG data obtained in a particular container size, and regressing them on the contemporaneous size of each fish (in this case, its length). The regression model we used for the pooled data is

$$SG_{ij} = b_0 + b_1 L_{1ij} + b_2 L_{1ij}^2 + \varepsilon_{ij}$$

where L_{1ij} = length of the ith fish at the start of week j, and ε is the normally distributed error term.

We define relative size-specific growth (RSSG) as the residual from this polynomial regression, namely

$$RSSG_{ij} = SG_{ij} - \widehat{SG}_{ij}.$$

Moving the fish from the smaller into the larger containers caused a second spurt of rapid growth (Fig. 2). The relationship between SG and body length is different in containers of different sizes, and the polynomial and RSSG calculations were made separately for each size (Fig. 1).

Results

The length-at-age curve is shown in Fig. 2. The two phases of the experiment, with separate cycles of sigmoidal growth in containers of each size, can be clearly distinguished.

The Pearson correlation coefficients for specific growth (SG) and relative size-specific growth (RSSG) are given in the upper and lower triangles, respectively, of Table 1. Five per cent of the correlations will be significant in such a large table by chance; however in the SG portion these show no pattern. Some significant negative correlations also appear in the upper triangle. The positive correlations in the lower (RSSG) part of the table fall into three clearly defined groups that are shown in relation to the length-at-age curve in Fig. 2. Within each period of compensatory growth, RSSG values are for the most part significantly correlated, and there is significant correlation of RSSG *between* these two periods as well. Outside the periods of decreasing SG, correlation of RSSG values was poor.

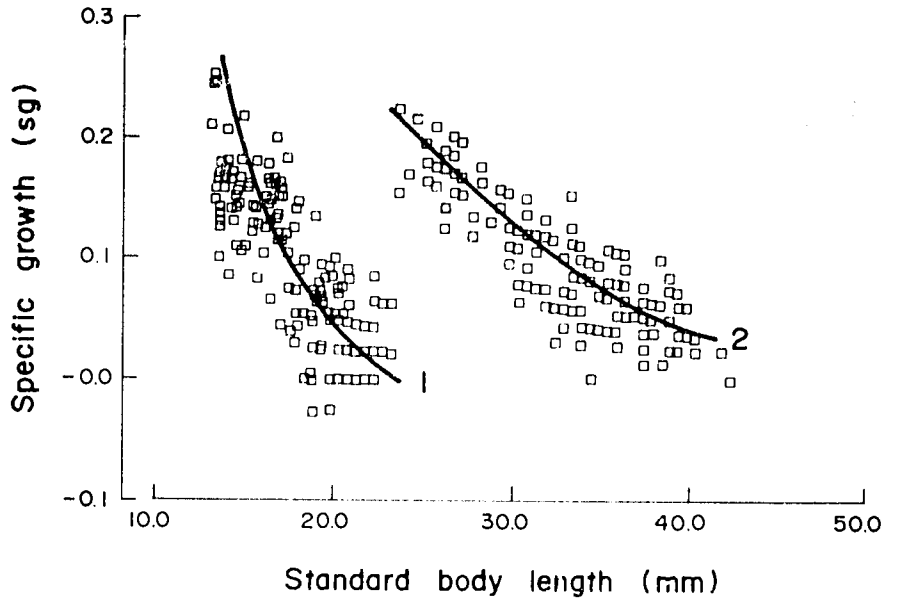

Fig. 1. Specific growth (SG) plotted against body length. Early data from each container (weeks 1-4 and 10-11) are excluded. Polynomial regression lines 1 and 2 are fitted to data taken during weeks 5-9 and 12-23.

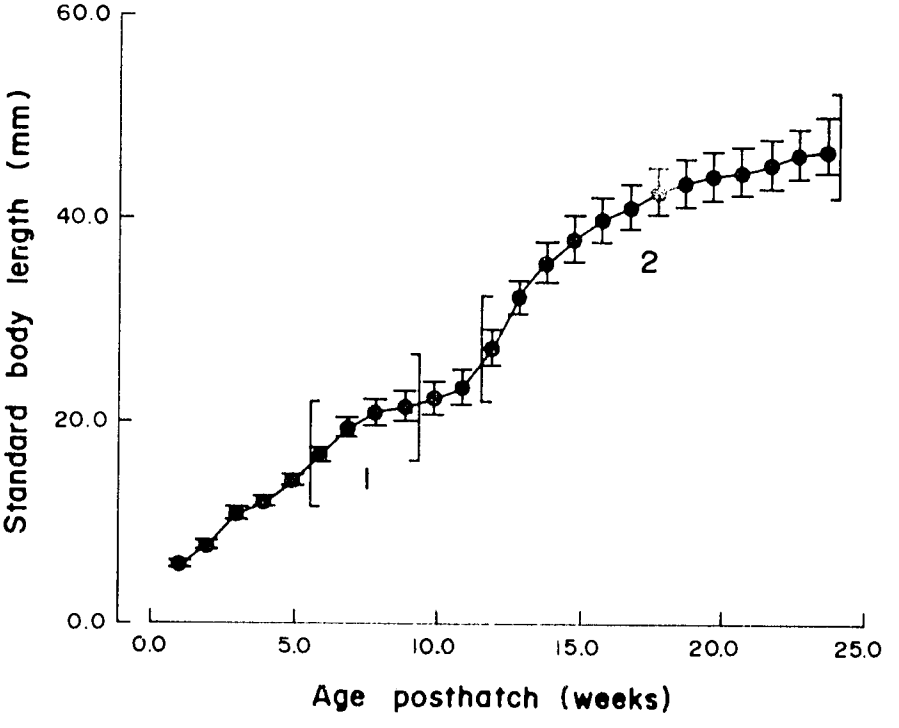

Fig. 2. Size-at-age of the experimental fish. Means and population standard deviations are shown. Most RSSG growth measurements are significantly correlated within and between the bracketed sections 1 and 2 of the growth trajectory (Table 1).

Table 1. Correlations among weekly growth increments. Upper triangle, length-specific growth (SG). Lower triangle, residual specific growth regressed on length (RSSG). Because of die-off during the experiment, the threshold of significance at $P < .05$ varies from 0.28 (weeks 1, 2; n = 47) to 0.37 (weeks 22, 23; n = 28).

Week	Week																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	1	−.26	−.29	−.09	−.19	−.04	−.03	−.02	−.13	−.13	.06	−.16	.11	.05	−.09	.27	0	.02	−.03	.14	.13	.23	−.20
2		1	−.24	−.33	−.13	.09	.13	0	.06	−.06	.19	−.06	−.21	.11	−.18	−.05	.14	.20	−.08	−.14	−.20	−.04	−.02
3			1	.29	.23	−.07	.11	.30	.20	.26	.11	−.16	.07	−.39	−.05	.05	.10	−.29	.07	−.04	−.02	−.34	−.11
4				1	−.02	−.22	.15	−.14	−.02	−.15	.22	−.03	.19	−.14	0	−.15	−.20	−.17	.25	−.01	−.30	.12	−.01
5					1	−.02	.21	.13	−.05	−.05	−.09	−.40	.09	−.01	−.03	.05	0	−.11	.29	−.11	.01	−.27	.11
6					.30	1	.14	−.06	.10	−.09	.01	−.14	−.45	−.15	−.05	.07	.13	.11	−.15	.24	.05	−.31	−.08
7					.39	.65	1	−.07	−.11	−.16	.06	−.34	.07	.07	−.14	−.31	.23	.11	.04	.09	−.25	−.12	−.04
8					.38	.51	.58	1	−.02	.27	−.21	−.34	.07	−.09	−.13	−.05	.22	−.18	0	.01	.04	−.07	.07
9					.18	.47	.45	.47	1	.05	.03	−.22	.12	.11	−.35	−.06	.11	.12	.14	−.29	.20	.03	.25
10										1	.24	−.02	.18	−.14	−.05	−.31	−.18	.02	−.15	.19	−.08	−.02	.39
11											1	−.04	.10	.16	−.09	−.13	.15	−.20	.13	−.07	−.07	−.13	.07
12					0	.38	.18	.24	.25			1	.06	−.18	.28	−.31	−.23	−.23	−.19	0	.03	−.03	.03
13					.15	.21	.38	.45	.49			.49	1	.20	−.25	−.23	−.22	−.31	0	−.23	−.15	.20	.13
14					.15	.37	.45	.43	.49			.40	.73	1	−.24	−.11	−.01	.08	−.02	−.01	−.16	.23	−.19
15					.14	.42	.37	.39	.30			.59	.51	.54	1	.10	−.27	−.26	−.03	−.19	−.13	−.25	−.36
16					.25	.54	.50	.50	.41			.30	.49	.59	.62	1	.11	.10	.10	−.17	.32	−.10	−.19
17					0	.48	.56	.58	.52			.25	.45	.56	.20	.58	1	.06	.16	.06	.06	−.15	.23
18					0	.44	.49	.30	.42			.09	.30	.55	.20	.45	.56	1	−.24	.12	.19	.17	.19
19					.40	.38	.49	.39	.46			.55	.74	.52	.50	.20	.60	.64	1	−.17	.06	−.13	.19
20					.16	.49	.49	.40	.16			.46	.60	.64	.58	.51	.58	.83	.61	1	−.10	.21	.01
21					.20	.33	.20	.27	.38			.42	.55	.75	.67	.62	.38	.67	.64	.78	1	−.27	.33
22					.06	.04	.15	.12	.17			.51	.66	.71	.66	.32	.29	.63	.72	.70	.73	1	−.20
23					.16	.06	.11	.20	.28			.58	.77	.72	.67	.43	.39	.74	.86	.79	.88	.90	1

Discussion

Repeatability of RSSG

The principal reason for the low repeatability of specific growth (SG) is the convergent, "compensatory" (Ricker 1975) or "targeted" (Riska et al. 1984) growth of tilapia. The decline in SG with size and age may be due to maturation, senescence (Gerking and Rausch 1979) or, as in this case, the limitations of the grow-out environment. A fish that reaches the targeted size rapidly, grows at first more quickly and then more slowly than other fish, so the serial correlation of SG in the population is poor. Slower growing fish tend to catch up. The phenomenon has long been recognized in fish (Ricker 1975) and in other animals (e.g., Laird 1965). It is superimposed on the statistical effects of correlated error and data dependencies which also tend to cause negative correlations among sequential growth measurements.

However, when measurements are made at a sequence of sizes, rather than of times or ages, the serial correlation of growth rates can become positive. A fish that has a larger size-at-age is growing faster *for its size* than a smaller fish will when it reaches the same size. This is the basis for using a residual, size-specific growth measurement (RSSG): if offers a more consistent description of what aquaculturists would generally call a faster-growing fish.

The low repeatability of RSSG during the early periods of growth in each environment may be a true observation concerning the biology of exponential growth. Growth may be more repeatable--and perhaps more heritable--during some phases of the growth curve in tilapia, as has been suggested for salmonids (McKay et al. 1986) and which appears to be true for laboratory mice and rats (Herbert et al. 1979; Riska et al. 1984). If this is the case, selective improvement should concentrate on these heritable phases of growth. The low repeatability of early RSSG may also be, in part, a statistical artifact of data and error dependencies during the periods of large week-to-week variation in growth rate and low week-to-week overlap in size distributions.

The correlation between RSSG in the two environments (container sizes) of the present experiment indicates that fish can go through a major disruption in the grow-out environment (such as "collimation"; see below) and then re-emerge with relative growth rates similar to what they had before. Thus, RSSG in our tilapia population was not only repeatable, it was *stable* in the engineering sense of returning to its original value after a perturbation. Stability is obviously important in aquaculture selection programs where selection is most conveniently applied during transfer from one type of grow-out environment to another.

Application to aquaculture: selection for RSSG

The usual procedure for stock improvement by mass selection--breed the biggest--is highly inefficient when there is variation in the age of the animals being selected (Doyle and Talbot 1986b). The confounding of growth rate and age variation greatly reduces heritability and the expected response to selection. The mouth-brooding habit of tilapia virtually ensures that confounding will be a problem whenever mass selection is attempted with this genus. One solution is to use within-family rather than mass selection (Uraiwan and Doyle 1986). Another possibility is to select for RSSG rather than size-at-age.

We now have two feasible methods for mass selecting RSSG. One is the "collimation" technique suggested by Doyle and Talbot (1986b), which consists of grading the population to obtain a sample of animals all of the same size, and then letting them grow for a time. Selection for the largest fish is performed after this grow-out. Since the fish were the same size but a mixture of ages at the start of the growth period, RSSG is being selected, not size-at-age. The procedure is being tried in Thailand with a reported realized

heritability of 0.2 (Jarimopas 1986). The second method is to select for RSSG indirectly, using the CIRC growth estimator (Doyle et al. 1987; Talbot et al., this vol.; Kamonrat and Doyle, this vol.).

Either method for selecting RSSG has the advantage that it can be used in small aquaculture establishments--at the minimum, in a single common pond where age classes coexist in a freely-interbreeding population.

Acknowledgements

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Growth and Sexual Development of 17α-Methyltestosterone- and Progesterone-Treated Nile Tilapia *(Oreochromis niloticus)* Reared in Earthen Ponds

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Abstract

Swim-up fry (average weight, 14 mg) were obtained from *Oreochromis niloticus* (Chitralada strain) held in concrete tanks. In experiment 1 (1985), fry were fed diets treated with 17α-methyltestosterone (MT) or progesterone at 60 mg/kg for 40 days. Control fish were fed untreated diet. Fingerlings (average weight, 2.86 g) were subsequently stocked at 1/m^2 in earthen ponds receiving septic tank slurry at 150 kg COD/ha/day and fine rice bran as supplementary feed for about 5 months. In experiment 2 (1986), similar procedures were followed but the fry were fed MT at 40 mg/kg for 40 days and pond stocking rates of 1, 3, 5 and 7 fingerlings/m^2 were compared.

Individual size, sex and stage of maturity were determined. In experiment 1, the average individual size was greatest in the MT-treated group (263 g), compared with the untreated (232 g) and progesterone-treated fish (189 g). The final size of fish in experiment 2 decreased significantly ($P < 0.05$) with density, from an

average of 209 g at 1 fish/m^2 to 94 g at 5/m^2; however those stocked at 7/m^2 gave the highest total biomass and were not significantly smaller ($P > 0.05$, mean weight, 88 g) than the fish stocked at 5/m^2. Sex could not be determined by external morphology alone, but sexing on gross gonadal structure agreed well with subsequent histological examination. The percentages of male fish were: MT-treated, over 93%; progesterone-treated, 72% and untreated, 57%. All treatments included fish at various stages of maturity, from undifferentiated to fully mature gonads. In MT-treated fish, females commonly showed abnormal enlarged ovaries: egg bound or distended with fluid. Some males, particularly those fed the higher level of hormone (60 mg/kg) showed some testicular degeneration which lowered GSI values.

Introduction

The effectiveness of synthetic steroid hormones for inducing functional sex-reversal in *Oreochromis* sp. has been well documented. The efficacy of certain androgens, particularly 17α-methyltestosterone (MT), for masculinization and, to a lesser extent estrogens for feminization, has been shown over a range of hormone levels and treatment periods (Tayamen and Shelton 1978; Guerrero 1982; Rothbard et al. 1983).

The use of steroids in feeds for fry has also been found to have a beneficial effect on the growth of *Oreochromis* spp. (Guerrero 1979; Hanson et al. 1983; Macintosh et al. 1985). However, there are no published data on the growout performance of androgen-treated *O. niloticus*, the most important cultured tilapia species in Southeast Asia.

This paper reports preliminary findings from pond trials to evaluate the long-term effect of hormone treatment on the growth and sexual maturation of *O. niloticus*. These trials, conducted in 1985 and 1986 at the Asian Institute of Technology (AIT), focused mainly on the performance of methyltestosterone-treated fish, but included one treatment with progesterone for comparison with previous work on the effects of other estrogens on *O. niloticus* (Tayamen and Shelton 1978).

Materials and Methods

Fry treatment

The Chitralada strain of *O. niloticus* produced and distributed by the Department of Fisheries, Royal Thai Government, was used for the trials. Electrophoretic analysis of this strain has shown it to be a pure strain (McAndrew 1981). Swim-up fry were obtained from broodfish held in a clear water, recirculating hatchery system. The fry were removed after draining a circular concrete spawning tank (4-m diameter) 10 days after stocking broodfish.

A fry feed (crude protein content 40%) was prepared from a mixture of a ground floating catfish pellet and fine fish meal, sieved to a size of less than 1 mm. Diets were prepared by the alcohol evaporation method (Shelton et al. 1978). The same feed mixture without hormone was used as a control diet. The hormones 17α-methyltestosterone (MT) and progesterone (Sigma Chemicals Ltd.) were incorporated in diets in trial 1 (1985) at 60 mg/kg feed for 40 days. Triplicate ponds were used for each treatment and control. In trial 2 (1986) MT was administered for 40 days at 40 mg/kg, the standard dosage that has proved consistently successful for sex reversal of *O. mossambicus* (Johnstone et al. 1983). In trial 2, MT-treated fish were stocked at 1, 3, 5 and 7 per m^2 in quadruplicate ponds. One pond at 1/m^2 and one at 5/m^2 received no rice bran. Diets were prepared every 5 days and were kept refrigerated as were the hormone stock solutions.

The fry were weighed and then released for subsequent hormone treatment into circular concrete tanks (1.5-m diameter) connected to a recirculated water system. Fry were stocked at a density of 12 l for hormone treatment over a period of 40 days when water temperature varied between 28 and 32°C. A random sample of fry was weighed every 5 days and feed weight adjusted accordingly; a feeding rate of 20% body weight/day was used for the first 20 days, declining to 10%

body weight/day for the final 20 days. The diets were fed four times daily.

Although received in the same condition, the MT-treated fry were larger on average than the controls after the hatchery period, while the progesterone-treated fry were significantly smaller. In experiment 1 the average individual weights of fry after 40 days were: controls 2.6 ± 0.3 g, MT-treated 2.8 ± 0.1 g, progesterone treated 1.3 ± 0.5 g.

Growout

Fry removed from the hatchery were first conditioned in a nylon hapa suspended in each experimental 200-m^2 earth pond for 12 hours before their release. The subsequent fry stocking was staggered for the different treatments over 7 days to facilitate staggered harvesting and stock analysis.

Dried sediments from previous use of the ponds were removed from the earth ponds before filling with canal water. The ponds were loaded with septage at a rate of 150 kg/chemical oxygen demand/ha/day at three times the daily loading rate on a twice weekly basis beginning on the day of fish release. In addition, the fish were fed fine rice bran (15% crude protein) on a daily basis at 2% body weight/day, declining to 1% body weight/day after 3 months. Feeding was estimated on a monthly basis from a seined sample of not less than 15% of the fish. Water quality in the ponds was monitored monthly following standard AIT practice (Edwards et al. 1984) Both trials were conducted in the monsoon season from May to October/November.

The ponds were drained and the fish were harvested and individually weighed and measured after 5 months. The fish were sexed by dissecting out of the gonads, which were weighed and preserved in buffered formalin for histological examination. Recruits were individually measured and sexed after dissection when possible. Recruits were clearly distinguishable in size from stocked fish in the hormone treatments. In the control ponds there was a strong bimodal length distribution of 20-25 cm fish (stocked) and 10-15 cm (recruits). On average, only 6 fish per control pond were in the intermediate length range 15-20 cm; these were assumed to be stocked fish.

Results

Survival rates ranged from 77 to 90% in trial 1 (Table 1). Average survival was not significantly different between treatments (t test, $P > 0.05$).

The sex ratio of the stocked control fish was close to the expected 1:1 ratio (46.4 to 62.5, overall average 54.5). The sex ratio among recruits from spawnings during the trial, large enough to be sexed reliably was also close to equality.

Table 1. Percentage survival, percentage of males and size at harvest of 17α-methyltestosterone (MT)-treated, progesterone (PROG)-treated and untreated control fry of Chitralada strain *Oreochromis niloticus* after growout for five months in septage-fed, rice-bran supplemented 200-m^2 ponds. For details of treatments, see text (experiment 1).

Treatment	Pond no.	Survival %	Males %	Size (mean and S.D.)			
				Weight (g)		Length (cm)	
				Male	Female	Male	Female
MT	2	81.0	95.7	276.1 ± 73.9	280.8 ± 59.9	24.1 ± 1.8	24.1 ± 2.0
MT	14	90.5	92.8	270.5 ± 58.2	278.5 ± 66.0	24.4 ± 1.6	23.7 ± 1.7
PROG	1	90.0	71.0	206.8 ± 65.2	191.0 ± 68.0	22.2 ± 3.0	21.6 ± 2.9
PROG	6	83.0	72.3	179.2 ± 40.8	159.5 ± 44.6	21.9 ± 1.8	20.8 ± 2.1
Control	3	89.5	46.4	207.9 ± 58.5	203.7 ± 58.5	22.8 ± 2.9	22.1 ± 1.8
Control	10	77.5	62.5	233.6 ± 54.7	189.0 ± 44.7	22.9 ± 1.7	21.6 ± 1.7
Control	13	83.0	60.8	260.3 ± 65.1	225.4 ± 40.0	24.0 ± 2.7	23.1 ± 1.2

The percentage of males harvested among the stocked fish was 95.7 and 92.8% in two of the ponds stocked with MT-treated fry. The third pond of this treatment and one of the progesterone treatments was discounted from the analysis because wild tilapia fry entered these two ponds during the early part of the trial, probably through holes in the dikes, and established a breeding population. This problem was prevented in trial 2 by rebuilding the ponds.

Survival rates in trial 2 were comparable to those of trial 1 although sex reversal was improved (Table 2). Climatic differences between 1985 and 1986 led to some variation in water quality between the two trials but a direct comparison between trials 1 and 2 is valid because some fish were stocked at the same density of 1/m^2 in both.

MT-treatment and subsequent stocking of sex-reversed fish (81-99% males) yielded fish averaging 270-280 g after 5 months pond culture using septage fertilization supplemented by rice bran in both trials (Tables 1 and 2). The largest individual fish were abnormal females; a large proportion of their body weight was gonadal tissue. This result was not due to better growth since average lengths were the same or slightly lower for MT-treated females compared to males. The significance of stocking density and supplementary feeding to final average size was indicated in trial 2; final individual size was inversely related both to stocking density and total yield. At the lowest stocking density (1/m^2), MT-treated fish reared without rice bran were less than half the weight of supplementary-fed fish (Table 2). The final size of untreated male fish in trial 1 was an average of 16% smaller (234 g and 273 g, respectively) than MT-treated male fish. The average size of females in the control group was only 206 g. The progesterone-treated fish were about 16% smaller than the controls (average weight below 200 g).

The two ponds containing progesterone-treated fish in trial 1 showed surprisingly high ratios of male fish (71 and 72.3%). The necessity for internal examination of gonads to verify the external sexual characteristics of the fish is shown in Table 3, e.g., in samples of 50 fish, several individuals exhibited abnormal gonads or genital papillae. Females from the MT-treated groups often had grossly abnormal gonads, as shown by

Table 2. Percentage survival, percentage of males, total biomass and mean weight of 17α-methyltestosterone-treated Chitralada strain *Oreochromis niloticus* after growout for five months in septage-fed, rice-bran supplemented (except where indicated) 200-m^2 ponds. For details of treatments, see text (experiment 2).

Stocking density (n/m^2)	Rice bran used	Survival %	Male %	Biomass (kg)	Mean wt. (g)
7	+	71.8	96.0	82.6	82.2
7	+	70.1	95.0	88.5	90.1
7	+	69.2	98.0	88.5	91.3
5	+	74.0	98.0	67.7	91.5
5	+	98.7	96.5	86.1	87.2
5	-	73.0	98.0	16.5	22.5
5	+	63.5	99.0	65.5	103.1
3	+	85.5	99.5	60.9	118.6
3	+	82.3	98.5	58.4	118.2
3	+	89.0	97.5	71.2	133.3
1	-	82.5	96.7	16.7	101.3
1	+	88.0	97.2	52.1	287.7
1	+	87.0	97.7	41.6	238.9
1	+	76.0	98.0	33.6	221.0

very high GSI values (Table 4). The genital duct was typically blocked and the ovaries were either egg bound or distended with fluid. Fluid retention in the ovaries or abdominal cavity comprised up to 20% of the body weight of some such females.

The MT-treated males had slightly lower average and maximum gonadosomatic index (GSI) values compared to normal males for each treatment in trial 1 (Table 4). Progesterone-treated fish showed no difference in GSI from the controls; there was no evidence of any effect of progesterone on the sexual development of treated fish. GSI values tended to increase with individual size (trial 2; Table 3) in the MT-treated fish. However, all stages of maturity were found in all sizes of fish.

Discussion

The results from this study suggest that a dose of 40 mg/kg MT for 40 days given to *O. niloticus* fry aged 10 days or less and held in clear water tanks is adequate for sex reversal. Indeed, at this lower dosage used in trial 2 compared to trial 1 (60 mg/kg), a higher degree of reversal was achieved. This supports the findings of several authors who treated fry for different species of *Oreochromis* over a range of hormone doses and found more effective sex reversal at lower doses of MT (Guerrero 1975; Obi and Shelton 1983).

Progesterone-treated fish were included in Experiment 1 to yield comparative data on the effect of growth by estrogen treatment, however, an all

Table 3. Average size, abnormalities and gonad development in phenotypic male *Oreochromis niloticus* (experiment 2).

Pond no.	Pond stocking rate (n/m^2)	Sample no.	No. of abnormal fish	Phenotypic males		
				Mean weight	Range	GSI
1	1	50	8	290.0	152-392	0.41
2	3	52	8	139.7	98-202	0.39
7	5	50	4	123.8	86-170	0.15

Table 4. Gonadosomatic indices (GSI), (means, standard deviations and ranges) for 17α-methyltestosterone-treated (MT), progesterone-treated (PROG) and untreated control Chitralada strain *Oreochromis niloticus* after growout for five months in septage-fed, rice-bran supplemented 200-m^2 ponds. For details of treatments, see text (experiment 1).

Treatment	Pond no.	Male				Female			
		GSI	SD	Max	Min	GSI	SD	Max	Min
MT	2	0.61	0.4	1.71	0.01	4.97	2.51	9.16	2.12
MT	4	0.52	0.32	1.90	0.00	4.87	5.15	17.62	0.19
MT	14	0.63	0.37	2.09	0.02	11.25	5.14	21.98	2.52
Control	3	0.87	0.43	1.91	0.02	1.85	1.10	5.55	0.05
Control	10	0.87	0.53	2.60	0.12	2.51	2.04	13.85	0.02
Control	13	0.82	0.42	2.12	0.10	1.62	0.78	3.37	0.57
PROG	1	0.82	0.59	2.71	0.01	2.06	2.70	19.39	0.37
PROG	5	0.71	0.67	4.76	0.01	2.13	1.27	5.81	0.11
PROG	6	0.86	0.77	5.08	0.01	1.82	1.17	5.44	0.01

female population was not expected as other estrogens have proved relatively ineffective for feminization (Hopkins et al. 1979; Jensen and Shelton 1979). The high ratio of male fish obtained has not been reported previously in the use of other estrogens. The structural similarity between progesterone and testosterone makes it likely that in the course of absorption and/or metabolism by the fry, conversion to testosterone occurred with a degree of masculinization (R.S. Wright, pers. comm.).

The improved final size of MT-treated male fish compared to untreated males (Table 1) agreed with the findings of several authors (Guerrero 1975; Katz et al. 1976; Owusu-Frimpong and Nijjhar 1981) as did the smaller size of females in the control group compared to males (Hanson et al. 1983). The former result suggested that MT had more than a temporary anabolic effect on growth, perhaps linked to reduced fertility and sexual activity. Histological analysis of the testes of MT-treated males revealed little evidence of viable sperm. The fish rarely exhibited breeding coloration at harvest, even when visibly mature, and individuals were docile and easier to handle than untreated males. The absence of females in the pond may also have contributed to the low level of testosterone found in MT-treated fish (Rothbard et al. 1983).

Variable effects of estrogen on growth in tilapias have been reported by other workers. Tayamen and Shelton (1978) found that diethylstilbestrol (25 mg/kg) caused an increased growth rate in *Oreochromis niloticus* but that estrone (100-200 mg/kg) had no appreciable effect. Jensen and Shelton (1979) reported a lack of response to estriol, estrone and estradiol (30-100 mg/kg) with *Oreochromis aureus*. In contrast, Majumdar (1984) reported a decline in growth of 28 and 42% in *Oreochromis mossambicus* and *Oreochromis aureus* after treatment with estradiol and an increase of over 20% in *Oreochromis niloticus*. Progesterone-treated fry achieved a lower final size than untreated or MT-treated fish in this study.

The interrelationships of stocking density, yield and final average size were clearly shown in trial 2. The limits to growth by reliance on natural food production alone were highlighted by the two ponds without supplementary rice bran; both average size and yield were lower at the higher stocking density (Table 2). This result demonstrated the problem of "stunting" usually caused by "wild spawning" in normal populations, but was produced here by stocking a pond above its carrying capacity. The lack of recruitment in trial 2, despite the presence of some viable females, suggested that less than total (100%) sex reversal may not be a problem in terms of pond production. Mires (1977) reported in Israel that a level of only 90% males prevented wild spawning (or at least recruitment).

The depressant effect of MT on testes, as observed here was reported by Macintosh et al. (1985) for both testes and ovaries. In this study, however, the average female GSI was much larger than controls, mainly because of the frequency of grossly abnormal ovaries which appeared to be associated with blocked oviducts in all of the females sampled; eggs were usually in various stages of degeneration and resorption. The absence of similar reports in the literature is probably because of the relatively long period of growout after treatment used here, which allowed fish to mature fully. Thassananukulkit (1979) observed a low level of gonadal abnormalities in MT-treated *O. niloticus* and remarked on hypertrophy of sperm ducts and degeneration of ova in testes and ovaries, respectively. Obi and Shelton (1983) reported a level of 5% abnormality in androgen-treated fish compared to approximately 13% in the sample presented here for trial 2 (Table 3); they concluded that urinogenital papillae were not an accurate indication of gonadal condition.

The gonadal abnormalities observed in this study seemed to be associated with a level of hormone received by the fish that was either too high or too low. The high level of abnormal male gonads observed in trial 1 was possibly induced by the higher MT dose (60 mg/kg). Conversely, the blocked duct syndrome exhibited by

females treated with MT was probably due to incomplete sex reversal at the time of differentiation. The same condition has been observed at AIT in young broodfish raised in a recirculatory system receiving wastes, and thus probably a low level of hormone, from fry MT-treatment tanks.

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Cannibalism Among Different Sizes of Tilapia *(Oreochromis niloticus)* Fry/Fingerlings and the Effect of Natural Food

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Abstract

Experiments were conducted in jars, tanks and aquaria to determine the occurrence of cannibalism among seven different size groups of Nile tilapia *(Oreochromis niloticus)* fry and fingerlings. Cannibalism became more intense as the size difference increased. Big fry were less susceptible to cannibalism than small fry. On the other hand, bigger fingerlings were highly cannibalistic compared with smaller ones. This was evident as early as the first 10 minutes after stocking when fingerlings which usually stayed at the bottom moved swiftly towards the surface and swallowed the small fry.

Availability of additional natural food in the growing medium affected survival of fry (mean weight = 9.3 mg) which were stocked with fingerlings (mean weight = 163.5 mg) in aquaria. Feeding with *Spirulina* proved more effective in reducing cannibalism than feeding with *Navicula*. After five days of rearing, fry survival was highest when fed with *Spirulina* (83.1%) followed by *Navicula* (16.6%) and the unfed control (5.6%).

Introduction

One of the major problems identified by small-scale tilapia hatchery operators in the Philippines is the suspected cannibalism among tilapia fry and fingerlings thus lowering their production. The demand for tilapia has been increasing among Asians as a cheap animal protein source.

Cannibalism among tilapias at the fry and fingerling stages has not been investigated previously. Territorial aggression among *Oreochromis mossambicus* breeders was reported by Bruton and Boltt (1975). In fact, removal of the

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premaxilla of the male fish to reduce aggression has been resorted to in aquarium experiments (Lee 1979). *O. niloticus* has been identified as an aggressive species compared with others (Mélard and Philippart 1980).

This paper presents the results of experiments on the occurrence of cannibalism among fry and fingerlings of tilapia *(O. niloticus).* The effects on cannibalism of feeding two specific phytoplankton, *Navicula notha* and *Spirulina platensis,* were also determined.

Materials and Methods

Different sized tilapia *(O. niloticus)* fry and fingerlings were harvested in spawning cages. Initial measurements (length and weight) were taken and the size groups were numbered as follows:

Fry	Mean weight (mg)	Mean length (mm)
#1	6.8	0.9
#2	7.1	1.0
#3	21.3	1.4
#4	73.1	1.6
Finger-lings	**Mean weight (mg)**	**Mean length (mm)**
#5	143.7	23.0
#6	654.5	35.0
#7	1,531.1	50.0

Fry and fingerlings were stocked in 4-l and 12-l plastic jars or tanks (30 l) as follows:

1. Cannibalism among varying sizes of tilapia fry/fingerlings.

Experiment 1. Two sizes of "small" tilapia fry belonging to groups #2 and #3 were stocked in the same container as fingerlings from groups 5, 6 or 7. Five fry and 3 fingerlings were stocked in plastic gallon jars containing 3 l of pond water. The experiment was conducted in complete randomized design with three replicates per treatment. Cannibalism was observed closely for the first 10 minutes, after 30 minutes and after one hour.

Experiment 2. Groups 2, 3 and 4 tilapia fry were stocked with fingerlings of groups 5, 6 or 7. The stocking density was increased to 8/l so that a total of 18 fry were grown together with 6 fingerlings in a 4-l jar containing 3 l of pond water.

The experiment was conducted in complete randomized design with three replicates per treatment. Cannibalism was observed within 10 minutes, 30 minutes and one hour after stocking.

2. Effect of natural feeding.

Glass aquaria (10 l) containing 4 l of tap water were stocked with tilapia fry/fingerlings of two different sizes, 9.26 mg and 163.5 mg at 6/1. Pure cultures of *Navicula* and *Spirulina* were supplied to the rearing medium giving a mean cell density of 8×10^3 cells/ml and 17.7×10^3 cells/ml, respectively. Cultures without phytoplankton served as controls. The experiment was conducted in complete randomized design with three replicates per treatment. Survival was determined daily for five days.

Additional natural food was prepared by harvesting and concentrating unialgal cultures of *Spirulina* from outdoor tanks and *Navicula* from laboratory cultures of one liter capacity. These were washed, mixed in one container, and apportioned equally in the different aquaria. The cell density was determined immediately after mixing with tap water.

Results and Discussion

There was an increasing occurrence of cannibalism as the tilapia fingerlings paired with small fry increased in size (Table 1). This was observed within the first 10 minutes after stocking. Survival rate of groups 2 and 3 fry decreased from 100% when stocked with group 5 fingerlings to 33% for group 2 fry and 77.6% for group 3 fry when paired with group 7 fingerlings.

Table 1. Mean survival (%) of two sizes of tilapia *(Oreochromis niloticus)* fry stocked with different sizes of fingerlings. Three fry were used for #2 and #3. All fingerlings survived.

Treatment	Mean survival (%) After 10 minutes #2	#3	After 30 minutes #2	#3	After 1 hour #2	#3
w/#5	100	100	77.6	100	66.6	100
w/#6	55.3	88.6	0	44	0	11
w/#7	33	77.6	0	11	0	11

After one hour there was no survival in treatments where group 2 fry were stocked with either group 6 or 7 fingerlings. Low (44% to 11%) survival was obtained among group 3 fry growing together with either group 6 or 7 fingerlings.

Observations showed that all big fingerlings swam after smaller fry upon stocking together. The fry remained on the surface while the fingerlings stayed at the bottom. The fingerlings then moved swiftly towards the small fry swallowing the latter. With moderate sized (group 3) fry, bitten-off portions were sometimes visible.

Based on the foregoing, it seems that the intensity of cannibalism depends on both size of fry as well as of fingerlings.

In the second experiment with 8 fish/l (Table 2), three sizes of fry were used to determine their susceptibility to cannibalism. Group 5 fingerlings were confirmed to be moderately cannibalistic. A high survival rate (83%) of group 3 fry was obtained. The larger fry, group 4, were not cannibalized by any of the fingerlings, possibly because of their fast movement.

Tilapia fry weighing 9.3 mg (slightly bigger than group 2 fry) were stocked with tilapia fingerlings with a mean weight of 163.5 mg (slightly bigger than group 5). These sizes were used because cannibalism between them is delayed so that the beneficial effect of sufficient natural food in the rearing medium could be demonstrated (Table 3). Without natural

Table 2. Mean survival (%) of three sizes of tilapia *(Oreochromis niloticus)* fry stocked with different sizes of fingerlings. Six fry were used for #2, #3 and #4. All fingerlings survived.

Treatment	Mean survival (%) After 10 minutes #2	#3	#4	After 30 minutes #2	#3	#4	After 1 hour #2	#3	#4
w/#5	66	94	100	0	83	100	0	83	100
w/#6	72	88	100	0	5	100	0	5	100
w/#7	27	66	100	0	0	100	0	0	100

Table 3. Mean survival rate (%) of small (group 2) tilapia *(Oreochromis niloticus)* fry given *Spirulina* and *Navicula* in the presence of group 5 tilapia fingerlings.[1]

Treatments	Culture period (days) 1	2	3	4	5
A. *Spirulina* feeding	100a	100a	100a	100a	83.1a
B. *Navicula* feeding	100a	100a	77.3a	52.5b	16.65b
C. Control, no feeding	100a	100a	63.87a	22.2c	5.56b

[1]% survivals with the same suffix letter in each vertical column are not significantly different ($P < 0.05$) using Duncan's Multiple Range Test.

food (control), cannibalism occurred on the third day with a fry survival of 64%. Almost all the small fry were eaten by the fingerlings at termination of the experiment. On the other hand, continuous supply of the *Navicula* was partially effective in preventing cannibalism, giving a mean survival rate of 77%. Finally, *Spirulina* feeding prevented cannibalistic behavior until after the fifth day. Survival was still high, 83%, compared to the treatment with *Navicula* where survival dropped to 16.6%.

Although earlier reports (Pantastico et al. 1985; Bowen 1982) showed that both diatoms and blue-green algae are acceptable to tilapias, high concentrations of *Spirulina* in the rearing medium seemed more effective in reducing cannibalism. Note, however, that the mean cell density of *Navicula* (8×10^3 cells/ml) was approximately that of the *Spirulina* (17.7×10^3 cells/ml).

With proper fertilization in tilapia nursery ponds, growth of sufficient natural food should ensure high production for hatchery operators because of the reduction of cannibalism among different sizes of fry and fingerlings.

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A Comparison of Overall Growth Performance of Tilapia in Open Waters and Aquaculture*

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Abstract

There are several indices for fish growth potential and growth performance comparisons available in the literature. One such index, found to allow meaningful comparisons for tilapia in the open waters of Africa and Asia, is: $\phi' = \log_{10}K + 2\log_{10}L_\infty$, where K and L_∞ are parameters of the von Bertalanffy growth equation. Overall, we found that tilapia in culture have higher values of ϕ' than in nature. The difference is highest in *Oreochromis aureus*, whose growth potential now appears to be fully realized. On the other hand, aquaculture systems based on *O. niloticus* appear to have hitherto failed to fully exploit the growth potential of this species. A discussion of the implications of our findings with regard to selecting suitable species and strains for aquaculture completes the paper.

*ICLARM Contribution No. 322.

Introduction

Tilapia growth, both in nature and in aquaculture, can be described by the von Bertalanffy growth function (VBGF), i.e.:

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

or

$$W_t = W_\infty (1-\exp(-K(t-t_0)))^3 \quad ...2)$$

where L_t (or W_t) is the size at age t, L_∞ (or W_∞) is the average size the fish of a given stock would reach if they were allowed to grow indefinitely, K is a growth constant (with dimension 1/t, i.e. not l/t or w/t, as implied by growth rates) and t_0 is the theoretical (usually negative) age of the fish at length zero (this parameter is of no relevance here and is not further discussed).

The present contribution is a followup to Moreau et al. (1986) in which 100 sets of growth parameters from natural populations of tilapia in Africa and Asia were used to identify the most useful of four different growth indices.

These most useful indices were:

$$\phi' = \log_{10}K + 2\log_{10}L_\infty \quad ...3)$$

and

$$P = \log_{10}K + \log_{10}W_\infty \quad ...4)$$

The algebraic relationships between P and ϕ' are given by Moreau et al. (1986, Table 3) as:

$$P = \phi' + (1/3)\log_{10}W_\infty + (2/3)\log_{10}a \quad ...5)$$

and

$$\phi' = P - (1/3)\log_{10}W_\infty + (2/3)\log_{10}a \quad ...6)$$

with "a" defined by an isometric length-weight relationship of the form

$$W = a \cdot L^3 \quad ...7)$$

In this contribution, these indices are used to compare the growth performance of tilapias belonging to various species, and of fish raised under culture conditions, with conspecifics that grew under natural conditions.

Materials and Methods

Table 1 summarizes the 150 sets of growth parameters, growth performance indices and related information assembled for the purposes of the present study.

The growth parameters were all estimated from size-at-age data in the literature cited in Table 1. All estimates refer to aquaculture growth experiments lasting at least three months and conducted with fishes stocked at weights usually not less than 10 g. Most estimates were obtained using the ETAL I computer program (Gaschütz et al. 1980), which allowed consideration of seasonal growth oscillations when these were manifest in the data.

In some cases, an alternative to the VBGF was used for describing growth, i.e., a logistic curve of the form:

$$W_t = W_\infty/(1 + \exp(-G(t-t_i))) \quad ...8)$$

where W_t is the weight as age t,

G a growth constant

and t_i the age of the fish at which $W_i = W_\infty/2$ (Moreau 1987).

This equation was fitted to data using the Microsimplex routine of Schnute (1983). Equation (8) implies that growth rate (dw/dt) is maximum when $W = W_\infty/2$, i.e., at t_i. Maximum growth rate can thus be computed from:

$$(dw/dt)_{max} = W_\infty G/4 \quad ...9)$$

In the VBGF, on the other hand, maximum growth rate is defined by:

$$(dw/dt)_{max} = (4/9) \cdot K \cdot W_\infty \quad ...10)$$

Table 1. Growth parameters and growth performance indices of 150 "stocks" of tilapia grown in controlled environments (ranked, within spp. according to ϕ').

Species	[A	B	C][a]	W_∞	L_∞	K	P	ϕ'	Area	Reference
O. andersonii	F&M	P	S	387.3	21.9	0.727	2.45	2.54	Zambia	Mortimer (1959)
	F&M	P	S	498.8	23.8	0.635	2.50	2.56	Zambia	Mortimer (1959)
	F&M	P	S	442.6	15.7	1.956	2.44	2.68	Zambia	Mortimer (1959)
	F&M	P	S	234.2	18.5	1.403	2.52	2.68	Zambia	Mortimer (1959)
	F&M	P	S	176.4	16.7	1.911	2.53	2.73	Zambia	Mortimer (1959)
	F&M	P	S	249.1	18.9	1.562	2.59	2.75	Zambia	Mortimer (1959)
	F&M	P	S	218.3	17.9	2.260	2.69	2.86	Uganda	Maar et al. (1966)
	F&M	P	S	230.5	18.4	2.474	2.76	2.92	Zambia	Mortimer (1959)
	F&M	P	V	196.1	17.4	8.204	3.20	3.40	Zambia	Lema et al. (1975)
O. aureus	F&M	P	V	89.4	13.0	4.038	2.56	2.83	El Salvador	Ramirios (1975)
	F&M	T	L	84.0	12.7	4.667	2.59	2.88	USA	Stickney et al. (1977)
	F&M	T	L	101.0	13.8	4.672	2.69	2.95	USA	Stickney et al. (1977)
	F&M	P	V	131.2	16.3	3.501	2.66	2.97	Costa Rica	Porras (1986)
	F&M	P	V	207.1	15.2	1.124	2.93	2.98	Costa Rica	Porras (1986)
	F&M	P	V	168.1	16.0	4.801	2.91	3.09	El Salvador	Ramirios (1975)
	F&M	P	V	147.1	15.3	5.239	2.80	3.10	El Salvador	Bowman (1975)
	F&M	P	L	384.2	25.3	2.761	3.03	3.10	USA	Henderson and Stickney (1981)
	F&M	P	V	269.4	18.7	4.016	3.03	3.15	El Salvador	Bowman (1975)
	F&M	P	V	108.4	13.8	7.478	2.91	3.16	El Salvador	Ramirios (1975)
	F	C	V	163.8	15.9	5.970	2.99	3.18	Belgium	Melard and Philippart (1981a)
	F&M	P	L	72.0	12.1	11.164	2.91	3.21	USA	Henderson and Stickney (1981)
	F&M	P	V	364.6	14.2	8.285	2.65	3.22	Costa Rica	Porras (1986)
	F&M	P	V	217.6	17.4	5.506	3.08	3.22	El Salvador	Bowman (1975)
	F&M	P	L	79.0	12.4	11.270	2.95	3.24	USA	Henderson and Stickney (1981)
	F&M	P	L	95.0	13.2	11.859	3.05	3.32	USA	Henderson and Stickney (1981)
	F&M	P	V	95.2	13.2	12.146	3.06	3.33	El Salvador	Bowman (1975)
	M	C	V	354.3	20.5	5.350	3.28	3.35	Belgium	Melard and Philippart (1981a)
	(M)	P	V	652.2	25.1	3.590	3.37	3.36	USA	Anderson and Smitherman (1978)
	M	P	V	496.4	23.0	5.027	3.40	3.42	USA	Anderson and Smitherman (1978)
O. esculentus	F&M	P	V	75.4	12.9	7.957	2.78	3.12	Tanzania	Payne (1971)
O. macrochir	F&M	P	S	2,250.9	39.3	0.235	2.72	2.56	Zambia	Mortimer (1959)
	F&M	P	S	104.2	14.0	2.603	2.43	2.70	Zambia	Mortimer (1959)
	F&M	P	S	3,669.9	46.3	0.274	3.00	2.77	Zambia	Mortimer (1959)
	F&M	P	V	104.8	14.0	3.113	2.51	2.79	Uganda	Maar et al. (1966)
	F&M	P	S	141.4	15.1	2.906	2.61	2.82	Zambia	Mortimer (1959)
	F&M	P	L	304.0	22.0	3.060	3.09	3.17	Cameroon	Bard (1960)
O. mossambicus	F&M	P	V	103.7	14.6	19.901	3.31	2.63	Philippines	Camacho et al. (1984)
	F&M	T	V	68.2	12.7	4.271	2.46	2.83	Zambia	Mabaye (1971)
	F&M	T	V	42.3	10.7	7.194	2.47	2.92	Thailand	Chotiyarnwong (1971)
	F&M	T	V	39.5	10.6	8.359	2.52	2.97	Thailand	Chotiyarnwong (1971)
	F&M	T	L	121.0	15.4	4.402	2.73	3.02	Zambia	Mabaye (1971)
	F&M	P	V	71.1	13.1	6.305	2.67	3.03	USA	Henderson and Stickney (1981)
	F&M	P	V	101.5	14.5	5.547	2.75	3.07	USA	Henderson and Stickney (1981)
	F&M	T	L	203.0	18.3	3.830	2.89	3.11	Zambia	Mabaye (1971)
	F&M	P	V	70.0	12.8	7.803	2.73	3.11	El Salvador	Bowman (1975)
	F&M	P	V	79.4	12.6	8.564	2.83	3.13	Philippines	O. Galman (pers. comm.)
	F&M	P	L	101.0	14.5	6.531	2.82	3.14	USA	Henderson and Stickney (1981)

Continued

Table 1. Continued

Species	[A	B	C]*	W_∞	L_∞	K	P	ϕ'	Area	Reference
	F&M	P	V	101.1	14.5	9.506	2.98	3.30	El Salvador	Bowman (1975)
	F&M	P	V	96.9	14.3	10.130	2.99	3.32	El Salvador	Bowman (1975)
	F&M	P	V	92.6	14.1	10.735	3.00	3.33	El Salvador	Bowman (1975)
	F&M	P	V	101.3	14.5	13.215	3.13	3.45	Philippines	Camacho et al. (1984)
	F&M	P	V	101.5	14.5	13.869	3.15	3.46	Philippines	Camacho et al. (1984)
	F&M	P	V	84.1	13.6	16.978	3.15	3.50	Philippines	Camacho et al. (1984)
O. niloticus	F&M	P	V	442.9	22.3	0.880	2.59	2.64	Burkina Faso	Traore (1985)
	F&M	P	V	123.7	14.6	2.385	2.47	2.70	Burkina Faso	Traore (1985)
	F&M	T	L	66.0	11.8	4.150	2.44	2.76	Thailand	Edwards et al. (1980)
	F	T	L	151.0	15.6	3.003	2.66	2.86	Centr.Afr.Rep.	Micha (1973)
	F&M	T	L	47.0	10.7	7.263	2.53	2.92	Thailand	Wee and Nag (1986)
	F&M	T	L	60.0	11.4	6.423	2.59	2.92	Thailand	Chotiyarnwong (1971)
	F&M	T	L	102.0	13.7	4.750	2.68	2.95	Thailand	Edwards et al. (1985)
	M	T	V	206.0	17.3	2.997	2.79	2.95	Centr.Afr.Rep.	Micha (1973)
	F&M	P	V	86.5	12.9	5.476	2.67	2.96	Thailand	Edwards et al. (1984)
	F&M	T	V	695.5	25.9	1.447	3.00	2.99	Centr.Afr.Rep.	Micha (1973)
	F	T	V	616.8	24.9	1.572	2.79	2.99	Centr.Afr.Rep.	Micha (1973)
	F&M	P	V	417.2	21.5	2.265	2.99	2.99	Belgium	Melard and Philippart (1981a)
	F&M	T	L	75.0	12.3	6.534	2.69	3.00	Thailand	Chotiyarnwong (1971)
	F&M	P	V	270.0	18.9	2.950	2.90	3.02	Sierra Leone	Iscandiri (1985)
	F&M	C	V	611.2	23.9	1.881	3.06	3.03	Côte d'Ivoire	Coche (1977)
	F&M	P	L	77.0	12.4	7.155	2.74	3.04	Philippines	Guerrero (1976)
	F&M	C	V	92.3	13.2	6.544	2.78	3.06	Philippines	Aquino and Nielsen (1983)
	F	P	V	2,848.4	40.8	0.716	3.31	3.08	Belgium	Melard and Philippart (1981a)
	F&M	P	V	129.3	14.9	5.585	2.87	3.09	Philippines	Cruz and Shehadeh (1980)
	F&M	T	L	89.0	13.3	7.093	2.80	3.10	Thailand	Wee and Nag (1986)
	M	P	V	144.2	15.3	5.342	2.89	3.10	Puerto Rico	Fram and Pagan-Font (1978)
	F&M	T	L	95.0	13.3	7.628	2.86	3.13	Thailand	Edwards et al. (1985)
	M	P	V	516.1	23.1	2.540	3.12	3.13	Belgium	Melard and Philippart (1981b)
	F&M	T	V	68.7	12.0	9.871	2.83	3.15	Thailand	Edwards et al. (1980)
	F&M	T	V	269.7	18.6	4.221	3.06	3.16	Belgium	Melard and Philippart (1981a)
	F&M	C	V	450.2	22.4	2.915	3.12	3.16	Philippines	Aquino and Nielsen (1983)
	F&M	P	V	114.6	14.2	7.291	2.92	3.17	USA	Anderson and Smitherman (1978)
	F	T	V	455.4	22.5	2.984	3.13	3.18	Belgium	Melard and Philippart (1981a)
	F&M	T	V	55.1	11.1	13.000	2.85	3.20	Thailand	Edwards et al. (1980)
	F&M	P	V	317.4	23.8	3.854	3.08	3.19	Philippines	Prein (1985)
	F&M	P	V	198.3	20.6	5.409	3.03	3.20	Philippines	Prein (1985)
	M	T	L	137.0	15.1	7.305	3.00	3.22	Centr.Afr.Rep.	Micha (1973)
	F&M	P	L	99.0	13.5	9.220	2.96	3.23	Philippines	Cruz and Shehadeh (1980)
	F	P	V	286.6	19.0	4.789	3.14	3.24	Belgium	Melard and Philippart (1981a)
	F&M	C	V	315.0	19.3	4.737	3.17	3.24	Côte d'Ivoire	Coche (1977)
	F&M	T	L	131.0	15.8	7.109	2.97	3.25	Thailand	Edwards et al. (1985)
	F&M	T	V	155.2	15.7	7.157	3.06	3.25	Thailand	Edwards et al. (1980)
	F&M	P	L	104.0	13.7	9.712	3.00	3.26	Thailand	Edwards et al. (1984)
	F&M	C	V	146.1	15.4	7.843	3.06	3.27	Philippines	Aquino and Nielsen (1983)
	M	T	V	1,073.3	29.9	2.076	3.35	3.27	Belgium	Melard and Philippart (1981b)
	F&M	T	L	245.0	18.3	5.881	3.16	3.29	Thailand	Edwards et al. (1980)
	F&M	P	L	82.0	12.7	12.348	3.00	3.30	Philippines	Cruz and Shehadeh (1980)
	M	P	V	513.0	23.4	3.690	3.28	3.31	Côte d'Ivoire	J. Lazard (pers. comm.)
	F&M	T	V	44.3	10.3	19.644	2.94	3.32	Thailand	Edwards et al. (1980)
	M	P	V	462.9	22.2	4.271	3.30	3.33	Belgium	Melard and Philippart (1981a)

Continued

Table 1. Continued

Species	[A	B	C]*	W_∞	L_∞	K	P	ϕ'	Area	Reference
	F&M	P	L	157.0	15.8	8.889	3.14	3.34	Thailand	Edwards et al. (1984)
	F&M	P	V	195.2	16.9	7.651	3.17	3.34	Thailand	Edwards et al. (1984)
	(M)	P	V	477.5	22.8	4.179	3.30	3.34	USA	Anderson and Smitherman (1978)
	F&M	C	V	85.3	12.9	13.933	3.07	3.36	Philippines	Aquino and Nielsen (1983)
	F&M	P	L	143.0	15.3	10.377	3.17	3.38	Philippines	Cruz and Shehadeh (1980)
	M	P	V	277.6	19.1	6.604	3.26	3.38	Côte d'Ivoire	J. Lazard (pers. comm.)
	F&M	P	V	444.0	21.9	4.329	3.28	3.38	Cameroon	Bard (1960)
	F&M	P	L	174.0	16.3	9.352	3.21	3.40	Philippines	Cruz and Shehadeh (1980)
	F&M	P	L	152.0	15.6	10.742	3.21	3.42	Philippines	Cruz and Shehadeh (1980)
	M	P	V	357.9	20.8	6.586	3.37	3.45	USA	Anderson and Smitherman (1978)
	M	T	V	509.2	23.3	5.419	3.44	3.47	Belgium	Melard and Philippart (1981a)
	F&M	P	V	183.3	16.6	10.942	3.30	3.48	Thailand	Edwards et al. (1984)
	F&M	P	L	210.0	17.4	10.631	3.35	3.51	Philippines	Cruz and Shehadeh (1980)
	M	T	V	205.6	17.3	12.654	3.41	3.58	Belgium	Melard and Philippart (1981a)
	M	T	V	270.3	18.9	10.665	3.46	3.58	Belgium	Melard and Philippart (1981a)
	F&M	P	L	539.0	23.6	6.879	3.56	3.59	Philippines	Cruz and Shehadeh (1980)
	M	P	V	474.2	22.8	8.028	3.58	3.62	USA	Shell (1968)
	M	T	V	233.4	13.2	13.207	3.49	3.63	Belgium	Melard and Philippart (1981a)
	M	P	V	456.7	22.5	10.458	3.68	3.72	USA	Shell (1968)
O. shiranus	F&M	T	V	53.3	11.0	9.870	2.72	3.08	Malawi	Msiska and Cantrell (1985)
O. spirulus niger	F	A	S	217.8	18.5	0.956	2.32	2.51	Uganda	Cridland (1965)
	M	A	S	166.0	16.9	1.240	2.31	2.55	Uganda	Cridland (1965)
	F&M	P	S	381.5	22.3	1.640	2.80	2.91	Kenya	Van Someren and Whitehead (1960)
	F&M	P	S	394.7	22.6	1.950	2.89	3.00	Kenya	Van Someren and Whitehead (1960)
O. variabilis	F&M	P	L	635.0	25.3	1.148	2.87	2.87	Kenya	Rinne (1975)
S. guineensis	F	C	V	200.0	17.4	1.224	2.89	2.56	Côte d'Ivoire	Cissé (1985)
	F	P	L	181.0	16.8	1.386	2.40	2.59	Côte d'Ivoire	Legendre (1983)
	F&M	P	V	221.9	18.0	1.314	2.47	2.61	Côte d'Ivoire	Legendre (1983)
	F&M	P	V	359.5	21.1	0.958	2.51	2.63	Côte d'Ivoire	Legendre (1983)
	M	P	L	174.0	16.6	1.756	2.48	2.69	Côte d'Ivoire	Legendre (1983)
	F	C	V	161.2	16.2	1.933	2.49	2.70	Côte d'Ivoire	Cissé (1985)
	M	C	V	297.4	18.2	2.151	2.69	2.85	Côte d'Ivoire	Cissé (1985)
	F&M	P	L	54.0	11.0	8.390	2.65	3.01	Côte d'Ivoire	Legendre (1983)
	M	C	V	240.6	18.5	3.004	2.86	3.01	Côte d'Ivoire	Cissé (1985)
S. melanotheron	M	P	V	367.1	21.3	0.298	2.01	2.11	Côte d'Ivoire	Legendre (1983)
	F	P	V	143.1	15.5	1.727	2.39	2.60	Côte d'Ivoire	Legendre (1983)
	F	P	V	200.5	17.1	1.373	2.44	2.60	Côte d'Ivoire	Legendre (1983)
	F	P	L	146.0	15.7	1.649	2.38	2.61	Côte d'Ivoire	Cissé (1985)
	M	P	L	338.0	20.7	1.244	2.62	2.73	Côte d'Ivoire	Legendre (1983)
	M	P	V	115.2	14.6	2.546	2.47	2.74	Côte d'Ivoire	Cissé (1985)
	F	P	V	175.2	16.7	2.188	2.58	2.79	Côte d'Ivoire	Cissé (1985)
	M	P	L	131.0	15.2	2.929	2.58	2.83	Côte d'Ivoire	Cissé (1985)
	F	P	V	329.7	20.7	1.678	2.74	2.86	Côte d'Ivoire	Cissé (1985)
	F&M	P	L	73.0	12.2	7.250	2.72	3.04	Côte d'Ivoire	Legendre (1983)

Continued

Table 1. Continued

Species	[A	B	C][a]	W_∞	L_∞	K	P	ϕ'	Area	Reference
T. rendalli	F&M	P	S	1,594.8	33.9	0.189	2.48	2.34	Zambia	Mortimer (1959)
	F&M	P	S	418.6	21.7	0.615	2.41	2.46	Zambia	Mortimer (1959)
	F&M	P	S	107.5	13.8	3.19	2.53	2.78	Zambia	Mortimer (1959)
	F&M	P	V	91.6	13.1	3.785	2.54	2.81	Uganda	Maar et al. (1966)
T. zillii	F&M	P	V	9,899.0	61.8	0.143	3.15	2.76	USA	Hauser (1975)
	F&M	P	V	141.1	15.0	3.434	2.69	2.89	Tanzania	Payne (1971)
	F&M	P	V	978.9	28.6	1.190	3.06	2.99	Uganda	Cridland (1962)

[a] A = sex (F or M); F & M refers to females + males; (M) refers to sex-reversed all-male broods.
B = culture type (P, C, T, A; P = ponds; C = cages; T = tanks; A = aquaria)
C = method of curve-fitting (V, S, L; V = ordinary VBGF fitting methods; S = seasonally oscillating VBGF fitted with ETAL 1)
L = Logistic growth curve fitted with microsimplex (Schnute 1983).

Hence, if W_∞ and G have been estimated using equation (8), $(dw/dt)_{max}$ can be estimated using equation (9), equated to equation (10) and the latter solved for K.

Growth parameters obtained via equations (8) to (10) are indicated as such in Table 1.

All weights are in g live weight, all lengths are standard lengths, in cm, and all values of K are in year^{-1}. The arithmetic mean and its standard error have been computed for the indices P and ϕ' in those tilapia species in which three or more data sets were available from both aquaculture systems and natural waters (Table 2).

The details of these computations, including the size-at-age data used and a listing of the ETAL 1 program are available on request from any of the authors.

Results and Discussion

Both Table 2 and Fig. 1 show that tilapia generally display, under culture conditions, a higher growth performance, as defined by the ϕ' index, than in nature.

Overall, P-values (referring to growth rate - dw/dt - at the inflexion point of the VBGF) of tilapia in aquaculture are equal to 2.951 while the mean P value of tilapia in nature is 2.681 (weighted means). Thus $\Delta P = 0.270$, corresponding (see equation 4) to a multiplicative increase of $10^P = 1.86$. Put differently, one may say cultured tilapia grow about 86% better than in nature (note that this percentage can be as high as 300 when, e.g., male monosex culture of a certain species is considered, see Table 1 and Fig 2). Fig. 1 shows, however, that $\Delta\phi'$ (roughly equivalent to ΔP) is very uneven among different tilapia species (at least with respect to the data sets compared here). Thus, in *T. rendalli*, $\Delta\phi'$ is only 0.149, while $\Delta\phi'$ reaches as much as 0.658 in *O. aureus*, the species in which the difference between aquaculture and natural growth performance appears to be highest. We attribute the high "realized domestication bonus" of *O. aureus* to the fact that this species is generally cultured under intensive or at least semi-intensive conditions (see Table 1); see for example the discussion on cage culture performance in the review by Coche (1982) in which *O. aureus* was ranked above *O. niloticus*.

This last point brings us to *O. niloticus* which, although it has the highest growth performance of any tilapia both in nature (Moreau et al. 1986) and in aquaculture (see Fig. 1), displays here a high degree of overlap between growth performance in nature and in aquaculture, with a $\Delta\phi' = 0.558$, less than *O. aureus* and even *O. mossambicus* (Table 2). We conclude from this that, as a whole, aquaculture systems based on *O. niloticus* have hitherto failed to fully exploit the

Table 2. Comparison of mean growth performance indices and related statistics of 7 species of tilapia grown in controlled environments (Table 1) with the corresponding statistics in conspecific wild stocks (data from Table 1 of Moreau et al. 1986).

Species	Stock[a]	n	$a_{(SL)}$[b]	S.E.M.	Mean P	S.E.M.	Mean ϕ'	Min	Max	S.E.M.
T. rendalli	A	4	0.409	0.01	2.49	0.05	2.60	2.34	2.81	0.12
T. rendalli	N	16	0.410	0.08	2.55	< 0.01	2.45	2.24	2.80	0.03
O. andersonii	A	9	0.372	0.02	2.63	0.04	2.79	2.54	3.40	0.09
O. andersonii	N	4	0.365	0.33	2.68	0.13	2.56	2.46	2.63	0.07
O. macrochir	A	6	0.382	0.06	2.73	< 0.01	2.80	2.56	3.17	0.08
O. macrochir	N	8	0.401	0.05	2.41	0.07	2.41	2.31	2.50	0.02
T. zillii	A	3	0.419	< 0.01	2.97	0.07	2.88	2.76	2.99	0.07
T. zillii	N	8	0.385	0.14	2.30	0.16	2.39	2.09	2.68	0.08
O. niloticus	A	65	0.407	0.10	3.05	0.02	3.21	2.64	3.72	0.03
O. niloticus	N	16	0.395	0.15	2.80	0.07	2.65	2.36	3.11	0.06
O. mossambicus	A	17	0.337	0.04	2.86	0.05	3.14	2.63	3.50	0.06
O. mossambicus	N	20	0.421	0.12	2.57	0.02	2.48	2.05	2.80	0.04
O. aureus	A	20	0.401	0.16	2.95	< 0.01	3.15	2.83	3.42	0.04
O. aureus	N	4	0.363	0.23	2.36	0.05	2.50	2.31	2.61	0.07

[a] A = Aquaculture; N = Natural stocks.

[b] $a_{(SL)}$ refers to multiplicative term of length-weight relationship (equation 7) based on standard length (SL).

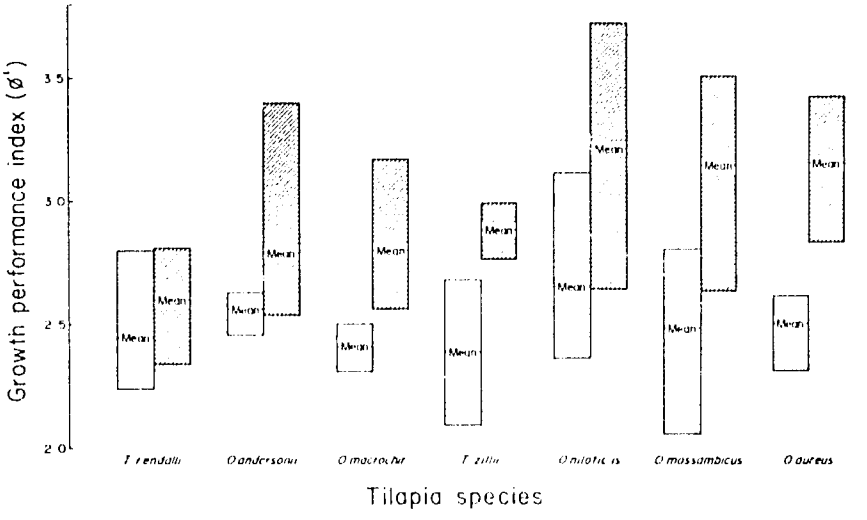

Fig. 1. Mean and range of the growth performance index ϕ' in 7 tilapia species for which at least three sets of growth parameters were available for both wild stocks (clear) and controlled environments (shaded). The 7 species are ranked according to their mean response to being cultured (i.e., according to the difference between their values of ϕ'_A and ϕ'_N, see Table 2).

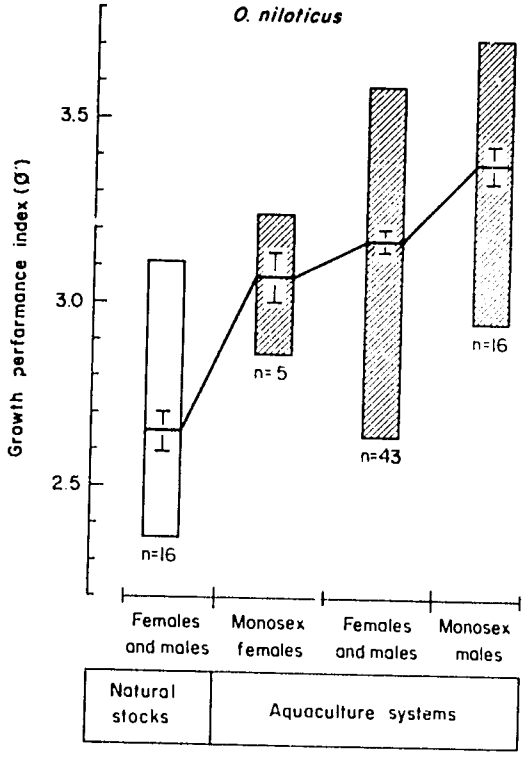

Fig. 2. Mean, standard error and range of the growth performance index ϕ' in various *O. niloticus* stocks (based on data in Tables 1 and 2).

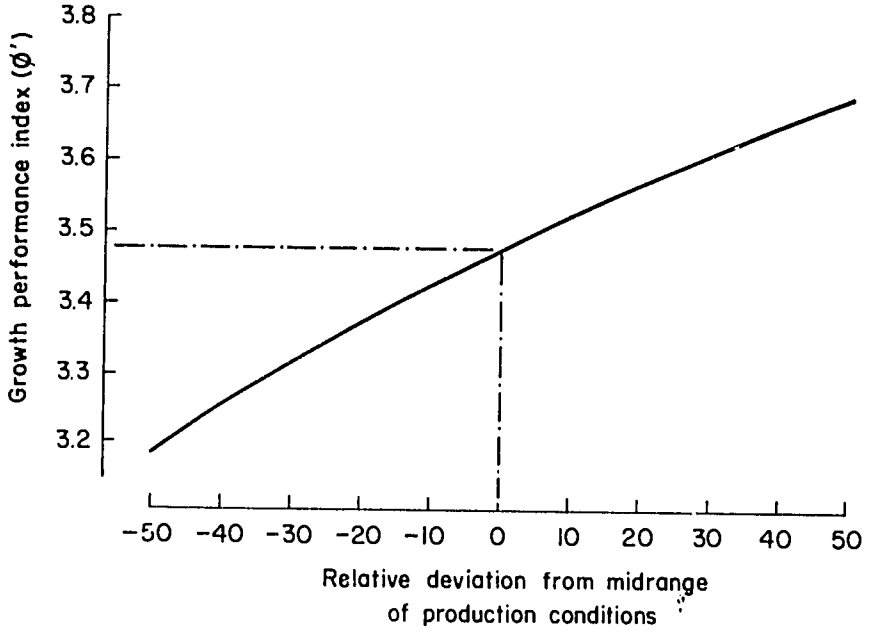

Fig. 3. Response of the growth performance index ϕ' to relative changes in pond condition in an integrated animal/fish system, Central Luzon State University, Philippines. Relative changes of pond condition refer to the aggregated effects of changes of 5 variables shown to affect *O. niloticus* growth (computed from data in Prein 1985).

growth potential of this species. This is also suggested by Fig. 3, based on data in Hopkins and Cruz (1982) and a multiple regression model in Prein (1985) which shows the response of *O. niloticus* growth performance to improvement of pond conditions, as well as by Fig. 2 showing the dramatic growth increases obtained in (male) monosex systems.

Future increases in growth performance will also come from the selection of improved strains and the identification of suitable hybrids - an area in which the growth performance indices used here may be useful. This is fully discussed by Pullin and Capili (this vol.).

Intuitively, the index P appears more useful than ϕ' given that it has the dimension ($\log_{10}$) w/t while ϕ' has the awkward dimension ($\log_{10}$) L2/t. However weight is, in fish, far more variable than length, as illustrated by the fact that fish may often lose weight but hardly ever "lose length". Therefore, the possibility of biases will tend to be greater in a weight-based index. Possibly, the best approach would be to rely, for future comparative studies of the type reported here, on both indices, and to devote attention to the species and/or strains whose growth performance is high in terms of both indices.

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The Influence of Salt (NaCl) Concentration and Temperature on the Growth of *Oreochromis spilurus spilurus, O. mossambicus* and a Red Tilapia Hybrid

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Abstract

The growth rates of *Oreochromis spilurus spilurus, O. mossambicus* and a red tilapia hybrid were studied using a standard 28-day assay with a diet of trout pellets fed at 2-3% body weight/day and with varying salt concentrations (0-28 g/l NaCl) and temperature (20-28°C). The growth rate of *O. spilurus spilurus* was similar at all salt concentrations from freshwater to 20 g/l but was depressed at 24 and 28 g/l. From the experimental groups of 10 fish/treatment, one fish died at 24 g/l and nine at 28 g/l. For *O. mossambicus*, growth patterns were fairly constant over the tested range of 0-14 g/l and increasing temperature in the range 20 to 28°C consistently increased growth rates. At 20°C, *O. mossambicus* growth rates declined with increasing salinity (6 to 14 g/l) but at 28°C there was a much improved growth rate at the highest salinity (14 g/l).

The salt concentration threshold for growth inhibition in *O. spilurus spilurus* was considerably greater than those shown from earlier work on *O. niloticus* x *O. aureus* hybrids (freshwater species) and a 'red tilapia' with probable affinities to *O. mossambicus* (fresh- and brackishwater species).

Introduction

There is a strong rationale for aquaculture of salt-tolerant fish. Around estuaries, there is less competition for water and land between agriculture and aquaculture. The water resources available for inland aquaculture, particularly in arid regions, are often in salt-laden areas. Irrigation of land for agriculture can also lead to salt accumulation in the soil to a point where

agriculture must cease. Incorporation of fishponds into flushing schemes for soil desalination can help in the rehabilitation of salt-laden soils whilst also producing a useful crop.

Many freshwater tilapias are moderately salt-tolerant (Chervinski 1982). Estuarine tilapias, such as *Oreochromis mossambicus* and *O. spilurus spilurus*, are euryhaline (Payne 1983; Trewavas 1983). Salt-tolerance is often equated with survival but, in a production system, survival *and* good growth performance should be the criteria.

Tilapias have a threshold salinity beyond which growth (Payne 1983; Stickney 1986) and reproduction (Watanabe and Kuo 1985) are inhibited. For saltwater tilapia culture, it is important to determine these thresholds and to establish the ways in which other factors influence them. Since changes in salinity affect osmoregulation (an active process) one probable modifier is likely to be temperature.

This paper describes a series of growth performance assays with three tilapias (*O.s. spilurus*, *O. mossambicus* and a red hybrid of uncertain parentage) fed a commercial pelleted feed under different conditions of temperature and salt concentration.

Methods

O.s. spilurus (originally from the stock of Baobab Farms Mombasa, Kenya) and *O. mossambicus* were obtained from Stirling University originally imported from Singapore. The red hybrid was obtained from Shearwater Fish Farms, UK. Meristic counts were made on 30 red hybrids to indicate their affinities. Their gill raker counts (16-18) and dorsal fin counts (XIV-XVI/10-12) were low and resembled *O. mossambicus* (14-19; XV-XVII) rather than other species known to be involved in red hybrids (Galman and Avtalion 1983). This hybrid may also have *O. aureus* and *O. urolepis hornorum* in its ancestry.

The experiments were carried out in a dual tank system (Fig. 1) with a capacity of 80 l. During an experiment, the fish were held in the upper tank whilst the lower acted as a fecal trap and assisted in the oxygenation of the water. To introduce the fish to a given salt concentration the appropriate quantity of salt (NaCl) was added to the bottom tank, gradually dissolved in the freshwater and circulated to the upper tank. It took around 24 hours to reach equilibrium and thus the fish were exposed to the increased concentration gradually. Freshwater used in these experiments was conditioned tapwater of 760 µS conductivity and 44 g/l calcium content.

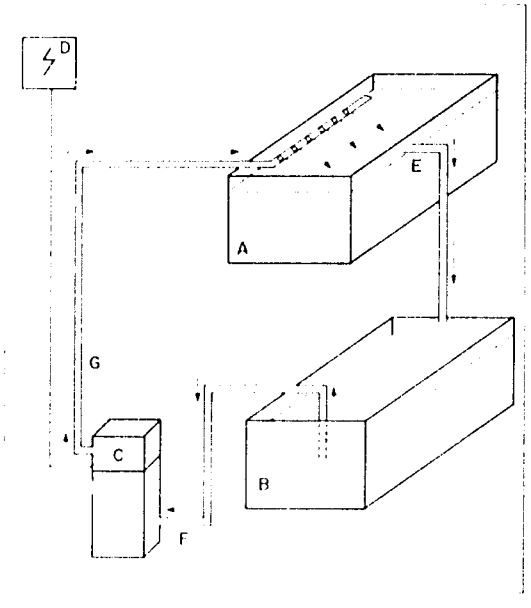

Fig. 1. Dual tank experimental system. A upper tank; B lower tank; C Eheim 2016 centrifugal pump/filter; D mains supply; E overflow; F inlet to pump; G outlet to pump.

The fish were acclimated for two days at the new concentration before the experiment started. They were then fed on commercial trout pellets for 28 days. Fish were weighed before and at the end of the experiment. Details of fish sizes, feeding rates and stocking densities and treatments are given in Table 1. Since the *O. mossambicus* experiment was intended to investigate the interaction of salt

concentration and temperature, a freshwater group was not included. Total dry weights of all fish were obtained by oven drying at 70°C for five days. The dry weight of the dead fish was determined rather than the wet weight because the latter is unreliable: the osmoregulatory system of the dead animal ceases to function and its weight changes due entirely to water flux. Initial percentage dry weights were estimated in the same way from random subsamples of 10 fish per group taken at the beginning of the experiment. Where energy determinations were carried out, whole dried fish were homogenized in a blender and samples ignited in a nonadiabatic bomb calorimeter.

Mortalities were accounted for by obtaining the total dry weight from each tank at the end of the experiment, including those which died during the experimental period, then dividing by the total number of fish survival days for each tank which gives the mean dry weight increment per fish per day. This was then expressed as a percentage of the initial dry weight and multiplied by a standard 21-day period. This does not allow for geometric increase in weight with time but over short experimental periods growth may be consisted linear (Winberg 1956).

Results

Oreochromis spilurus spilurus

O.s. spilurus received less feed than other species (Table 1) and its growth rates were generally lower. Only one individual survived the entire experimental period at 28 g/l. One fish died at 24 g/l.

Growth in *O.s. spilurus* was maintained up to 16 g/l at a rate comparable to that in freshwater (Fig. 2), but between 16 and 20 g/l it was depressed and declined rapidly at 24 g/l. Changes in wet and dry weight showed similar patterns (Fig. 2) except at the

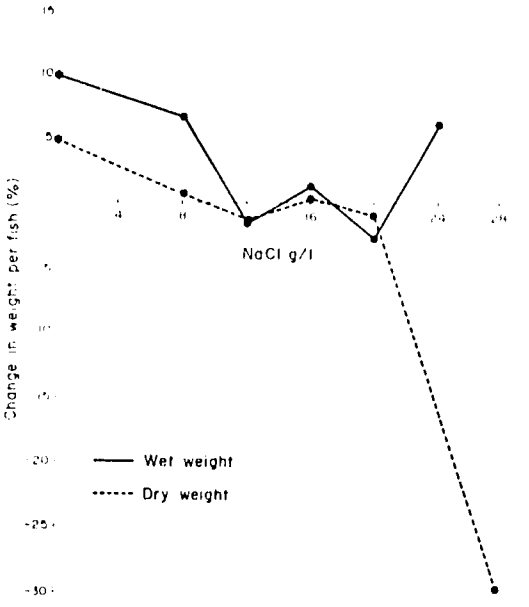

Fig. 2. Change in wet and dry weight of *O. spilurus* over 21-day period. Each point represents the mean of 10 fish.

Table 1. Details of stocking density, feeding regimes and treatments (different salt concentration/temperature combinations) for growth experiments with various tilapias.

Fish	No./tank	Mean wet wt (g)	Feeding % body wt/day	NaCl concentration g/l	Temperature (°C)
O.s. spilurus	10	4.3	2	0, 8, 12, 16, 20, 28	20
O. mossambicus	10	1.2	3	6, 10, 14	20, 24, 28
Red hybrid	5	16.1	3	0, 4, 8, 16, 20, 24	20

higher salinities where wet weight increased and dry weight declined precipitously. The increased wet weight was entirely due to fluid retention. All the fish were bloated at the end of the experiment, even the cornea of the eye was swollen. Piercing the abdominal wall released considerable fluid. This was an extreme case of the elevation of the water content of fish at relatively high salt concentrations observed by Payne (1983). In such circumstances, wet weight can be unusually high and misleading as a measure of growth.

Dry weights were converted into energy equivalents for *O.s. spilurus* (Fig. 3). The initial mean energy content was 28,360 ± 1,432 j/g and final energy contents ranged from 28,493 j/g in freshwater to 26,607 j/g at 24 ppt. There was no significant difference between energy values from each treatment.

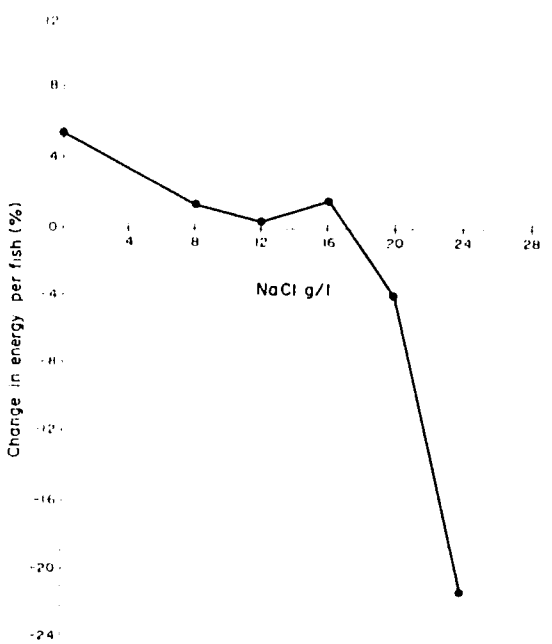

Fig. 3. Change in energy content of *O. spilurus* over 21-day period. Each point represents mean of 10 fish.

Oreochromis mossambicus

There were no mortalities during the experiment, suggesting that both the temperature and salt concentration ranges employed were well within the tolerance limits. At all salinities, growth increased with temperature (Fig. 4). At the lowest temperature (20°C) growth rates declined with increasing salinity but

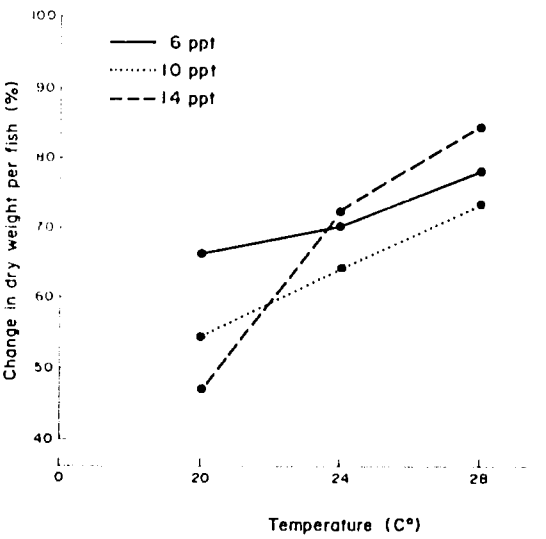

Fig. 4. Change in dry weight of *O. mossambicus* at various salinities and temperatures over 21-day period. Each point represents mean of 10 fish.

at higher temperatures growth rate became more rapid and was greatest at the highest salinity.

ANOVA showed that increases in growth rate due to temperature were significant ($P = 0.05$) whereas differences due to salt concentration were not significant.

Red Hybrids

The red hybrid maintained its growth rate from freshwater up to 12 g/l, after which growth declined. Wet weight changes paralleled dry weight changes (Fig. 5). Only three mortalities occurred towards the end of the experimental period at 24 g/l.

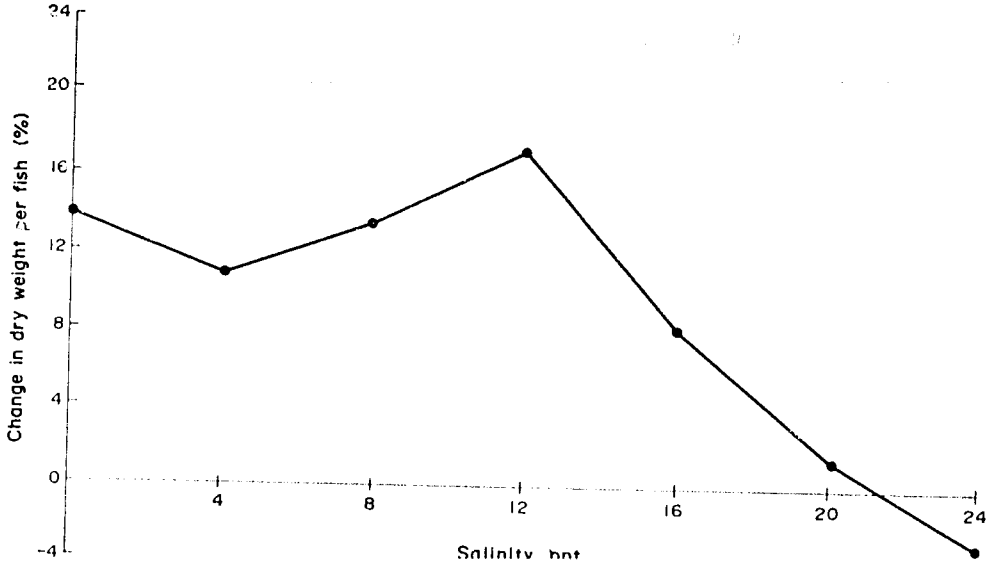

Fig. 5. Dry weight changes in 'red tilapia' at various salinities over 21 days. Each point represents mean of five fish.

Discussion

Both *O.s. spilurus* (Figs. 2 and 3) and the red hybrid (Fig. 5) showed an optimum range over which growth rates were more or less similar. Beyond this, growth was inhibited, presumably because more energy was diverted into osmoregulation. Towards the upper salt-tolerance limits this energy demand for osmoregulation became so great that growth became negative as body reserves were also utilized. A similar pattern has been demonstrated in *O. niloticus* x *O. aureus* hybrids and common carp, *Cyprinus carpio* (Payne 1983).

Since the response to salt concentration involves osmoregulation and water exchange with the external medium, it is essential that dry weight be used as an index of growth performance; note the fluid retention at the highest salt concentration by *O.s. spilurus* (Fig. 2). One reason for this may be fish drinking the surrounding medium to replace water loss. In such a case, there could be a point, particularly with fish of freshwater origin, where the salt intake with the water exceeds the fish's capacity to excrete it and the concentration of the body fluid rises. The normal response to this could be to drink even more resulting in more salt accumulation and ultimately greater hydration of tissues. In a farming situation, this could lead to weight increases but a poor quality product.

The optimum range for growth of *O.s. spilurus* was found to be between freshwater and 16-20 g/l NaCl. This species has been used very successfully for intensive culture at Baobab Fish Farms, Mombasa, in brackishwater which occasionally reached 15 g/l (J.D. Balarin, pers. comm.). However, good survival and moderate growth of *O.s. spilurus* (imported from Baobab Farms) have been obtained in full-strength seawater in Kuwait (Hopkins et al. 1985). The growth rate of *O.s. spilurus* is reportedly unaffected by salinity at 40 g/l in the Red Sea (Osborne 1979). Survival and growth were poor at 28 g/l in the present experiments. These differences could be due to a number of factors including the different salt composition of the waters, the warmer temperatures used in Kuwait and the Red Sea and acclimation factors.

Within the range of 6 to 14 g/l, there was no significant variation in growth rate of *O. mossambicus*. Since *O. mossambicus*, like *O.s. spilurus*, can be an estuarine species, it is probable that 14 g/l lies well within its optimum range for growth. Whilst renowned as one of the most salt-tolerant of all tilapias its growth in saline waters has not been well studied (Payne 1983; Stickney 1986). It appears to grow better in brackishwater than in very low or high salinities. The optimum range evidently extends above 14 g/l. Paloheimo and Dickie (1966) deduced that salinity affected the partition of energy between growth and other metabolic processes, whereas temperature influenced the general rate and efficiency of processes. Between 20 and 28°C there was a significant increase in the growth of *O. mossambicus* at all salinities tested here (Fig. 4).

Job (1969) suggested that the growth rate of *O. mossambicus* increases up to 30°C and declines at higher temperatures. The highest salt concentration used in our experiments did not go beyond the optimum range so it is difficult to comment on salinity/temperature interaction in detail. However, there was a change in the ranking of the growth rates at different salt concentrations with changes in temperature within the optimum salinity range. At 20°C, the lowest salt concentration supported the highest growth rate and the highest salt concentration the lowest growth rate. This was reversed at the higher temperatures. This is precisely the situation seen in experiments on the desert pupfish *Cyprinodon macularius* by Kinne (1960). A possible explanation is that higher temperatures increase the efficiency of osmoregulation and thereby liberate more energy for growth.

For the red tilapia hybrid, there is the suggestion that growth may reach a peak at some intermediate salinity within the optimum range, 12 g/l in this case (Fig. 5). There have been similar indications for both *O. niloticus* x *O. aureus* hybrids and *Cyprinus carpio* (Payne 1983). The optimum range for growth of the red hybrid used here was between freshwater and 16-20 g/l. After reviewing the influence of salinity upon the growth of red tilapia hybrids Stickney (1986) concluded that it would be appropriate to grow them in up to 50% seawater, that is about 17.5 g/l, which is in accordance with the results presented here. However, it should be borne in mind that there are many different red tilapias and their salt-tolerance and responses to salinity changes may be different.

Finally, the upper thresholds of the optimum range of salt concentrations for growth of the species examined here are rather greater than that observed for *O. niloticus* x *O. aureus* hybrids for which the major reduction in growth rate occurs between 8 and 10 g/l compared to freshwater controls (Payne 1983).

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Aluminium Toxicity to Tilapias Based on Experiments with *Oreochromis aureus*

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Abstract

Aluminium has been recognized as an extremely important toxic metal in many temperate freshwater environments, but has received very little attention in tropical aquatic ecosystems. This paper briefly reviews and presents new data on the occurrence of aluminium in aquatic ecosystems, with particular reference to the sources, chemistry and concentration of aluminium in tropical environments. The paper then describes a series of experiments on the toxicity of aluminium to *Oreochromis aureus* in relation to pH, water hardness and fish size. The results are compared to previous studies on aluminium toxicity in temperate environments and some tentative water quality guidelines for aluminium in tilapia culture systems are presented.

Introduction

Aluminium is the third most common element within the earth's crust (Garrels et al. 1975), occurring widely in aluminosilicate minerals and their weathering products. However, aluminium is not universally abundant in freshwater, largely because of the insoluble nature of its hydroxides and its tendency to form com-

plexes and polymerize (Burrows 1977). Aluminium is more soluble in acid waters and has now been implicated in fish mortalities in temperate regions affected by acid rain (Howells 1983). Hence reappraisal of the importance of aluminium toxicity in acid waters is beginning.

No data have been found on the toxicity of aluminium to tilapias and other tropical fish species, but there are some data on the tolerance of tilapias and acid waters. *Tilapia congica* has been found at pH 4.5-5.0 (Dubois 1959). Lowe-McConnell (1982) notes that tilapias lived in extremely acidic (pH 2.0-4.0) ponds in Malacca. Reite et al. (1973) found that pH < 3.5 resulted in 100% mortality of *Oreochromis grahami* in less than 2 hours, and Wendelaar Bonga et al. (1984) found low mortalities in *O. mossambicus* exposed to pH 4.0. Bhaskar and Govindappa (1985) also found some physiological changes in the muscle of *O. mossambicus* at pH 5.0. It is highly likely that some tilapias exposed to acidic environments will also be exposed to aluminium and therefore, an understanding of their response to aluminium is necessary to interpret fully their physiology and behavior in acid waters. In this study, the toxicity of aluminium to *Oreochromis aureus* was examined.

Materials and Methods

The test fish were juvenile *Oreochromis aureus* (mean weight 5.2 g ± S.D. = 0.7 g) bred at the Institute of Aquaculture, University of Stirling, from a genetically homogeneous stock (McAndrew and Majumdar 1983) and raised in a recirculating aquarium at 24.5-26.0°C and pH 6.6-6.8.

The experiments were performed in a flowthrough toxicity testing system comprising 12-, 25-l tanks fed at 2.2 l/hour from a 150-l reservoir. Aluminium was dosed into the experimental tanks as dilute aluminium nitrate via a peristaltic pump and mixing chambers. The experimental tanks and reservoir were continuously mixed by gentle aeration and kept at 24.5-26.0°C.

To investigate the effects of calcium concentration and pH on aluminium toxicity and to maintain uniform basic water quality, two synthetic dilution waters were formulated from deionized water and Analar grade chemicals, according to the guidelines given by HMSO (1969), with total hardnesses of 2 and 20 mg/l as $CaCO_3$ (0.60 and 5.85 mg/l of calcium, respectively). pH was controlled within the systems by appropriate dosing of H_2SO_4 and NaOH.

Five concentrations of aluminium plus a control tank were replicated and tested in each experiment. The experiments were initiated by taking ten fish at random from the stock tank and placing them into each experimental tank, and then allowing them to acclimatize to the test apparatus for 24 hours. The aluminium solutions were then pumped into the tanks and the experiments were run for 96 hours. Mortalities were counted and removed from each tank at 12-hour intervals, cessation of opercular movements being used to characterize death.

A close check was kept on water quality within the tanks during the experiments. Aluminium and pH were analyzed every 12 hours and calcium and magnesium every 24 hours. Two fractions of aluminium were measured, total acid reactive aluminium and dissolved acid reactive aluminium using the pyrocatechol violet method described by Dougan and Wilson (1974) and HMSO (1980). Calcium and magnesium were analyzed using Atomic Absorption Spectroscopy and pH with a glass electrode and pH meter. These analyses showed that water quality remained relatively stable throughout the tests (Table 1).

The median lethal concentration (LC_{50}) was calculated from the measured concentrations of dissolved aluminium and mortality using the trimmed Spearman-Karber method (Hamilton et al. 1977). The statistical differences between LC_{50}'s were tested using the methods outlined by APHA (1980).

Table 1. Experimental treatments used for assessing the effects of pH and calcium on the toxicity of aluminium to *Oreochromis aureus* (brackets indicate the mean and range measured during the experiments.

pH	Total hardness (mg/l as $CaCO_3$)	Calcium concentration (mg/l)	Dissolved aluminium concentration (mg/l)
4.0 (4.03) (3.79-4.21)	2.0 (2.2) (1.6-2.6)	0.6 (0.7) (0.5-0.8)	0.0 (0.0) (0.0-0.0)
5.0 (4.97) (4.79-5.12)	20.0 (20.4) (20.2-21.7)	6.4 (6.5) (6.4-6.9)	0.3 (0.31) (0.29-0.32)
6.0 (6.05) (5.87-6.30)			0.6 (0.6) (0.55-0.62)
			0.8 (0.8) (0.76-0.82)
			1.0 (1.01) (0.97-1.09)
			1.5 (1.49) (1.43-1.55)
			2.0 (1.98) (1.24-2.01)
			2.5 (2.48) (2.44-2.51)

Results

Aluminium was found to be toxic to *Oreochromis aureus*, although toxicity was significantly modified by the pH and calcium concentration (Fig. 1; Table 2). The toxicity of aluminium increased with time, and the asymptotic lethal threshold was not reached over the 96-hour period in any of the trials. Aluminium was significantly ($P < 0.05$) more toxic to juveniles at pH 5 than at pH 4 and pH 6 in both calcium concentrations and was least toxic at pH 6 in 6.4 mg/l calcium. The effect of calcium was most marked in the first hours of the trials when there were significantly ($P < 0.05$) lower median lethal concentrations in the low hardness waters. The difference between the LC_{50}'s in longer trials was only significant at 72 and 96 hours at pH 4 and after 96 hours in pH 5, suggesting that the effects of calcium on aluminium toxicity were most marked in more acidic media.

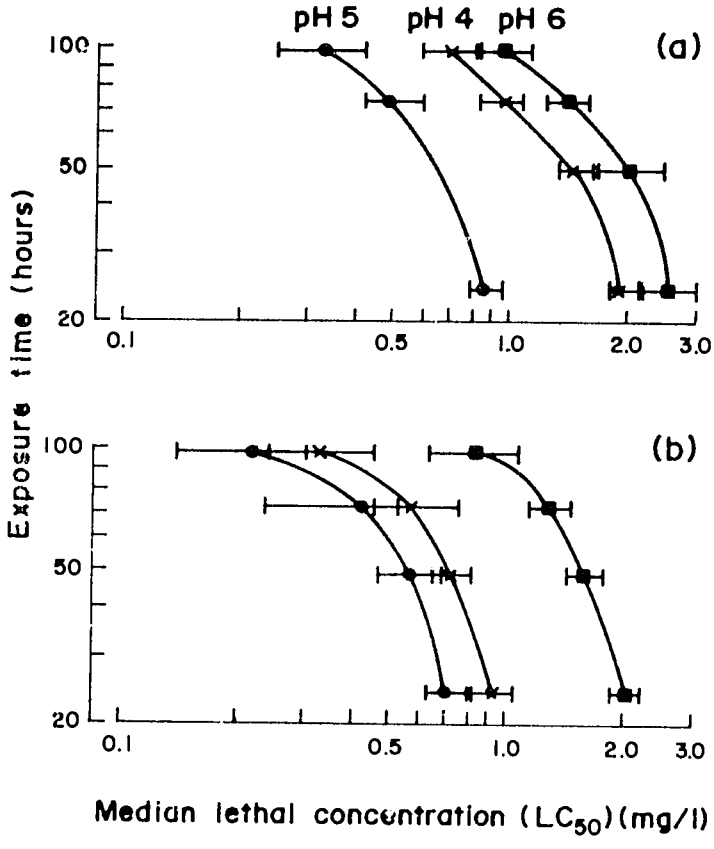

Fig. 1. Toxicity of dissolved aluminium to *Oreochromis aureus* at different pH and calcium concentrations. (a) calcium = 6.4 mg/l; (b) calcium = 0.6 mg/l. Bars indicate 95% confidence limits.

Table 2. Aluminium toxicity to *Oreochromis aureus* in relation to pH, calcium concentration and exposure time.

pH	Calcium (mg/l)	Median lethal concentration (mg/l measured dissolved Al) 24 hours	48 hours	72 hours	96 hours
6.0	6.4	2.54 (2.17-2.99)	2.02 (1.65-2.47)	1.41 (1.23-1.59)	0.97 (0.83-1.13)
	0.6	2.00 (1.84-2.17)	1.58 (1.42-1.74)	1.28 (1.13-1.45)	0.82 (0.63-1.07)
5.0	6.4	0.87 (0.79-0.96)	no data	0.50 (0.42-0.59)	0.33 (0.25-0.42)
	0.6	0.70 (0.62-0.78)	0.56 (0.46-0.68)	0.34 (0.23-0.52)	0.22 (0.14-0.30)
4.0	6.4	1.93 (1.82-2.14)	1.48 (1.33-1.65)	0.96 (0.86-1.07)	0.70 (0.59-0.82)
	0.6	0.92 (0.81-1.02)	0.72 (0.64-0.81)	0.58 (0.45-0.75)	0.33 (0.24-0.45)

There were some noticeable changes in fish behavior during the experiment. There was a rapid loss of the normal striated coloration and increase in the ventilation rate of fish in acutely toxic concentrations, followed by a gradual loss of color and excessive mucus production and coughing. The final acute responses were frenzied activity, followed by death. These behavioral patterns are characteristic of fish exposed to acutely toxic acids (Alabaster and Lloyd 1982).

Discussion

The results of these experiments clearly show that dissolved aluminium is toxic to *Oreochromis aureus* at concentrations that can be found in tropical freshwater (Fig. 2), suggesting that there is a risk to tilapias in such environments, dependent on pH and calcium concentration.

The aluminium concentrations that are toxic to *O. aureus* fall within the range reported as being toxic to other fish species (Table 3), although such comparisons are difficult because of differences in experimental procedures and test water quality.

The results of this study also show that *Oreochromis aureus* is tolerant to pH 4 for at least 96 hours, close to the limits for other tilapias.

Calcium has also been found to ameliorate aluminium toxicity in experiments with salmonids (Muniz and Leivestad 1980; Brown 1983) and Brown (1983) has recommended a minimum calcium concentration of 2.0 mg/l for protection of fish from aluminium toxicity. The exact mechanism of this protective effect has not been clarified, although calcium may limit toxicity by controlling gill permeability (McWilliams 1983; Marshall 1985).

pH has also been shown to significantly affect aluminium toxicity. Muniz and Leivestad (1980) found that alu-

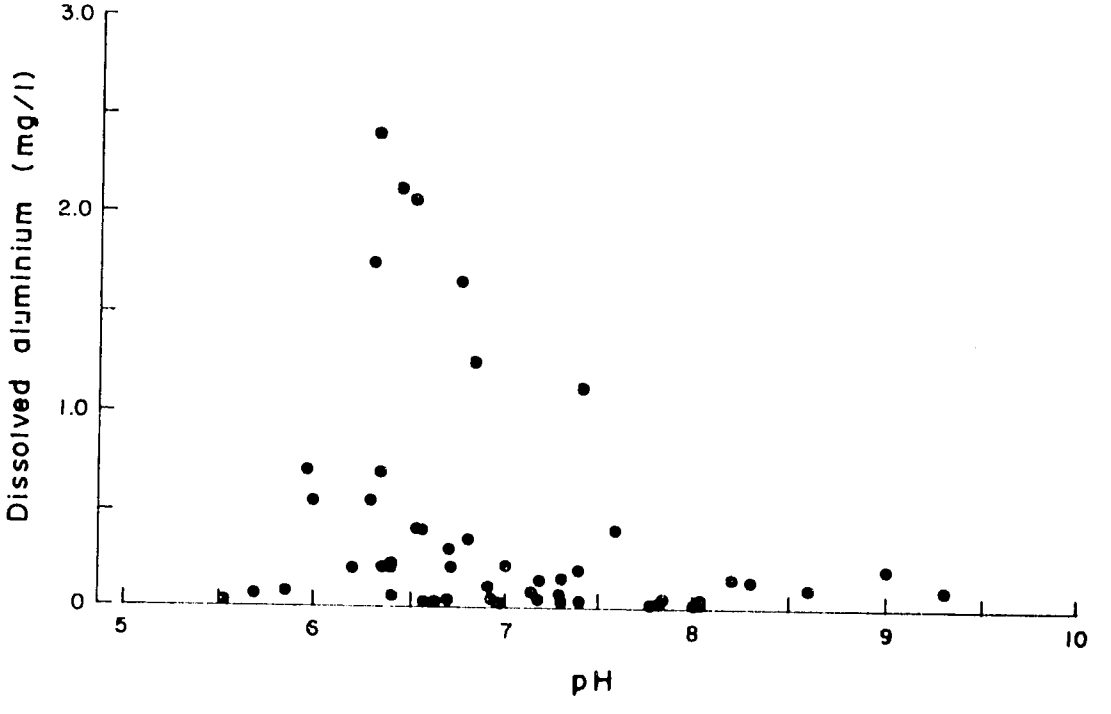

Fig. 2. Dissolved aluminium concentrations relative to pH in a wide range of freshwaters in Southeast Asia.

Table 3. A summary of selected references on the toxicity of aluminium to fish in acid waters.

Aluminium (mg/l)	Calcium (mg/l)	Fish species, notes and reference
0-0.50	2.2	*Salvelinus fontinalis* and *Catostomus commersoni* postlarvae mortality increased at 0.1 mg/l and 0.2 mg/l, respectively (Baker and Scholfield 1980) (pH 4.2-5.6)
0.2		*S. fontinalis* mortality during acid pulse in field (pH 5) (Cronan and Scholfield 1979)
0.19		pH 5.0; fatal to *Salmo trutta*
0.38		pH 4.0; sublethal effect on *S. trutta*
0.90		pH 6.0; no toxic effect on *S. trutta* (Muniz and Leivestad 1980)
0.5		pH 5.2; most toxic to *S. fontinalis* fingerlings (50% survival after 1.6 days) (Schofield and Trojnar 1980)
0.22-0.45	3.4-5.5	*Salmo trutta* > 50% mortality of juvenile fish in acid streams in 17 days (Stoner et al. 1984)
0.374	2.0	*Salmo salar* LC_{50} (14 g fish) (pH 5.2)
0.183	2.0	*Salmo gairdneri* LC_{50} (57 g fish) (pH 5.2) (unpublished)
0.13	4.0	*Notropus cornutus* mortality greater than 90% at pH 5.0 (Kramer et al. 1986)
0.075	1.3	*Salmo salar* smolts (38 g); 50% mortality in 108 hours (Skogheim and Rosseland 1986)

minium was most toxic to brown trout at pH 5.0 and less so at pH 4.0 and pH 6.0. Driscoll et al. (1980) and Scholfield and Trojnar (1980) also found that aluminium was most toxic to brook trout at pH 5.0 than in more acid media, where aluminium may offer some protection against low pH (Baker and Scholfield 1980). pH affects both the solubility and speciation of aluminium in water and it is probable that the, as yet unidentified, toxic forms are most abundant at pH 5.0-5.2 (Odonnell et al. 1984). This is the reason why many acid waters are more toxic to fish than would appear from a consideration of pH alone.

The main site of aluminium toxicity in fish is thought to be the gills (Karlsson-Norrgren et al. 1986). Structural gill damage, excessive mucus production and resulting anoxia, impaired ion exchange and loss of body salts have all been implicated in mortalities (Odonnell et al. 1984) and are diagnostic of pH and aluminium toxicity. Lamellar hyperplasia, fusion, necrosis and inflammation were also found in *O. aureus* in the present experiments (Saleh and Phillips, unpublished data).

It is difficult to recommend a "safe" level of dissolved aluminium for tilapia from the present results, because a lethal threshold was not reached and because of the likely variation in tolerance between different species. The tolerance may also depend on prior exposure. Salmonids can

become acclimated to high aluminium concentrations (Orr et al. 1986). It has been recommended that 0.5 mg/l is a safe aluminium concentration in fishponds over acid sulfate soils (Singh 1985), but the present study experiments show that this concentration could result in mortalities of *O. aureus* in certain water quality conditions. Odonnell et al. (1984) showed that dissolved aluminium could be acutely toxic to a range of fish species at 0.1 mg/l between pH 4.0 and 5.2. EPA (1986) has also recommended that, between pH 6.5 and 9.0, the 4-day average concentration of dissolved reactive aluminium should not exceed 0.15 mg/l and the 1-hour average concentration should not exceed 0.95 mg/l more than once every 3 years. We recommend, therefore, that the present results be used as a possible indication of acute toxicity in relation to pH and calcium concentration, but that future work should aim to quantify the long-term effects, particularly, if possible, under field conditions, and that attention should also be paid to the possible amelioratory role of organic compounds and silica, both of which may reduce the toxicity of aluminium (Odonnell et al. 1984; Birchall and Espie 1986).

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A Comparison of the Quality of Hatchery-Reared *Oreochromis niloticus* and *O. mossambicus* Fry

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RANA, K.J. and D.J. MACINTOSH. 1988. A comparison of the quality of hatchery-reared *Oreochromis niloticus* and *O. mossambicus* fry, p. 497-502. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture ICLARM Conference Proceedings 15, 623 p. Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines.

Abstract

A comparison was made between growth, feeding capability and survival of *Oreochromis niloticus* and *O. mossambicus* fry. Three age-classes of hatchery-reared females were used as broodstock and their eggs and fry reared in an artificial incubation system consisting of 0.7-l containers.

The ages of fish from the 0+, 1+ and 2+ year-classes were 8-10, 12-14 and 23-25 months for *O. niloticus*, and 9-10, 13-14 and 24-25 months for *O. mossambicus*. Mean egg sizes of *O. niloticus* females were significantly ($P < 0.05$) larger than those from *O. mossambicus* females of a similar age. The dry weights of *O. niloticus* eggs from 0+, 1+ and 2+ females averaged 1.70 ± 0.14 (X ± S.E.), 2.91 ± 0.09 and 3.74 ± 0.09 mg, respectively, compared with 1.01 ± 0.04, 1.52 ± 0.04 and 1.87 ± 0.06 mg for eggs from *O. mossambicus* females of equivalent age-classes.

The times to mass hatching were neither species-specific nor egg-size dependent. Mean body length and weight of fry developing solely on their yolk reserves were significantly ($P < 0.05$) higher for *O. niloticus* when fry from corresponding age-class females were compared. The maximum dry body weights of *O. niloticus* fry from 0+, 1+ and 2+ females averaged 1.00 ± 0.12 (X ± SD), 1.81 ± 0.22 and 2.50 ± 0.08 mg, respectively, compared with 0.36 ± 0.06, 0.58 ± 0.05 and 0.78 ± 0.10 mg for *O. mossambicus* fry.

Onset of feeding of developing fry was not species-specific and feeding commenced within 5-6 days of hatching at 28°C. Overall *O. mossambicus* fry were more resistant to starvation. Survival times (ST_{50}) of unfed developing fry from 0+, 1+ and 2+ *O. niloticus* females were 13.5, 16 and 17.5 days after hatching, respectively, compared to 15, 16 and 19 days for *O. mossambicus*.

The initial advantages of larger egg size persisted through to 60 days posthatching in both species. However, *O. niloticus* fry at this age were significantly ($P < 0.05$) heavier than *O. mossambicus* fry.

Introduction

It is now generally accepted that tilapias can make a significant contribution to aquaculture, but a current problem facing the tilapia farmer is the choice of species for culture. Schoenen (1982) mentions eight tilapia species with aquaculture potential: *Oreochromis niloticus* (L.), *O. mossambicus* (Peters), *O. aureus* (Stein-

dachner), *O. urolepis hornorum* (Trewavas), *O. macrochir* (Boulenger), *Sarotherodon galilaeus* (Hasselquist), *Tilapia niloticus* and *O. mossambicus* are now widely cultured (Caulton 1979; Pullin 1983).

Species may be chosen on the basis of their biological or economic traits (Pullin 1983). Evaluation of these traits between species, however, is usually made difficult because of many other variables operating that affect the performance of the species, such as management, nutrition and environmental conditions. Thus, one neglected area of study in tilapia hatchery research has been the question of how different biological traits in broodstock affect early fry quality. An obvious starting point is to compare fry quality resulting from broodfish of different genotype or age/size.

A trial was conducted to compare the performance of *O. niloticus* and *O. mossambicus* fry from known ages of broodstock under controlled hatchery conditions identical for both species. Fry performance was evaluated by monitoring their mean egg size, growth and survival on yolk reserves and growth and survival of fry fed for 60 days.

Materials and Methods

Egg supply and incubation

Three age-classes (0+, 1+ and 2+) of *O. niloticus* and *O. mossambicus* females from a heterogeneous gene-pool were reared under similar conditions in 1-m² tanks within a common recirculatory system. Broodstock were hand-sexed and males were discarded to avoid future sib-matings. Females representing each age-class were selected randomly and tagged. They were then stocked in 2-m² diameter circular fiberglass spawning tanks at a sex ratio of 3 females: 1 male and fed a commercial diet containing 40% protein at a rate of 2% body weight/day. Broodstock were allowed to spawn naturally except when small 0+ females were used. Broodstock sizes are given in Table 1. These were stripped manually and artificially

Table 1. Mean growth traits of *Oreochromis niloticus* and *O. mossambicus* fry from three age-classes of females. Values shown are means, with standard deviation in parenthesis, unless otherwise stated.

Trait	Age of maternal parent (months)[1,2]					
	O. niloticus			*O. mossambicus*		
Year class	0+	1+	2+	0+	1+	2+
Range in age (months)	(8-10)	(12-14)	(23-25)	(9-10)	(13-14)	(24-25)
Broodstock size:						
Mean weight (g) (range)	53.2 (31-90)	192 (160-220)	316.2 (218-486)	47.9 (25-110)	126.9 (107-151)	220.9 (168-290)
Mean length (cm) (range)	11.8 (9.5-14.6)	17.6 (16.7-18.8)	21.9 (18.4-23.7)	11.3 (9.2-13.6)	15.5 (14.1-16.6)	18.9 (17.0-20.2)
Unfed fry[3]						
Egg size (mg)	1.70a1 (0.14)	2.91b1 (0.69)	3.74c1 (0.09)	1.01a2 (0.04)	1.52b2 (0.05)	1.87c2 (0.06)
Survival time ST_{50} - days after hatching	13.5 (1.2)	16 (1.4)	17.5 (1.2)	15 (1.0)	16 (0.8)	19 (2.0)
Maximum growth:						
Dry body weight (mg)	1.00a1 (0.12)	1.81b1 (0.22)	2.50c1 (0.08)	0.36a2 (0.06)	0.58b2 (0.05)	0.78c2 (0.10)
Standard length (mm)	6.6a1 (0.44)	7.9b1 (0.10)	8.5c1 (0.12)	5.3a2 (0.05)	6.0b2 (0.16)	6.7c2 (0.13)
Fed fry[4]						
Mean egg size (mg)	1.93 (0.16)	2.79 (0.06)	3.68 (0.13)	1.30 (0.06)	1.70 (0.09)	2.43 (0.10)
Weight at 60 days (g)	1.96a1 (0.24)	2.50b1 (0.32)	2.98c1 (0.16)	1.02a2 (0.04)	1.52b2 (0.06)	1.98c2 (0.06)

[1]Different letters within rows and species denote significant difference at 5% level.
[2]Different numbers within columns and between species of the same age-class are significantly different at the 5% level.
[3]Fry developing solely on their yolk reserves (n = 5).
[4]Means of each age-class based on fry from four individual females.

fertilized with milt from conspecific males. Egg batches were obtained from at least four individual females from each age-class. The eggs were collected from the buccal cavity of the females within 12 hours of spawning and incubated artificially in round-bottomed containers (Rana 1985). In addition, a random sample of 50 eggs was removed from each batch, dried on absorbent paper, oven dried at 50°C and weighed (± 0.1 mg).

Trial 1. Survival, growth and delayed feeding ability of unfed fry

The three-day old hatchlings from each batch were transferred from the incubators into 2-l glass containers held in a covered water bath maintained at 27-28°C. To maintain water quality, at least half the water in each container was replaced every 2 days with preheated filtered water; aeration was used to provide water circulation. These fry, which were not fed, served as a 'supply' for the studies described below.

To determine the onset of feeding, a sample of 20 fry was removed from the supply container and transferred to a 500-ml beaker. They were then fed finely ground (< 300 µm) broodstock diet dyed with Carmosine, E122. After 2-3 hours the number of fry with dyed food in the guts were counted; onset of feeding was recorded as the time when 50% of fry were able to ingest food.

For survival studies, 30 randomly sampled 6-day old fry were transferred from the supply into 2-l glass containers holding aerated filtered water. Mortalities were monitored daily. The time to 50% survival (ST_{50}) was determined for each batch.

To estimate the capacity of yolk reserves to support fry growth, 20 fry from the supply containers were randomly sampled at 3-day intervals from hatching and their dry body weights determined after removal of the yolk-sac (if present).

Trial 2. Growth of fed fry

To compare the early growth performance of the two species, 5-day old yolk-sac fry were stocked at 2/l in 20-l plastic tanks linked to a recirculatory system. The fry were fed in excess, 6 times a day on a commercial diet containing 50% protein (No. 3, Edward Baker, Bathgate, Scotland). Aeration of the water in the header tanks ensured a minimal oxygen level of 4.5 mg/l. The tanks were kept clean by daily siphoning of uneaten food.

The fry were sampled at 20, 40 and 60 days posthatching to determine their mean weights. Prior to weighing the fry were starved for 12 hours. All the fry were then carefully removed with a hand net and after removal of surface moisture on absorbent paper, weighed on a tared balance.

Statistical analysis

Interspecific differences in growth and survival were computed using the Students 't' test (Sokal and Rohlf 1969). Intraspecific comparison of egg size and growth was carried out by one-way ANOVA technique using a computerized statistical package (Minitab, Pennsylvania State University) and Duncan's Multiple Range Test (Duncan 1955).

Results and Discussion

The mean size of *O. niloticus* and *O. mossambicus* eggs used in Trial 1 shown in Fig. 1 and Table 1 suggest species-specific differences. For each age-class *O. niloticus* produced significantly ($P < 0.05$) larger eggs than *O. mossambicus*. In *O. niloticus* egg size was significantly ($P < 0.05$) different between all age-classes, the older females producing the largest eggs. Furthermore, in contrast to total batch weight, there was no significant ($P > 0.05$) correlation between female size and mean egg size for females within the same age-class. In *O. mossambicus*, however, only

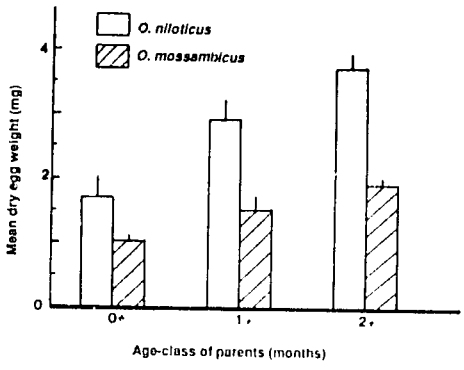

Fig. 1. Comparison of mean egg size within and between three age-classes of *Oreochromis niloticus* and *O. mossambicus* broodstock. Means and S.D.

eggs from 0+ females were significantly ($P < 0.05$) smaller than those of older fish. Comparable results for *O. mossambicus*, where age structure is well defined, are not available but those of *O. niloticus* are in close agreement with those of Siraj et al. (1983).

These differences in egg quality between age-classes and between species were also reflected in the quality of fry.

The time to onset of feeding was not species-specific. In both species initial exogenous feeding commenced at 5-6 days posthatching at 28°C and in all cases, the proportion of fry feeding in a sample increased with time. In both species, however, the maximum mean proportion of feeding fry was lower ($P < 0.05$) for fry from 0+ females compared to fry from 1+ and 2+ females (Fig. 2).

The mean survival times, ST_{50} (time to 50% survival) of *O. niloticus* and *O. mossambicus* fry developing solely on their yolk reserves are shown in Fig. 3 and Table 1. Within each species the larger yolk reserves of fry from older females helped them to tolerate starvation for a longer period. Even though *O. niloticus* produced larger eggs, the survival times for *O. niloticus* fry were lower than those for *O. mossambicus* fry from 0+ and 2+ females. The reasons for these differences are unclear, but two possibilities are suggested:

(1) Although every effort was made to remove dead fry from the containers as soon as they were noticed, some carcasses may have been exploited for food by *O. mossambicus* fry.

(2) Casual observations during these trials indicated that *O. niloticus* fry were more active than *O. mossambicus* fry especially after the period of onset of feeding.

Fig. 2. The maximum proportion of *Oreochromis niloticus* and *O. mossambicus* fry in a sample that were capable of exogenous feeding. Means and S.D.

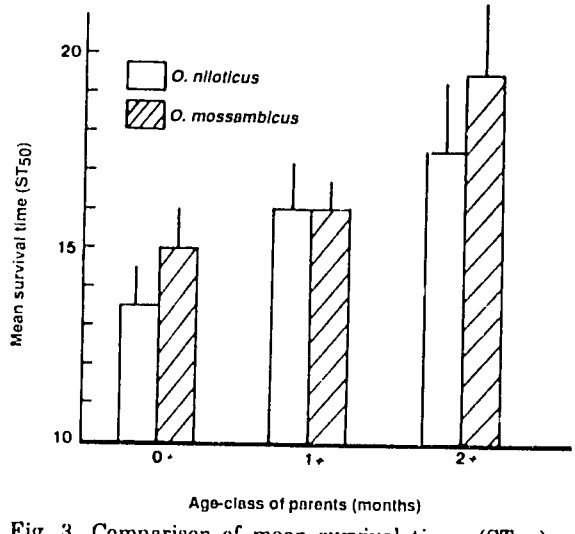

Fig. 3. Comparison of mean survival times (ST_{50}) between *Oreochromis niloticus* and *O. mossambicus* unfed fry from 3 age-classes of broodstock. Survival times are for unfed fry developing solely on their yold reserves. Means and S.D.

Whilst it is appreciated that under ideal conditions in green water spawning systems the survival times may not be critical, the results do show the susceptibility of tilapia fry to starvation during mouth brooding. The duration of mouth brooding to the time of initial fry release varies between females and also between spawnings of the same individual (Rana 1986). In addition, observations in 2-m^2 diameter spawning tanks suggest that initial fry release depends on the ability of the brooder to secure and defend a territory (Rana 1985) Therefore, under suboptimal conditions, e.g., crowding, the time to initial fry release may be delayed and thus fry survival limits may be approached.

In both species the larger eggs from older females resulted in larger fry. The mean maximum growth traits of fry developing solely on their yolk reserves are shown in Fig. 4 and Table 1. In both species older females produced significantly heavier ($P < 0.05$) and longer ($P < 0.05$) fry. These differences were more clearly seen when weight is considered. For example, *O. niloticus* fry from 2+ females were 150% heavier than those from 0+ females. In *O. mossambicus* this difference was about 100%. For all three age-classes, however, *O. niloticus* females produced significantly heavier ($P < 0.05$) and longer ($P < 0.05$) fry than *O. mossambicus*. The observation that longer and heavier fry result from bigger eggs is well documented for many fish species (Blaxter and Hempel 1963; Bagenal 1969; Pitman 1979; Thorpe et al. 1984; Rombough 1985).

In these trials the initial advantages of larger eggs on growth persisted through to the end of the 60-day trial period, by which time the fry had reached 1-3 g average weight (Fig. 5, Table 1). The final weights of *O. niloticus* fry were significantly higher ($P < 0.05$) than those of *O. mossambicus* for fry from parents of each age-class. In contrast to these results, Siraj et al. (1983) reported that the initial advantages of larger eggs from older *O. niloticus* brooders became obscured after the first 20 days.

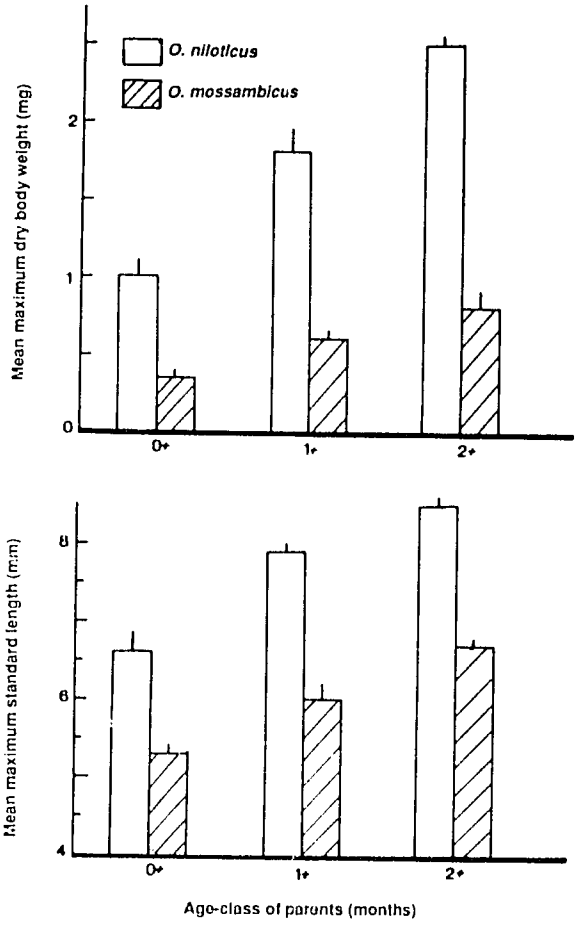

Fig. 4. Comparison of maximum weight and length of *Oreochromis niloticus* and *O. mossambicus* fry from three classes of females. Means, and S.D.

In the past the interest of scientists and farmers alike has focused mainly on the effect of broodstock size on fecundity. The results of the research reported here show that maternal age as well as genetic differences between species are also important factors affecting fry quality because of their influence on egg size. Overall, the biological traits reported here suggest that *O. niloticus* fry are of superior quality when compared to *O. mossambicus*. Trials by Majumdar (1984) who reared tilapias up to a size of 30 g under controlled hatchery conditions have confirmed the superior performance of *O. niloticus* over *O. mossambicus*.

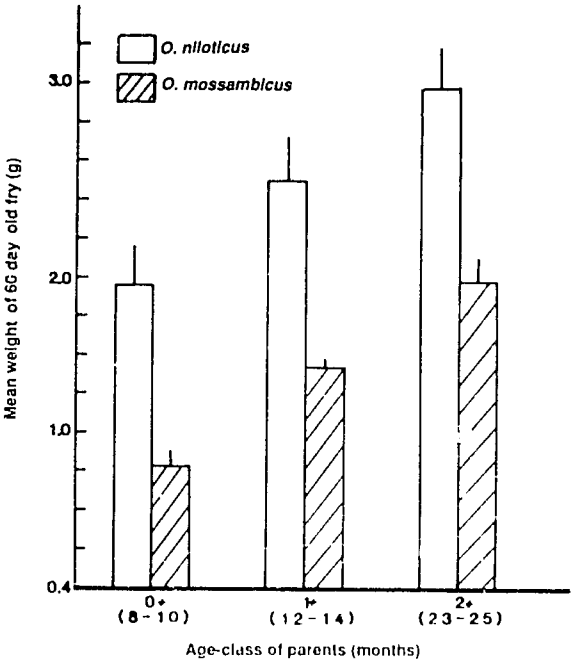

Fig. 5. Comparison of growth between 60-day old *Oreochromis niloticus* and *O. mossambicus* fry originating from 3 age-classes of broodstock females. Means, and S.D.

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Behavior Phenotypes of Juvenile Tilapia and Their Correlations with Individual Growth Rates*

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Abstract

Quantitative observations were made on interactive behavior and growth in juvenile *O. mossambicus/O. urolepis hornorum* hybrids. Test fish were observed first in the presence of smaller, then of larger fish. This period was followed by a grow-out phase in the presence of equal-sized fish. A second observation period identical to the first completed the experiment.

Nine behavioral traits were measured for each test fish from video recordings during competitive feeding trials. Two traits (latency and the number of feeding bites) are reported on in this paper. ANOVA was used to test for the significance of relative size, time of day and other environmental parameters in explaining the observed behavior. The behavior traits are correlated with each other and with size-specific growth rate. Individual fish differed in their sensitivity to larger competitors and the effect of competition on growth. The usefulness of artificial selection using behavioral criteria to indirectly select for improved growth is addressed.

Introduction

A principal concern of fish geneticists is whether size-selection will improve the growth rate of cultured fish. If this process indirectly selects for increased competitive ability (i.e., if growth is highly correlated with competitive success), then the mean competitive ability of all conspecifics will increase and no net improvement in growth rate will be realized (Kinghorn 1983). Doyle and Talbot (1986) have argued that this will not occur under laboratory or aquaculture conditions managed to minimize competitive interaction. A well-managed system will select for decreasing competitive interaction and improved growth rates, if maximum growth is achieved by fish that minimize energy expended in competition.

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A survey of the literature provides evidence that fish displaying the highest levels of aggressive or competitive interaction do not always grow as fast as their less dominant contemporaries in experimental or aquaculture environments (Newman 1956; Jenkins 1969; Yamagishi et al. 1974; Fernet and Smith 1976; Li and Brockson 1977; Gibbard et al. 1979; Wise 1983; Nelissen 1985; Jobling and Reinsnes 1986; Metcalfe 1986). The link between growth and behavior under culture conditions clearly warrants further examination.

This research had two objectives. Firstly, to examine the nature of the competitive behavior expressed by a lab population of juvenile tilapia particularly in relation to individual relative size, and secondly, to determine how such behavior correlates with individual growth rate.

Materials and Methods

The origin of the *O. mossambicus*/*O. urolepis hornorum* hybrid used is described by Doyle and Talbot (this vol.). Video recordings of 32 competitive feeding trials were made of 8 observed test fish and 8 unobserved companion fish over a 52-day experiment (replicated twice). Competitive feeding was encouraged by suspending a single large food pellet on a wire just below the surface at one top corner of the 60 litre aquarium. The experiment was divided into two 16-day observation periods separated by a 20-day grow-out period. In each observation period, the group of test fish was placed first with a group of smaller, then with a group of larger companion fish for 8 days in each size class. During this period, 16 competitive feeding trials were video-recorded; 8 trials with each size class following 4 days of acclimation. The 10 minute feeding trials were recorded both in the morning and afternoon. Following each such trial the fish were fed non-competitively and *ad libitum*. Weight measurements were taken on all fish at the beginning and end of each size class phase of the experiment.

The two behavioral characters discussed here were measured on each test fish at every feeding trial: The number of BITES at the food, and LATENCY or the elapsed time from the presentation of the food pellet to first successful bite.

Two preliminary analyses have been performed to date. The first uses a linear model to test for the importance of the experimental parameters: feeding trial, observation period, recording time (AM/PM), relative size of test fish, fish number and interaction effects of these last two (Inter.), in explaining the variance in each transformed behavior character (Behav.). The model is

$$1)\quad \text{Behav.} = C + \text{Trial} + \text{Period} + \text{AM/PM} + \text{Size} + \text{Fish} + \text{Inter.} + E$$

where, C is a constant and E is the error.

The second analysis tests the significance of a linear combination of the transformed behavior characters at explaining the observed size-specific growth rates of each fish:

$$2)\quad \text{GROWTH} = C + \text{BITES} + \text{LATENCY} + E$$

where, GROWTH = ln [(weight at time T) / (weight T-1)].

This model was evaluated separately for observations on the test fish when they were relatively larger and smaller than the companion fish.

The two traits were Poisson distributed, with highest frequencies at or near zero. Because of this, 0.5 was added to each observation allowing logarithmic transformations. Plots of residuals against fitted values for each model revealed these transformations to be acceptable.

Results

The ANOVA results of the experimental parameters on the transformed behavior characters (model #1) are given in Table 1. The R^2 for BITES and LATENCY are respectively .558 and .459.

Table 1. ANOVA tables of the experimental parameters in explaining the observed behavior traits.

Behavior	Source of variation	df	ss	Fs
Bites	Feeding trial	1	4.9	2.3
	Observation period	1	768.8	362.2**
	AM or PM recording	1	114.3	53.9**
	Relative size	1	1.2	.57
	Individual fish	15	150.7	4.7**
	(Size) x (individual)	15	86.1	2.7*
	Error	477	1,012.3	
	Total	511	2,138.3	
Latency	Feeding trial	1	55.7	33.4**
	Observation period	1	324.1	193.9**
	AM or PM recording	1	79.9	47.8**
	Relative size	1	43.5	26.1**
	Individual fish	15	67.2	2.7*
	(Size) x (individual)	15	64.0	2.6*
	Error	477	797.3	
	Total	511	1,431.7	

*P-value $< .001$
**P-value $<< .001$

BITES and LATENCY are negatively correlated with each other both when the fish were smaller ($r = -.755$) and larger ($r = -.708$) than the other fish with which they were competing. The direct relationship (model #2) between BITES and growth is best represented by the standardized coefficients of .188 (when smaller; $P < .044$) and .407 (larger; $P << .001$). For LATENCY and growth these coefficients are -.071 (when smaller/ $P < .448$) and -.220 (larger; $P < .003$). Fig. 1 is a path model (Li 1975) of this causal system. The variance/covariance matrix is given in Table 2 together with the Pearson correlations.

Discussion

Table 1 shows that feeding trial, first or last observation period, and time of day are statisticaly significant in explaining both traits. Relative size, although not significant in explaining the number of BITES, was significant for LATENCY.

The direct relationships between BITES and growth, and LATENCY and growth were strongest when the fish were relatively large than their competitors as seen in the path diagram (Fig. 1). However, both characters are indirectly (through the other) as well as directly related to growth. The overall Pearson correlation between a trait and growth takes this into account as the sum of the two pathways (the indirect pathway's strength being the product of its steps).

This net relationship between feeding behavior and growth became more significant with relative size as well. The Pearson correlations presented in the lower triangle of Table 2 between each behavior trait and growth for relatively larger fish are twice those of the smaller fish.

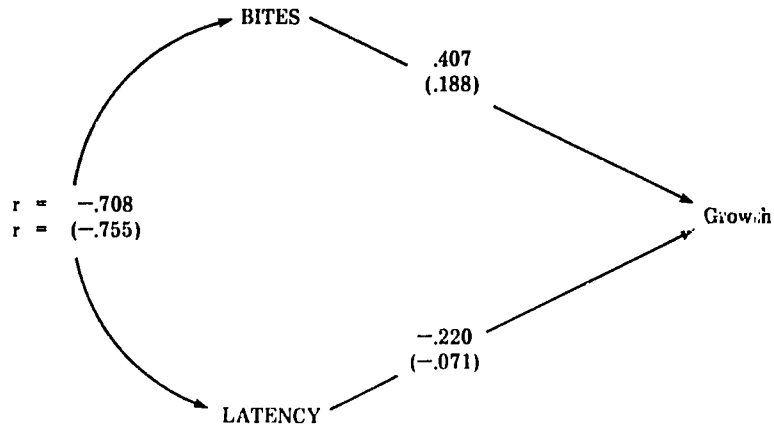

Fig. 1. Hypothetical model of correlated causes between BITES and LATENCY and their relationships to growth described by standardized regression coefficients (unbracketed and bracketed values correspond to when the test fish were larger and smaller, respectively).

Table 2. Variance/covariance matrix for the two traits and growth. The upper and lower triangle values excluding the diagonal are covariance and Pearson correlation estimates respectively (unbracketed and bracketed values correspond to when the test fish were larger and smaller respectively).

	Growth		Bites		Latency	
Growth	(.0013)	.0064	(.0197)	.0865	(−.0144)	−.0600
Bites	(.241)*	.563*	(5.144)	3.690	(−3.240)	−2.014
Latency	(−.212)*	−.508*	(−.755)*	−.708*	(3.580)	2.193

*P-value << .01

The individual fish and individual by relative size interaction terms were both significant in the ANOVA. This indicates that the presence of relatively larger or smaller competitors did not have the same effect on the feeding behavior of each fish. Some individuals were more sensitive than others, and the affected behavior was related to growth. The objective of this study was to determine which behavior phenotypes might be optimal under competitive and non-competitive feeding regimes. Our preliminary results suggest that those fish whose growth-related behavior is least affected by their competitors will probably achieve better growth rates than other more severely affected fish.

High correlations between behavior and growth (Brett 1979, Villegas and Doyle 1986) indicate the possibility of good response to selection on behavior traits (as suggested by Purdom 1979; Doyle and Talbot 1986).

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"Current Growth" Estimators in Tilapia*

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Abstract

The exact age of individual tilapia is often unknown in aquaculture systems, which makes it very difficult to estimate growth rates. Yet information about relative growth rates is essential in selection programs. The difficulty can be avoided if a technique can be found to estimate growth rate when the age of the fish is not known. We have investigated experimentally 3 methods of estimating individual growth rates from scales of an *Oreochromis mossambicus/O. urolepis hornorum* hybrid tilapia (black and red morphs) reared in aquaria. These methods are (1) [14C]-glycine uptake *in vitro* (GLY), (2) staining of the calcified portion of the scale to estimate the amount of uncalcified tissue (STAIN), and (3) measurements of the distance between circuli on the outer anterior field of the scale (CIRC). The linear correlations between change in size and the growth estimators are all highly significant (0.82, 0.67 and 0.75 for GLY, STAIN and CIRC, respectively). The usefulness of these techniques for genetic, nutritional and other studies requiring information on growth rates in "non-research" aquaculture environments is discussed.

Introduction

Fish growth estimators are useful in situations where mark-recapture is difficult or impossible, and where the studied population consists of mixed age classes, thus making size-at-age impossible to determine. Several growth estimators have been described in the literature. Otoliths have recognizable daily rings (see Campana and Neilson 1985) which have been used to estimate growth (e.g., Taubert and Coble 1977). RNA/DNA ratios are a good predictor of growth rate (Bulow 1970) and have been used to relate growth to other variables such as prey density (Buckley 1979). The liver somatic index (Adams and McLean 1985), as well as enzymatic activity (Smith and Chong 1982) have also been used with success. However, all these techiques are destructive. This disadvantage is critical in some practical applications, especially selective breeding.

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This paper compares three methods of estimating growth rates in tilapia from scales: 1) *in vitro* [^{14}C]-glycine uptake, 2) the amount of cartilaginous (uncalcified tissue) on the anterior edge of the scale as estimated by specific stains and 3) the spacing of bony ridges (circuli) on the anterior edge of the scale The species used was a mixture of black and red morphs of tilapia, probably a hybrid of *Oreochromis mossambicus* and *O. urolepis hornorum* (Behrends et al. 1982). These fish originated from a supplier in Florida. Various generations were used in these experiments but always from spawnings in our laboratories.

Methods

Glycine

The incorporation of [^{14}C]-glycine into scales *in vitro* has been used first to estimate growth by Ottaway and Simkiss (1977). It operates on the principle that uptake is related to the metabolic activity of the scale, which is related to its own growth rate, and thus to the growth rate of the fish.

We used Adelman's (1980) technique. A live scale was removed from a specific area on all fish, immersed immediately in a [^{14}C]-glycine/saline solution for 2 hours, and the amount of glycine incorporated into the scale was recorded. The following recipe for fish saline was used: 7.014 g l^{-1} NaCl, 0.200 g l^{-1} KCl, 0.222 g l^{-1} $CaCl_2H_2O$, 1.260 g l^{-1} $NaHCO_3$, 6.505 g l^{-1} HEPES (sodium salt; Product H-7006, Sigma Chemical Co., St. Louis, Missouri, USA) and enough HCl to bring the solution to pH 7.5.

Two hundred µl of this solution was dispensed into a 0.5 ml plastic vial in which [^{14}C]-glycine was added to make a final dilution of 1 µC ml^{-1} activity. One scale was placed in each vial and vials were incubated in a water bath at 27°C. The dry weight of each scale was measured to the nearest 10^{-5} g, digested in Protosol (New England Nuclear, Boston, Massachusetts), suspended in scintillation fluid (Aquasol, NEN, Boston, Massachusetts) and the activity measured using a scintillation counter. Five scales per fish were counted individually. The average uptake per fish was related to change in length and weight over a 1 month period.

Staining

As scales grow, pre-osseoid tissue is laid down on the outer margin, and is gradually calcified (Neave 1940; Sire and Géraudie 1983). The distance between the edge of the scale and the calcification 'front' is proportional to the rate at which the scale is growing. We used a staining procedure adapted from Hanken and Wassersug (1981). Formalin-fixed scales were thoroughly washed in water to remove all traces of fixative, then immersed for 2-3 hours in a solution of 1 g Alcian Blue 8GX, 80 ml 95% ethyl alcohol and 20 ml glacial acetic acid. (This step is required to stain collagen uncalcified tissue). After a series of graded alcohol/water washes, terminating with 100% water to remove all traces of acetic acid, scales were immersed for 24 hours in a solution of 0.5% aqueous KOH with enough Alizarin Red S to turn the water a deep purple. Scales were then rinsed and wet-mounted on microscope slides, and the distance between the edge of the red (calcium) stained tissue and the edge of the scale was measured in two locations on 4 scales per fish. All measurements were on the anterior (insertion into the body) end of the scale at 100x magnification. Measurements were averaged within fish. These data were related to change in weight and length over 25 days.

Circuli spacing (CIRC)

This method is based on the well-known fact that spacing between bony ridges (circuli) on scales of temperate fishes is less during periods of slow growth (winter) than during rapid growth (summer) (Bugaev 1984). Doyle et al. (1987) have confirmed the validity of this tech-

nique for tilapia and describe the technique and its statistical interpretation in detail. McNaughton (1986) has used it extensively to compare the growth of carp from various farming environments in Indonesia.

Scales are removed from a specific area on the fish and preserved in formalin to protect the fragile outer margin. Scales are then wet mounted on microscope slides and examined at 100x magnification with a light microscope. The spacing of the outer 4 circuli on the anterior field of the scale was recorded along 5 rays per scale and 2 scales per fish. In the analysis presented here, an overall average circuli spacing per fish was used to relate to the weight and length change of tilapia over 22 days. The fish were raised in two groups, the first fed *ad libitum* and the other half that calculated amount.

Results and Discussion

All three methods of estimating growth rates give significant correlations with change in size (Table 1). GLY was better correlated with change in weight and STAIN and CIRC were better correlated with growth in length. Our results with GLY compares favorably with published reports (e.g., Goolish and Adelman 1983). The ability of CIRC to discriminate between the two feeding regimes (full and half ration) is represented by the histograms in Fig. 1. Although CIRC is not as powerful as change in length itself (F ratio 14.99 compared to 43.60 with 1,84 df), it is much more efficient to increase the sample size with CIRC than with change in length (Doyle et al. 1987). Nevertheless, the probabilities in both cases were very high ($P<0.0005$), and CIRC is more normally distributed.

Although GLY gave the best correlation, its intricate procedure and strong dependence on temperature, pH and incubation time render it difficult for field use. It is not allometrically independent of body size, an important consideration when relationships to other variables which are related to body size must be estimated. Staining is not as reliable as an estimator of growth, but probably requires further experimentation. CIRC is by far the simplest technique to use, gives reliable correlations when compared to growth measured on a time axis, and is potentially a diary of a fish's growth rate throughout ontogeny. CIRC has the additional advantage of not being expressed as a ratio of body size (unlike GLY).

CIRC appears to open new avenues of research that were previously unavailable or costly to undertake. For example, CIRC could be used to monitor fish growth rates

Table 1. Observed product-moment correlations (with 95% C.I.) between the growth estimators and change in size. GLY: [14C]-glycine uptake *in vitro*, STAIN: calcification staining technique, CIRC: scale circuli spacing. See text for description of methods. For all correlations except those with CIRC, the following transformations apply: (log$_e$) for growth estimators and $(\quad)^{0.3}$ for change in size.

Growth estimator	N	Change in weight	Change in length
GLY	28	0.824 (.651, .915)	0.769 (.555, .888)
STAIN	28	0.551 (.224, .766)	0.670 (.396, .834)
CIRC	86	0.735 (.619, .818)	0.750 (.640, .828)

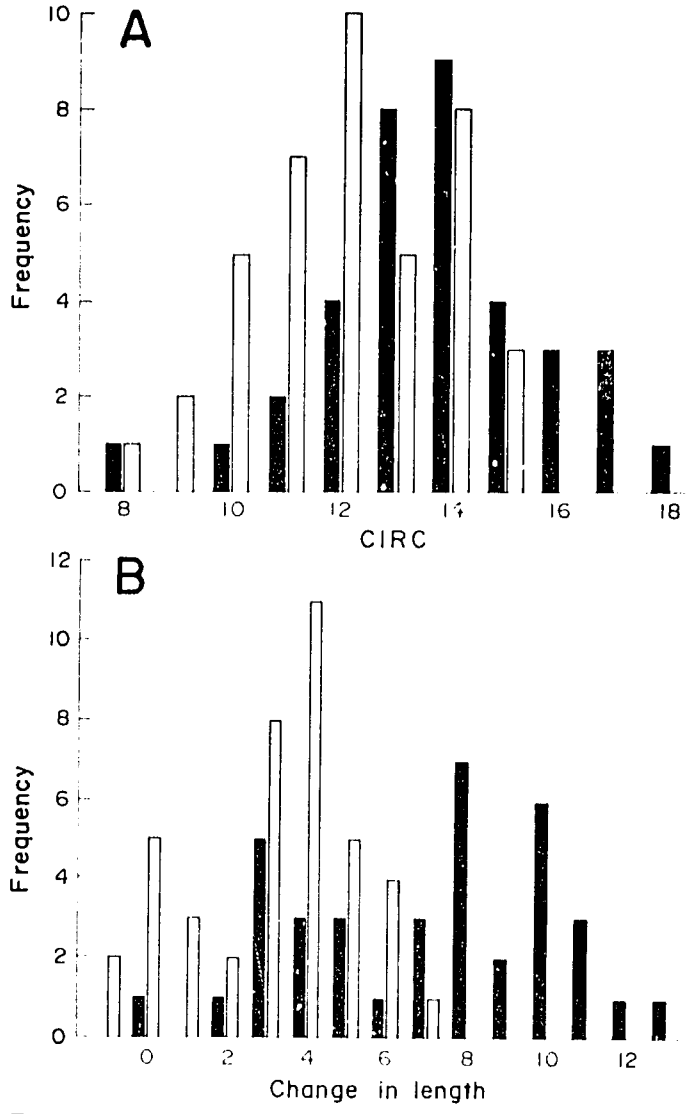

Fig. 1. Histograms of frequency distribution of (A) scale circulus spacing (CIRC) and (B) change in length, showing the different observed growth rates between *ad libitum* (solid bars) and half (open bars) feeding rations. Results of ANOVA for both groups are reported in the text.

in commercial ponds as an indication that feed levels, population density or parasite infestation (etc.) are affecting yield. Because individuals, not ponds or aquaria, are the statistical units, measurement of the effect of experimental diets, ration levels, etc. can be much more quickly achieved and with greater statistical efficiency than at present (Doyle et al. 1987). CIRC can also be used in broodstock management and selection programs to reduce the error caused by confounding growth rate variation and age variation in the population (Doyle and Talbot, this vol.).

Acknowledgements

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The Effects of Salinity on Growth, Food Consumption and Conversion in Juvenile, Monosex Male Florida Red Tilapia

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Abstract

The effects of salinity on the growth of juvenile, all-male, sex-reversed Florida red tilapia hybrids *(Oreochromis urolepis hornorum-O. mossambicus)* were studied under controlled photoperiod (12 L : 12 D) and temperature (28 °C). Fish (0.72 g mean initial weight) were grown in 200-l aquaria at salinities of 1, 10, 19, 28 and 36 ppt at a density of 15 fish per tank. Mean specific growth rates, daily food consumption, food conversion ratios and condition factors were determined at each salinity.

Growth at 10 ppt and greater was significantly higher than that at 1 ppt, and there was a clear trend towards an increase in growth with salinity. Increased growth with salinity was attributed to increased food consumption and lowered food conversion ratios with increasing salinity.

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Introduction

It is well known that some freshwater tilapias are generally able to survive and grow over a wide range of salinities. However, the effects of salinity on growth of tilapias, studied in relatively few species, are not well understood. For most species, optimal ranges of salinities for growth have been inferred from data on natural distributions or fragmentary experimental evidence. The variety of conditions (e.g., temperature, photoperiod and stocking densities) used in previous studies further precludes meaningful comparisons of results.

Payne (1983) reported that the optimum salinity for growth of *(Oreochromis niloticus* x *O aureus)* hybrids (6 ppt) was considerably less than the maximum allowing survival (16 ppt) and suggested that knowledge of optimum salinities for growth is important toward selection of appropriate species for culture in saline waters. At present, there is considerable interest in extending the culture of red tilapia hybrids to brackishwater or seawater, due to the high salt tolerance exhibited by certain strains and their high marketability and excellent growth rates in freshwater. The ability of Taiwanese red tilapia hybrids *(O. niloticus-O. mossambicus)* to survive and grow in brackishwater (Meriwether et al. 1984) and seawater (Hopkins et al. 1986) has been demonstrated; relative growth at various salinities was determined in one previous study (Liao and Chang 1983).

The potential for saltwater culture of Florida red tilapia is currently being investigated in the Bahamas (Watanabe et al., in press). Although preliminary studies have suggested this strain to be highly adaptable to seawater, the influence of salinity on growth is unknown. The objectives of this study were to determine the effects of salinity on growth, food consumption and conversion in Florida red tilapia, under controlled conditions of photoperiod and temperature.

Materials and Methods

This study was conducted at the Caribbean Marine Research Center on Lee Stocking Island (Exuma Cays, Bahamas) from August to October 1986.

Experimental animals

The Florida red tilapia broodstock used was a hybrid: descendents of an original cross of *O. urolepis hornorum* (female) with *O. mossambicus* (male) (Sipe 1979). Breeders were maintained in above ground plastic pools containing groundwater at 2 ppt salinity (freshwater), where spawning occurred naturally.

Yolk-sac-absorbed fry were collected from brood pools following release by mouthbrooding females (approximately 7-14 days posthatching) and transferred to raceway tanks (55 l) in freshwater. Fry were sex-reversed by feeding a commercial diet (ground Purina Trout Chow) treated with 17 α - ethynyltestosterone (60 mg/kg feed) (Guerrero 1975). Fry were fed *ad libitum* 3 times daily for 28 days. After sex-reversal, they were passed through 12/64" and 16/64" graders and medium grades selected for growth studies.

Experimental procedures

Growth experiments were conducted under controlled laboratory conditions in 200-l glass aquaria (122 x 46 x 51 cm). Light for each tank was supplied by a fluorescent lamp (20 watt) controlled by an automatic timer to provide a 12 L : 12 D daily photoperiod. Water temperature was maintained at 28 ± 1°C by controlling room temperature.

Growth of sex-reversed fry was compared at 1, 10, 19, 28 and 36 ppt with each treatment consisting of 5 replicate aquaria. Aquaria were stocked at a density of 15 fish/tank and growth monitored for 43 days.

To begin an experiment, sex-reversed fry were anesthetized (0.3 ppt 2-phenoxyethanol) in freshwater, weighed and measured and placed in experimental aquaria. Initial mean body weights and lengths averaged 0.72 g and 34.7 mm, respectively, and were not significantly different among salinities (Table 1). On the day after stocking (day 1), fish were acclimatized to their respective experimental salinities at a rate of 5 ppt per day. At 7-8 day intervals throughout the experiment, individual fish were weighed and measured following anesthetization under isosaline conditions.

Fish were fed to satiation twice daily (0800 and 1500 hours) a commercial tilapia diet (Insta Pro, 30% protein) with the daily ration based on a specified percentage of the tank biomass determined at the last sampling. At each feeding, a level of food was provided such that an excess remained after 30 minutes. Fish were not fed prior to weighing, and only 1/2 of the daily ration was provided following sampling.

Various salinities were prepared by mixing seawater (36 ppt), collected adjacent to the laboratory, with freshwater (1 ppt), obtained by reverse-osmosis of seawater. Each aquarium was supplied with an airstone, and water was continuously recirculated through an external filter containing ceramic, activated carbon, and nontoxic foam as media. Daily, feces were siphoned and approximately 80% of the water exchanged.

Salinity and water temperature were measured daily. Dissolved oxygen was measured on alternate days and pH, total NH_4-N and NO_2-N monitored weekly.

Analytical procedures

Preliminary analysis showed a clear departure in growth rates from the first 15 days till the end of the experiment. Thus, specific growth rates were determined from days 0-15 (phase 1) and from day 16 till the end of the experiment (phase 2). During these periods, linear relationships were observed when mean weight was plotted against time on semilogarithmic paper; thus, growth was assumed to be exponential. Specific growth rate (G), expressed as percentage body weight per day, was calculated from $G = 100 \times (\ln W_f - \ln W_i)/t$, where W_f = mean weight at the end of the period, W_i = mean weight at the beginning of the period, and t = time in days (Ricker 1975).

Condition factor (K) was calculated from $K = W/L^3 \times 100$, where W = weight in grams and L = total length in centimeters (Weatherly 1972).

Food consumption during a sampling interval was expressed as a percentage of average daily consumption to average biomass during the interval with consumption for the duration of an experimental period being the average over all sampling intervals. Food conversion was expressed as the ratio of total food consumed (dry weight) to growth (wet weight).

Treatment means were compared by analysis of variance (ANOVA, df 1 = 4, df 2 = 20). If the overall ANOVA was significant, differences between treatment means were further analyzed by Student-Newman-Keuls test (SNK) for equal sample sizes. Level of significance in all tests was $P < 0.05$.

Results

Mean body weights at each salinity were closely similar at the end of the acclimatization period (day 8) but diverged thereafter (Fig. 1). A clear trend was evident after day 22 with mean body weights increasing with salinity. On day 43, all comparisons of mean weights between salinities were significant, except for that between 10 and 19 ppt (Table 1).

Mean specific growth rates during phase 1 (days 0-15) increased with salinity from 6.5% at 1 ppt to 7.9% at 36 ppt (Fig. 2). Specific growth rates at salinities of 19 ppt and above were significantly higher than that at 1 ppt. Specific growth rates were lower during phase 2 at all salinities

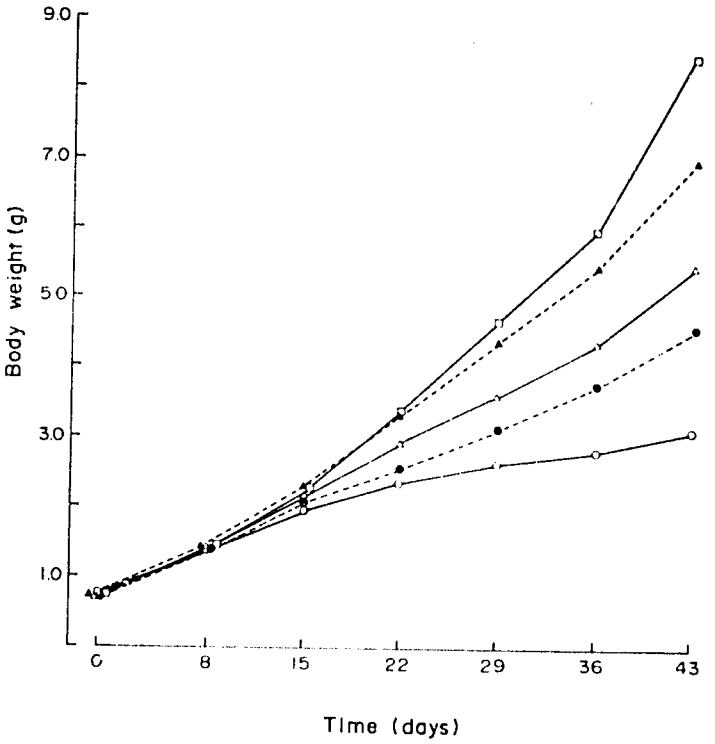

Fig. 1. Growth in wet weight of juvenile, monosex male Florida red tilapia at different salinities (○ 1, ● 10, △ 19, ▲ 28, □ 36 ppt). Plotted points represent means (n = 5).

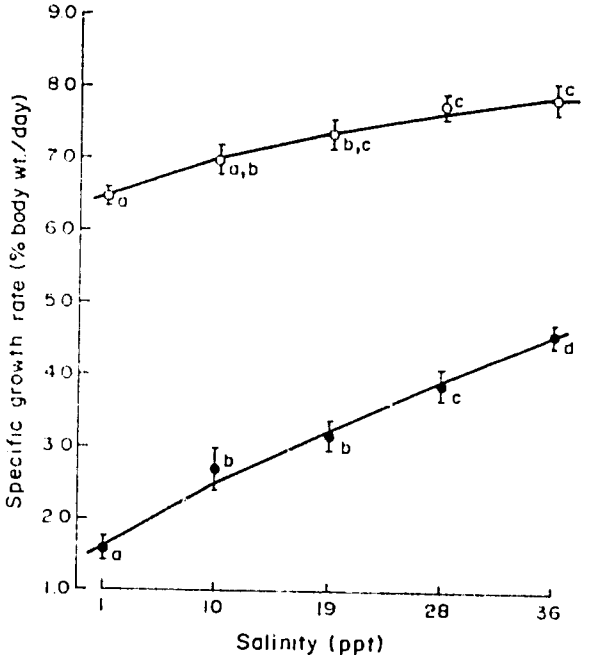

Fig. 2. Relationships between specific growth rate and salinity in Florida red tilapia during phases 1 (open symbols) and 2 (closed symbols). Plotted points represent means ± S.E.M. (n = 5). For each phase, means followed by the same letter are not significantly different ($P > 0.05$, SNK). Regression analysis defined these relationships as follows: phase 1, $y = 6.4451 + 0.0638x - 0.0007x^2$; $r^2 = 0.706$; phase 2, $y = 1.5739 + 0.0993x - 0.0005^2$; $r^2 = 0.865$.

Table 1. Initial and final body weights and lengths and survival rates of juvenile, monosex male Florida red tilapia grown for 43 days at different salinities.

Treatment (ppt)	Body weight (g)		Body length (mm)		Survival (%)
	Initial	Final	Initial	Final	
		($P < 0.001$)[β]		($P < 0.001$)	($P < 0.01$)
1	0.73 ± 0.02[α]	3.06 ± 0.15 a[‡]	34.9 ± 0.1	46.3 ± 0.5 a	85.3 ± 4.4 a
10	0.72 ± 0.01	4.47 ± 0.46 b	34.7 ± 0.2	51.9 ± 2.5 b	86.7 ± 2.1 a
19	0.71 ± 0.01	5.41 ± 0.44 b	34.7 ± 0.2	55.7 ± 1.3 b	90.7 ± 1.6 a, b
28	0.72 ± 0.01	6.90 ± 0.53 c	34.8 ± 0.2	62.2 ± 2.5 c	98.7 ± 1.3 b, c
36	0.71 ± 0.01	8.40 ± 0.47 d	34.6 ± 0.2	68.5 ± 1.9 d	94.7 ± 1.3 a, b

[α] Mean ± S.E.M. (n = 5).
[β] Probability level when overall ANOVA was significant.
[‡] Means followed by the same letter are not significantly different ($P < 0.05$, SNK).

and ranged from 1.6% at 1 ppt to 4.6% at 36 ppt. All comparisons of mean values between salinities were significant, except for that between 10 and 19 ppt. During both phases, specific growth rates increased with salinity, and these relationships could be expressed by curvilinear regressions (Fig. 2).

Final (day 43) mean body lengths ranged from 46.3 mm at 1 ppt to 68.5 mm at 36 ppt (Table 1). All comparisons of mean lengths between salinities were significant, except for that between 10 and 19 ppt.

Survival rates over the 43-day experimental period were relatively low at lower salinities, declining from 98.7% at 28 ppt to 85.3% at 1 ppt (Table 1). Survival rates at 1 and 10 ppt were significantly lower than that at 28 ppt.

Feeding and food conversion

Average daily food consumption over the 43-day experimental period increased with salinity from 5.93% at 1 ppt to 8.71% at 36 ppt (Fig. 3a). All comparisons of mean values between salinities were significant except for that between 28 and 36 ppt. The relationship between average daily consumption and salinity could be expressed by a curvilinear regression (Fig. 3a).

Mean food conversion ratios generally declined with salinity from 1.94 at 1 ppt to 1.50 at 36 ppt (Fig. 3b). Mean conversion ratios at 10 ppt and above were significantly lower than that at 1 ppt. The relationship between food conversion ratio and salinity could be expressed by a linear regression (Fig. 3b).

Condition factor

Initial condition factors averaged 1.72 and did not differ significantly among salinities (Table 2). Final (day 43) condition factors were significantly greater than initial values and were significantly higher at 1-19 ppt (range = 3.07 - 3.26) than at 36 ppt (2.61).

Mean dissolved oxygen concentrations were maintained near saturation (range = 6.8 - 8.0 ppm) at all salinities. Mean pH ranged from 7.79 to 7.90. Mean NH_4-N levels ranged from 0.06 to 0.20 ppm and mean NO_2-N levels ranged from 0.018 to 0.095 ppm.

Discussion

Canagaratnam (1966) reported that *Tilapia mossambica (O. mossambicus)* fry (0.2 g initial weight) fed *ad libitum* grew faster in brackishwater and seawater (35 ppt) than in freshwater, with highest growth at 17.5 and 26.3 ppt. Growth at 9 ppt (near iso-osmotic) was lower than at

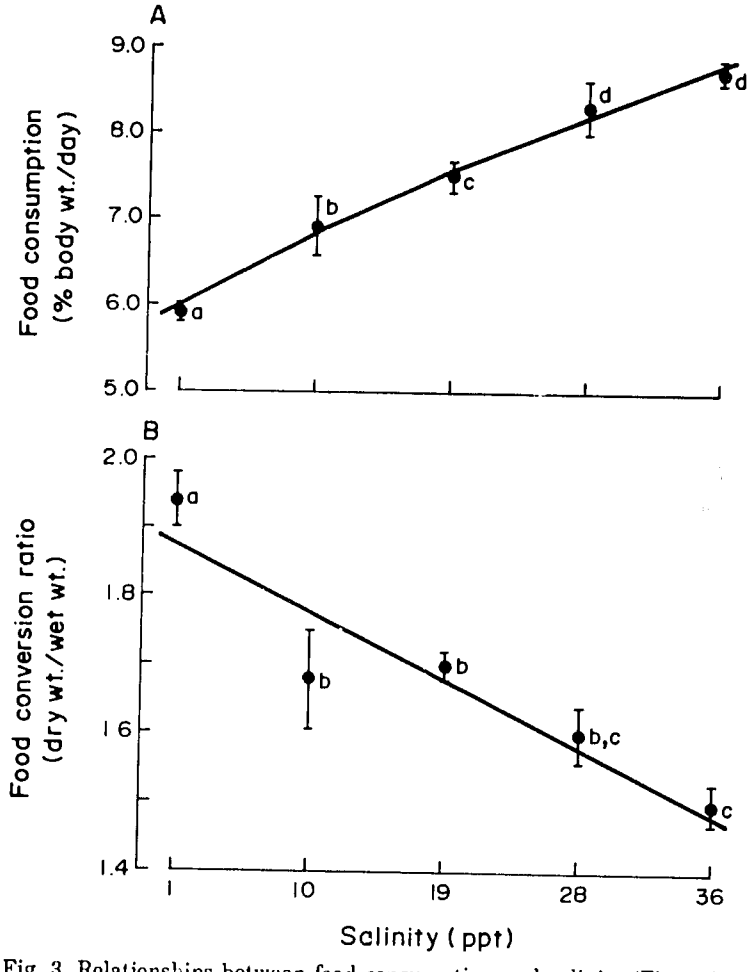

Fig. 3. Relationships between food consumption and salinity (Fig. 3a) and between food conversion ratio and salinity (Fig. 3b). Plotted points represent means ± S.E.M. (n = 5). For each figure, means followed by the same letter are not significantly different (P > 0.05, SNK). Regression analysis defined these relationships as follows: food consumption, $y = 5.8476 + 0.1035x - 0.0007x^2$; $r^2 = 0.877$; food conversion ratio, $y = 1.890 - 0.011x$; $r^2 = 0.654$.

Table 2. Condition factors (mean ± S.E.M., n = 5) of juvenile, monosex male Florida red tilapia grown for 43 days at different salinities.

Treatment (ppt)	Condition factor	
	Initial	Final[α]
		(P < 0.01)[β]
1	1.73 ± 0.04	3.07 ± 0.08 a[†]
10	1.73 ± 0.02	3.26 ± 0.16 a
19	1.72 ± 0 01	3.11 ± 0.04 a
28	1.70 ± 0.01	2.87 ± 0.15 a, b
36	1.71 ± 0.02	2.61 ± 0.11 b

α All final values were significantly different from initial values (P < 0.001, t-test).
β Probability level when overall ANOVA was significant.
† Means followed by the same letter are not significantly different (P > 0.05, SNK).

higher salinities. Jurss et al. (1984) reported that growth of *O. mossambicus* juveniles (12.1 - 13.0 g initial weight) on restricted rations was equal under iso-osmotic (10 ppt) and hyper-osmotic (33 ppt) conditions and better than that in freshwater. The effects of starvation on weight loss were equivalent at all three salinities, suggesting that the metabolic advantage under iso-osmotic conditions was negligible.

In this study, growth of Florida red tilapia was higher in brackishwater and seawater than in freshwater with growth at 10 ppt (near iso-osmotic) being significantly lower than that at 28 or 36 ppt. Evidence for the common assumption that growth of euryhaline teleosts is increased at salinities near iso-osmotic, since osmoregulation costs are minimal under these conditions, is largely inconclusive (Brett 1979). Growth of Florida red tilapia was unimpaired over a wide range of hyperosmotic conditions.

Febry and Lutz (1987) found in an *O. mossambicus* x *O. urolepis hornorum* hybrid that osmoregulation costs were higher in freshwater (0 ppt) than in seawater (35 ppt) and were lowest in iso-osmotic seawater (12 ppt). They noted, however, that salinity-related differences in total metabolic rates could not be solely attributed to changes in osmoregulation costs and concluded that other non-osmoregulatory factors must also affect metabolic rate. It follows that relative growth at different salinities may not necessarily reflect the cost of osmoregulatin at these salinities, depending on the magnitude of nonosmoregulatory effects on metabolism. In this study, salinity-related differences in the incidence of fish with damaged fins (due to agonistic encounters) indicated behavioral differences among fish grown under different salinities (see Watanabe et al.; this conference). In Florida red tilapia, growth response to salinity is modified by stocking density (Watanabe et al., unpublished data) also suggesting that growth is influenced by nonosmoregulatory (i.e., behavioral) effects on metabolism.

Liao and Chang (1983) reported that growth of mixed-sex stocks of Taiwanese red tilapia (*O. niloticus* x *O. mossambicus* hybrid) was faster in brackishwater (17 ppt) and seawater (34 ppt) than in freshwater (1.5 - 2.0 ppt). Growth of all-male stocks, however, was faster in freshwater. They attributed these differences to the inhibition of male aggression (which decreased feeding in females and weaker males in mixed-sex stocks) by increasing salinity. The important influence of nonosmoregulatory factors (i.e., aggression) on growth response to salinity, is clearly suggested. Territorial aggression can account for one-third to one-half of the active metabolic rate in teleosts during intense contesting (Brett and Groves 1979). Experimental evidence that growth response to salinity in Florida red tilapia is modified by aggression is discussed elsewhere in this volume (Watanabe et al.).

In contrast to all-male Taiwanese red tilapia which exhibited faster growth in freshwater than in saltwater (Liao and Chang 1983), growth of monosex male Florida red tilapia was faster in saltwater, suggesting a relatively high adaptability to seawater of the Florida red hybrid strain.

Results of this study suggest that increased growth with increasing salinity was related to increased food consumption (appetite) with salinity. Increased food consumption with increasing salinity was reported by Kinne (1960) in the freshwater euryhaline pupfish *(Cyprinodon macularius)*, by De Silva and Perera (1976) in the marine euryhaline mullet *(Mugil cephalus)* and by Dendrinos and Thorpe (1985) in the marine euryhaline bass *(Dicentrarchus labrax)*. Contrary observations were reported by Peters and Boyd (1972) in the freshwater euryhaline hogchoker *(Trinectes maculatus)*.

Food conversion ratios declined with increasing salinity. Jurss et al. (1984) attributed better growth of *O. mossambicus* at 10 and 33 ppt to a lower conversion ratio than that in freshwater. In fish, food conversion ratios decline with

increasing consumption to a minimum at an optimal rate of consumption (Brett 1979). Hence, declining conversion ratios with increasing salinity reflected the trend toward more optimal consumption rates, as well as the lowering of nonosmoregulatory (i.e., behavioral) metabolic costs, with increasing salinity.

Final condition factors were significantly increased at all salinities, indicating allometric growth increasing body fatness. That differences in condition factors between salinities were not simply related to differences in growth rates was evidenced by relatively lower condition factors in faster growing fish (i.e., those grown at higher salinities). Salinity, therefore, affected degree of fatness as well as growth in size. Dendrinos and Thorpe (1985) similarly reported that growth was more allometric at lower salinities in *D. labrax*, possibly related to changes in the biochemical composition of the fish. In contrast, Liao and Chang (1983) reported that condition factors of mixed-sex stocks of red tilapia reared in brackishwater and seawater were significantly higher than those of fish reared in freshwater.

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Further Investigations on the Effects of Salinity on Growth in Florida Red Tilapia: Evidence for the Influence of Behavior

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Abstract

In experiments on growth of Florida red tilapia (an *Oreochromis urolepis hornorum-O. mossambicus* hybrid) at different salinities, observations on the frequency of agonistic encounters among fish and on the incidence of fish with damaged fins (due to agonistic encounters) suggest that growth response to salinity is influenced by inhibitory effects of territorial aggression, which is mitigated by increasing salinity.

Introduction

Liao and Chang (1983) reported that mixed-sex stocks of Taiwanese red tilapia grew faster in brackishwater and seawater than in freshwater and suggested that these results may have been attributed to inhibitory effects of aggressive behavior which varied among different salinities. Watanabe et al. (this

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vol.) similarly reported that monosex male Florida red tilapia (an *Oreochromis urolepis hornorum-O. mossambicus* hybrid) grew faster in brackishwater and seawater than in freshwater and, based on salinity-related differences in the incidences of fish with damaged fins (due to agonistic encounters) as well as density-dependent differences in growth responses to salinity, hypothesized that behavioral factors may have influenced these results. In this paper, evidence relative to this hypothesis is presented.

Materials and Methods

This study was conducted at the Caribbean Marine Research Center on Lee Stocking Island (Exuma Cays, Bahamas).

Experimental animals

The Florida red tilapia broodstock used was a hybrid: descendants of an original cross of *O. urolepis hornorum* (female) with *O. mossambicus* (male) (Sipe 1979). The procedures used for collection, sex-reversal and grading of fry are described by Watanabe et al. (this vol.).

Experimental procedures

In two separate experiments conducted from August 1986 to January 1987, growth of all-male, sex-reversed juveniles was compared at different salinities under controlled laboratory conditions. In experiment I, fish were stocked at a density of 15 fish/tank and growth compared at salinities of 1, 10, 19, 28 and 36 ppt for 43 days (11 September to 25 October 1986). Details of the experimental procedures used are described by Watanabe et al. (this vol.).

In experiment II, fish were stocked at a density of 15 fish/tank and growth compared at salinities of 1, 19 and 37 ppt for 48 days (9 December 1986 to 24 January 1987). Each treatment consisted of four replicate aquaria. Water temperatures were normally maintained at 28 ± 1°C; however, mean temperatures ranged from 26.1-26.4°C during days 26-29 when control was temporarily lost. The experimental conditions used in the experiments were otherwise identical. Individual fish were weighed and measured on day 8, and at 10-day intervals thereafter. Initial mean body weights and lengths averaged 0.77 g and 35.2 mm, respectively, and did not differ significantly ($P > 0.05$, ANOVA) among salinities (Table 2).

Beginning one day after stocking, each tank was observed twice daily for 3 minutes, and the number of agonistic encounters (chases and/or bites) among fish was recorded. Observations were made prior to feeding and were begun at 0800 and 1400 hours.

The percentage of fish with damaged caudal and pectoral fins (due to agonistic encounters) in each replicate was observed on day 43 in experiment I and on day 48 in experiment II.

Analytical procedures

Food consumption rates and conversion ratios over the experimental period were calculated as described in Watanabe et al. (this vol.).

At each salinity, total number of agonistic encounters observed among replicate tanks was computed over all observation periods.

Statistics

Data on percentages of fish with damaged fins were analyzed by analysis of variance (ANOVA) following arcsine transformation If the overall ANOVA was significant, differences between treatment means were further analyzed by Student-Newman-Keuls test (SNK) for equal sample sizes. In experiment II, treatment means were compared by *t*-test. Relative frequency of agonistic encounters among salinities was analyzed by the chi-square test for goodness of fit. Level of significance in all tests was $P < 0.05$.

Results

In experiment I, the mean percentage of fish with damaged fins on day 43 declined with salinity from a maximum value at 1 ppt to a minimum value at 36 ppt (Fig. 1). The percentage of fish with damaged fins was significantly higher at 1 ppt than at 10 ppt or above, and significantly higher at 10-19 ppt than at 36 ppt. In experiment II, a trend similar to that observed in experiment I was evident on day 48, with mean percentage of fish with damaged fins declining with salinity from a maximum value at 1 ppt to a minimum value at 37 ppt. The percentage of fish with damaged fins was significantly higher at 1 ppt than at 19 or 37 ppt (Fig. 1).

In experiment II, total frequency of agonistic encounters declined with salinity from 1,211 at 1 ppt, to 785 at 37 ppt (Fig. 2). Total frequencies of encounters were significantly different among salinities.

In experiment II, mean weights were closely similar through day 17, thereafter increasing with salinity until final sampling (day 48). Mean lengths showed similar changes, being closely similar through day 8, and increasing with salinity thereafter. Final mean weights and lengths increased from minimum values at 1 ppt, to maximum values at 37 ppt (Table 1), although differences in final weights and lengths among salinities were not significant ($P > 0.05$).

Mean survival rate increased with salinity from 81.7% at 1 ppt to 86.7% at 37 ppt (Table 1), although differences among salinities were not significant ($P > 0.05$).

In experiment II, a trend of increasing food consumption with salinity was evident (Table 2). Average daily consumption over the experimental period was significantly higher at 37 ppt (6.54%) than at 1 ppt (5.88%).

Food conversion ratios ranged from 1.59-1.60 and did not differ significantly among salinities (Table 2).

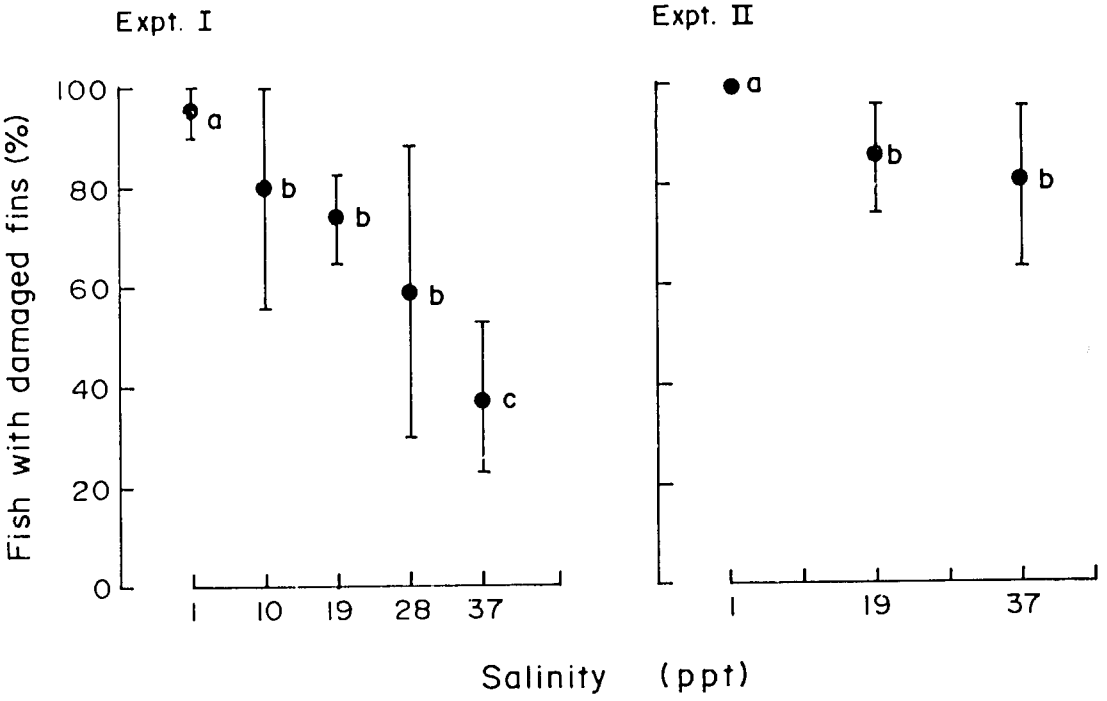

Fig. 1. Percentages of Florida red tilapia with damaged fins at different salinities in experiments I (day 43) and II (day 48). Plotted points represent means n = 5, experiment I; n = 4, experiment II) and vertical bars represent 95% confidence limits. For each experiment, means followed by the same letter are not significantly different ($P > 0.05$, SNK).

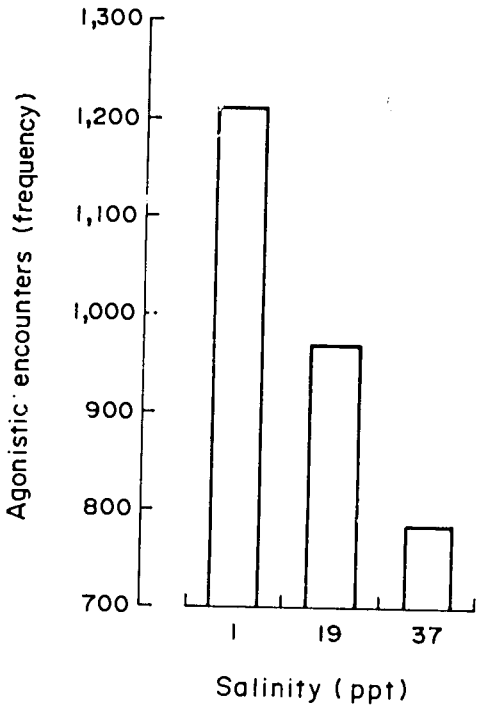

Fig. 2. Total frequencies of agonistic encounters among Florida red tilapia grown at different salinities, observed during 88 three-minute periods. At each salinity, fish were grown in four groups of 15 fish per tank. Frequencies of encounters were significantly different among salinities ($P < 0.005$, chi-square goodness-of-fit test).

Table 1. Initial and final body weights and lengths and survival rates of juvenile, monosex male Florida red tilapia grown at different salinities for 48 days in experiment II.

Treatment (ppt)	Body weight (g) Initial	Final	Body length (mm) Initial	Final	Survival (%)
1	0.80 ± 0.01[α]	4.60 ± 0.43	35.5 ± 0.2	42.4 ± 1.7	81.7 ± 5.7
19	0.75 ± 0.01	4.86 ± 0.43	34.8 ± 0.3	43.0 ± 1.7	83.3 ± 7.9
37	0.76 ± 0.02	5.27 ± 0.27	35.3 ± 0.2	44.2 ± 1.2	86.7 ± 3.1

α Mean ± S.E.M. (n = 4).

Table 2. Average daily food consumption and food conversion ratios of juvenile, monosex male Florida red tilapia grown at different salinities for 48 days in experiment II.

Treatment (ppt)	Food consumption (% body wt./day)	Food conversion (dry wt./wet wt.)
1	5.88 ± 0.19[α] a*	1.60 ± 0.06 a
19	6.37 ± 0.15 a, b	1.59 ± 0.07 a
37	6.54 ± 0.16 b	1.60 ± 0.04 a

α Mean ± S.E.M. (n = 4).

* Values within a column followed by the same letter are not significantly different ($P > 0.05$, t-test).

Discussion

In tilapias, territorial aggression by dominant males can result in damage to fins, scales and eyes of other fish, sometimes leading to death (Balarin and Haller 1982; Philippart and Ruwet 1982). It was observed during experiment I that the incidence of fish with damaged caudal and pectoral fins (due to agonistic encounters) declined with increasing salinity, suggesting that aggression was reduced by increasing salinity. To further investigate this possibility, the relative frequency of agonistic encounters among fish grown at different salinities was determined in experiment II under conditions identical to those used in experiment I. Results of experiment II showed that the frequency of agonistic encounters among fish as well as the incidence of fish with damaged fins declined with increasing salinity, supporting the idea that aggression was inhibited by increasing salinity. Relatively low survival rates at lower salinities in both experiments I and II were also consistent with this idea. It is well known that environmental perturbations cause behavioral changes in fish (see review by Olla et al. 1980). Olla et al. (1978) reported that elevated (although sublethal) temperatures reduced interfish spacing and aggression in the tautog *(Tautoga onitis)*.

It is recognized that behavioral interactions can adversely affect growth in tilapias. Territorial and reproductive activity were reported to reduce growth of *O. mossambicus* in laboratory aquaria (Turner 1986). Growth inhibition during intensive culture of tilapias at high stocking densities has been attributed to behavioral interactions (Balarin and Haller 1982). Fishelson (1983) suggested that techniques which limit aggressive behavior under intensive culture should improve production by reducing energy expenditure in aggressive interactions.

Intraspecific aggression acts to restrict the food consumption of subordinate fish and may occur even when food is not limiting (Brett 1979). Disproportional acquisition of food by dominant fish was reported to mediate the size heirarchy effect in juvenile *Tilapia zillii* (Koebele 1985). In experiment I, increased growth with increasing salinity was attributed to increased food consumption (appetite) and lowered food conversion ratios with increasing salinity (see Watanabe et al., this vol.). In experiment II, the changes in mean weights and lengths relative to salinity were similar to those observed in experiment I, and a trend toward increased food consumption with increasing salinity was again evident, although food conversion ratios were not significantly different among salinities. This suggests that aggression impairs growth in Florida red tilapia by lowering food consumption (appetite) and increasing food conversion ratios, this effect increasing with declining salinity. Hence, as aggression is mitigated by increasing salinity, growth is improved.

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SESSION VII: ECONOMICS AND SOCIOECONOMICS

Development Planning for Tilapia Farming in Africa

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Abstract

Tilapia farming in Africa has a long but fragmented tradition. Pond culture activities became widespread from 1940 to 1960, but subsequently declined. Recently there has been renewed interest. This paper gives a historical account of tilapia farming in Africa and puts forward factors to be considered when planning tilapia culture. Socioeconomic, agroclimatic, economic and social constraints to aquaculture development are discussed. Attention is paid to the rationale for the selection of appropriate systems (particularly integrated farming), species, and degree of intensification. Marketing, resource appraisals, manpower availability and infrastructural support are considered. Climatic data are used to present an indicative tilapia farming zonation of Africa.

Introduction

The most recent meeting of the Committee for Inland Fisheries of Africa (CIFA) held in Lusaka, Zambia in October 1985 reviewed the state of the art of aquaculture in Africa and concluded that, "The lack or inadequacy of qualified personnel with sufficient practical experience remains one of the main handicaps for aquaculture development in Africa" (CIFA 1985). The lack of organized aquaculture extension services was considered a major constraint to the development of aquaculture.

Country reviews, published by FAO in the Fisheries Circular 770 series, confirm this view. These studies took a holistic view of food supply. Many interrelated factors decide the success or otherwise of aquaculture endeavors and the degree of intensification of appropriate systems. This paper draws upon these macro-analytical FAO studies and other sources of development options for tilapia farming in Africa (Balarin and Hatton 1979; Balarin 1984a, 1984b, 1984c, 1984d, 1984e, 1985a, 1985b, 1985c, 1985d, 1985e, 1986a, 1986b, 1986c, 1987, 1988a, 1988b; FAO 1984a).

The history of aquaculture development in Africa is rather fragmented. Basreliefs on walls of tombs in Egypt as early as 2500 BC depict the beginnings of fish keeping in Africa. Reference is also made in the Bible to the "fish sluices" of the

River Nile (Isaiah, 11 v. 10). This may have been a reference to the extensive area of drain-in ponds or howash systems of the Nile Delta which cover an estimated area of 30,000 to 50,000 ha (Balarin 1986a). Examples of other such traditional, semi-aquaculture systems are the brushparks or "acadja" in Bénin (Balarin 1984e); fish holes or "whedos" in Cameroun (Balarin 1985a); clam stocking in the Lower Volta in Ghana (Balarin 1988b) and mother of pearl culture in Sudan (Balarin 1988a). It is easy to imagine how fishing techniques could have evolved into aquaculture systems in Africa. For example, barrier traps as used in floodplain fisheries could, with a little modification, be turned into pen culture. However, the development of such systems would be restricted to areas with adequate water resources such as rivers, lakes or coastal zones. Generally, these areas have existing fisheries. Aquaculture could develop as a secondary industry in order to supplement the market demand for fish.

More modern aquaculture systems, such as ponds, cages and tanks (Balarin 1988c) were introduced to Africa this century by colonial settlers. Initially, for reasons of familiarity, exotic species such as common carp, trout and bass were the main species farmed. Only after World War II did scientists show an interest in indigenous fish as a potential food crop for rural farmers. Over 150 species have been tested, of which 25% are tilapias. By 1960, nearly 320,000 ponds were reported to have been built in Africa. For various reasons, as described in Balarin and Hatton (1979) and Balarin (1986b), this activity declined.

Recently, renewed interest has given rise to an overall aquaculture production of around 45,000 t/year, mainly from the pond culture of tilapias (Ruddle and Balarin, in preparation). The significance of this resurgence of interest is perhaps that aquaculture is now being promoted as an integrated part of farming systems. This paper therefore attempts to elaborate those factors which are important to future development of tilapia farming.

Factors Affecting the Development of Tilapia Farming in Africa

Demographic and nutritional factors

Africa's population, currently estimated at 560 million is likely to reach almost 900 million by the year 2000 at its current growth rate of 2.0 to 4.2% per year (Grant 1987). African per capita food production indices FAO (1986) suggest that agricultural production cannot keep up with demand in Africa. Further, African protein consumption levels are considered amongst the lowest in the world, averaging 57.9 g/capita/day; 16% below the world average. Consequently expenditure on imports of agricultural products to sustain African food consumption was increasing in 1983 at an annual rate of 14%: US$694 million was being spent on fish and fish products.

Adopting the rationale developed by Balarin (1986b, 1988c), Africa would require by the turn of the century about 6.8 million tonnes of fish to maintain present consumption levels. This would have to rise to as much as 9 million tonnes in order to achieve a theoretical optimum intake of 10 kg per capita per year. Such figures are of course based on assumptions that take no account of alternative sources of protein for human consumption such as vegetable protein. However, they may provide an indication of demand *vs.* supply. Current African fish supply is estimated at 75-80% of the predicted maximum sustainable yield of 5.0 million tonnes. Therefore, even if maximum sustainable exploitation were possible, there may be a shortfall in fish supply *vs.* demand from 1.8 to 4.0 million tonnes. Alternative sources of animal protein production would therefore have to be found and it is feasible that aquaculture could go some way toward meeting this demand. However, attention must also be given to the environmental impact of aquaculture. For example, the develop-

ment of integrated small-scale farming systems incorporating aquaculture while improving nutrition and livelihood could also help to spread water borne diseases such as bilharzia and malaria.

Environmental factors

Temperature is perhaps the single most important factor determining tilapia production in ponds. The limits of temperature tolerance, growth and breeding for various species are summarized in Fig. 1. For a tilapia farm, the days with temperatures above the minimum required for growth determine the growing period. Generally, tilapia stop growing between 15 and 20°C, do best when temperatures are between 25 and 30°C and have a Q_{10} of about 2.5. Winter water temperature decides the growing season for a tilapia pond. There is only a narrow band across the equatorial region where water temperatures are unlikely to go below the optimum range for tilapia growth.

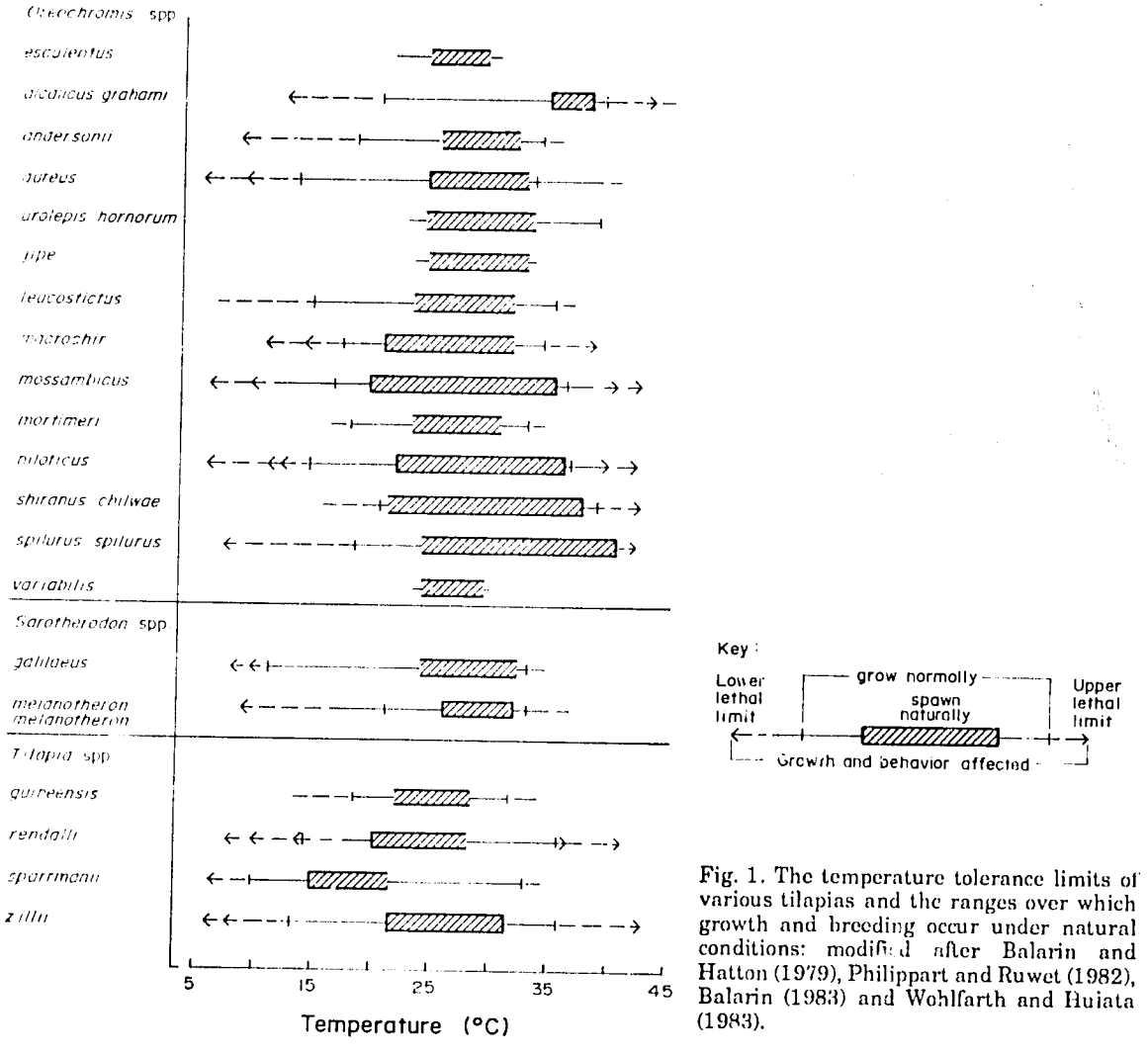

Fig. 1. The temperature tolerance limits of various tilapias and the ranges over which growth and breeding occur under natural conditions: modified after Balarin and Hatton (1979), Philippart and Ruwet (1982), Balarin (1983) and Wohlfarth and Ruiata (1983).

Air temperature decreases between 0.4 and 0.8°C for every 100-m increase in altitude. Tilapia production in ponds shows a decrease with increasing altitude in Malawi and Madagascar (Fig. 2). Thus not only does the growing season for tilapia decrease north and south of the equator but there is also a likelihood of a 250 to 300 kg/ha/year decline in pond production for every 100-m increase in altitude. The normal distribution range for tilapias stops at about 1,500 m above sea level perhaps due to low temperatures precluding reproduction. Moreover, whereas within the tropics in areas below 500 m temperatures may be suitable for near year-round growth of tilapia, the influence of higher altitudes on water temperature may adversely affect growth. Above 1,000 m, there is likely to be a 3- to 6-month period of no growth or breeding.

For tilapia ponds, between 30,000 and 70,000 m^3 of water per year are required per tonne of fish yield. Seepage losses may be 5 to 10 mm/day or 2.1 to 4.2 m^3/hour water replacement/ha while evaporation can account for 1,500 to 2,400 mm/year or 1.7 to 2.7 m^3/hour water replacement per hectare. Intensive tank culture systems require 8 to 10 m^3 of water per hour per tonne of fish while cages and pens need permanent water. The use of irrigation water is feasible for tilapia production either in rice paddies, storage dams or flow-through systems such as raceways or tanks. For integrated crop-fish systems, yields of approximately 50 to 500 kg/year of fish are feasible per hectare of crop irrigated (Balarin 1984f). Fig. 3 provides an overall zonation of the potential for tilapia culture in Africa based on altitude, temperature and water resources.

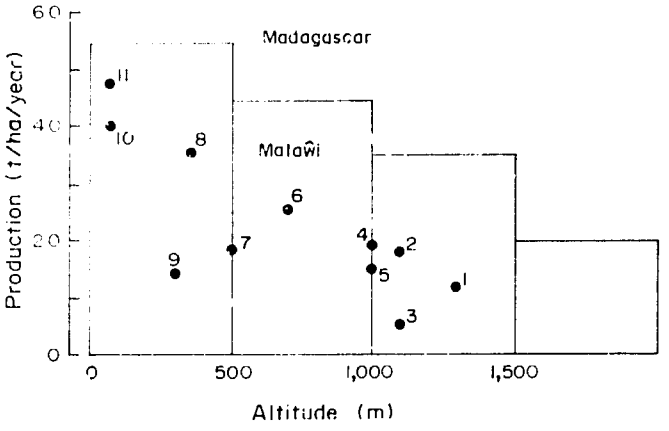

Fig. 2. Relationship between yield of selected tilapia ponds and altitude: redrawn from data for Malawi (points 1-11) and Madagascar (histogram blocks), Balarin and Hatton (1979) and Balarin (1987).

Large tracts of Africa are arid or semi-arid and therefore unsuitable for aquaculture except perhaps along permanent watercourses or where pastoralists may have developed water storage for watering livestock. Many areas have a long dry season. Where rainfed ponds are possible, their design depends on the patterns of rainfall and runoff. A catchment area of approximately 10 ha is needed per hectare of rainfed pond in a rainfall zone of 1,400 to 1,800 mm/year with 20% runoff rate. However, periodic water supply is necessary to top up evaporation, seepage and drainage losses.

Preferably water supply should be by gravity. Pumping may only be economical for tilapia culture if the total head is less than 10 m. Pond siting, therefore must take topography into account to optimize cost-effective water supply. Soil type is also important. Clay soils reduce seepage losses and facilitate construction. They also avoid the problems of more acidic soils that reduce aquatic productivity. Salinity is another important variable. Fig. 4 summarizes available data on salinity tolerance and growth and reproduction in saltwater for various tilapias. Such data must be interpreted

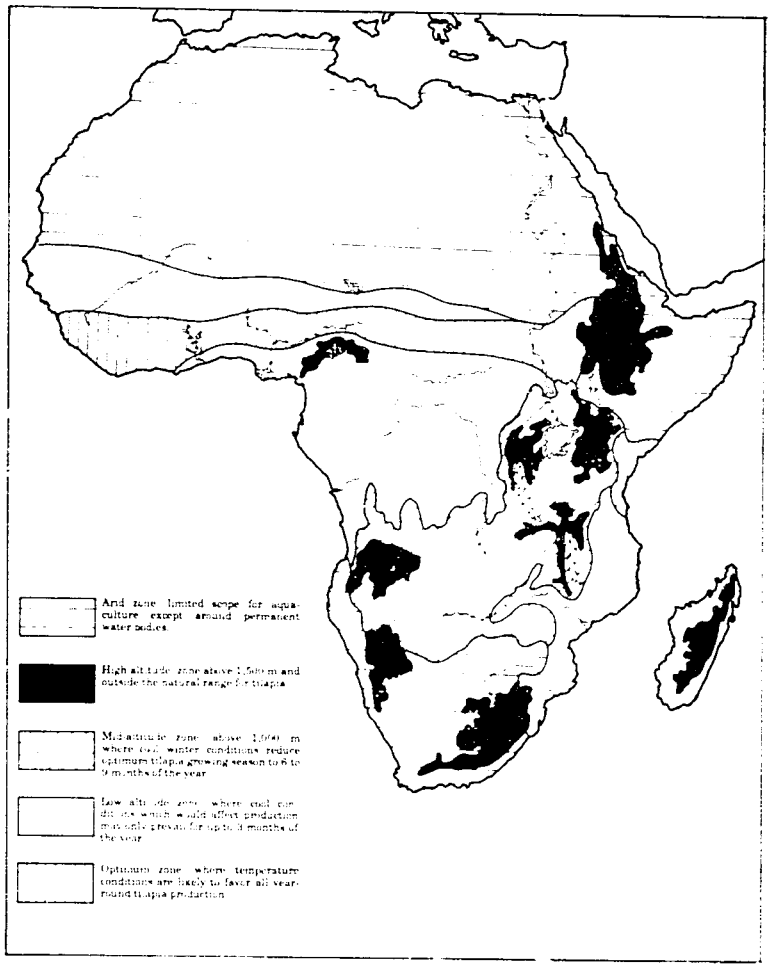

Fig. 3. An overall zonation of the potential for tilapia culture in Africa based on altitude, temperature and water resources.

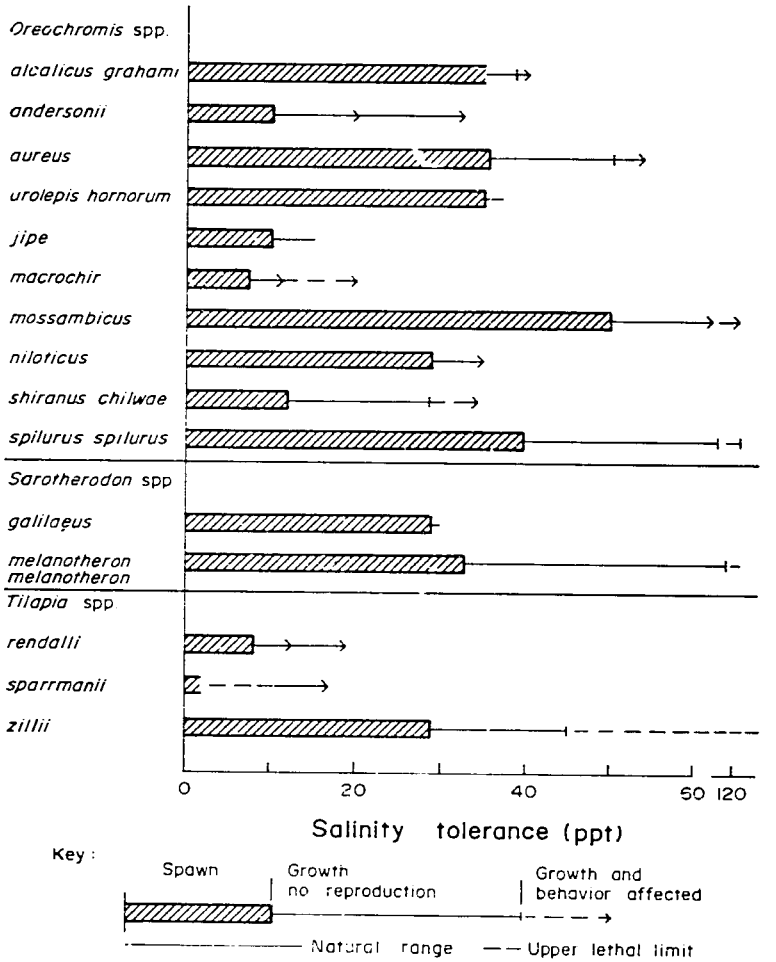

Fig. 4. The salinity tolerance of some tilapias and the range of salinities over which breeding occurs: adapted from Balarin and Hatton (1979), Wohlfarth and Hulata (1983) and Balarin (1983).

with caution. For example *O. niloticus* succumbs to bacterial infections in West African lagoons at salinities around 8 ppt.

Economic and social factors

Over 40 species of tilapia have been tested for aquaculture in Africa. The most important, in order of frequency of culture trials, appear to be *Oreochromis niloticus, Tilapia rendalli, O. macrochir, T. zillii, Sarotherodon galilaeus* and *O. mossambicus*. Most countries choose endemic species but there has been a tendency to introduce exotics. Tilapias satisfy most bioeconomic criteria. Endemic to Africa, they are readily accepted by the markets and fetch a premium price. More is known about this group than any other in Africa and few major disease problems of farmed tilapia have been recorded.

The range of systems available for tilapia farming in Africa are listed in order of increasing intensity and complexity in Balarin (1986b, 1988c). Maximum performance of each, however, requires optimal environmental conditions. Viability is also dependent upon socioeconomic conditions. Given the short history of modern aquaculture in Africa, development projects have often been carried out by foreign technical assistance. The shortcomings of this have been that few of the experts were familiar with the social background of the target groups, resulting not only in communication problems but misconceptions of capabilities. Ideally livestock farmers or people with a fishing background are quicker to appreciate concepts of fish husbandry at subsistence or semi-intensive levels. More intensive commercial fish farmers require a more technical background. The caliber of expertise available among prospective farmers and extension workers depends very much on social and monetary incentives. Fish farming is often considered to be novel and of low priority and its career benefits have not been attractive enough to attract high caliber personnel.

Most governments in Africa provide basic extension services in aquaculture. FAO (1984b) lists 426,000 trained agricultural personnel in Africa: 12% at 'professional' level and 19,000 fisheries oriented, but with a lower average of 9% 'professionals'. For aquaculture development, Africa needs many more trained support staff. A total of 130 aquaculturists were trained by FAO through the African Regional Aquaculture Centre, Nigeria (CIFA 1985). However, development of rural aquaculture, by virtue of its current dispersed nature, requires a high ratio of extension support. At present this is in the order of one field agent per 30 ponds. Greater mobility and consolidation of effort may, however, improve efficiency of extension. Aquaculture is generally considered a low priority by African governments and thus receives low budgets for extension. Consequently, external financial assistance has been necessary. Moreover, there are very few financial institutions willing to advance credit for tilapia culture in Africa in view of the perceived risk in such a novel enterprise. Current development costs for tilapia culture in Africa are therefore high. They often depend upon foreign technical and financial support for new infrastructure and materials.

Integrated farming systems

Integrated farming systems incorporating tilapia culture could be the most energy efficient and beneficial for rural African farmers. However, not all such systems in use in Asia (Edwards 1986) may be appropriate for Africa. For example, the use of human excreta to fertilize ponds is taboo (Balarin 1988d). The use of crop residues to feed livestock and then the use of their manure as a pond fertilizer is theoretically an efficient option for rural farmers. However, in Africa, few farmers keep large numbers of livestock; the few that are kept range freely. What little manure is collected finds priority use on crops or vegetables.

There is also limited scope for integrated duck-fish or pig-fish systems in Africa because, unlike Asia, these animals are not widely farmed. Integration of fish culture with cattle, sheep and goat husbandry is feasible but there has been little basic research on this. The only livestock-fish system which seems appropriate and has been tested widely is chicken-tilapia culture, but here again chickens are generally allowed to range freely in African farmyards. Any livestock-fish integration would have to involve penning, housing and feeding animals: features not commonly practiced in rural Africa.

The major crop residues in Africa are maize stover, sorghum or millet stalks, cassava and yam leaves and peels. These are of low nutrient value as fish feeds and pond fertilizers. Composting such residues for addition to ponds although practiced has yet to be significantly quantified. Since African agriculture in general is a "slash and burn" activity, ash from crop residues as well as domestic cooking fires should be evaluated as pond inputs, for example to raise the water pH in acid soil areas. The more nutritious crop residues, such as cereal brans find ready use in livestock feeds or as export products. Their use as fish feeds in integrated farming would have to compete with these established practices.

The Future of Tilapia Culture in Africa

Africa has always exploited fishery resources and fish as an accepted and relatively cheap form of animal protein in Africa. Aquaculture in Africa must compete not only with fish supply from capture fisheries, but also other forms of animal protein, such as poultry. However, rural aquaculture could be encouraged where it will lead to improved nutrition and income. Too often African rural aquaculture projects have been initiated with exaggerated commercial objectives; misconceptions which often lead to dissatisfaction and abandonment. The rural African farmer is a very shrewd economist who cannot afford to risk investing resources beyond the traditional crops that insure survival of the household unless the result is sure and there is an economic buffer for such risk taking. Consequently, unless aquaculture can be shown to work in rural Africa without massive technical support, it will be difficult to convince farmers to try diversification into producing fish with other produce. As Africa has few examples of successful rural fish farmers and, with rare exceptions, a nonviable economic climate for tilapia farming by corporate concerns, it is perhaps too early in the evolution of African aquaculture to assess the prospects for widespread tilapia culture development. When fishery resources will become further over-exploited, thus reducing fish supply and increasing prices the picture will change. These issues are further discussed by Balarin (1988d, 1988e).

All things being equal, it is the state of development of a nation, its development policies and especially the status of its agricultural development, that will determine the future of tilapia farming in Africa. Support services are vital to development. It may well be that the initial target market should be the major cities where dwellers would pay premium prices for fresh fish. Satellite fish farms would benefit from the urban support infrastructure and transport service. Such developments would reduce demand for fish destined for such centers, these could then find outlets in the rural areas.

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Philippine Tilapia Economics: Industry Growth and Potential*

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Abstract

The tilapia farming industry of the Philippines began slowly with the initial introduction of *Oreochromis mossambicus* in the 1950s. The more recent introduction of *O. niloticus*, however, has resulted in extremely rapid development of tilapia farming in lakes and ponds. Currently, tilapia is second only to milkfish in terms of annual production; the industry is estimated to produce over 50,000 tonnes of tilapia annually.

Rapid inflation beginning in 1983 has led to over a 20% decline in the real prices of tilapia. Margins between wholesale and retail prices are narrowing, implying more competition in the industry as volume has increased. Declining purchasing power of consumers has also put additional pressures on producers to become more efficient. Future market potential thus depends not only on future incomes of consumers but also on the ability of producers to reduce average production costs. This can be done not only through increasing average farm efficiency but also through stock improvement.

Introduction

The successful expansion during the 1980s of the tilapia farming industry in the Philippines is not only of considerable interest in that country but also for its implications for tilapia industries elsewhere. This recent industry growth came after several decades of experimentation by Philippine scientists and fishfarmers with *Oreochromis mossambicus* using farming systems such as rice-fish culture which over time have proven less attractive for the private sector than pond and cage culture systems using *O. niloticus* (Guerrero 1985, 1986). Also of major importance for the industry have been the levelling off of fisheries

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catches from marine and inland waters and an apparent shift in consumer preference in certain parts of the country towards tilapia and away from the premier cultured fish of the Philippines, the milkfish *(Chanos chanos)*. The real price of milkfish declined rather significantly during the decade ending 1983 (Smith and Chong 1984). Real prices of tilapia also declined but at a lower rate than those of milkfish (real prices of milkfish since 1983 have again begun to increase). From negligible levels of production fifteen years ago (although statistics are somewhat incomplete) the Philippine tilapia industry is now estimated to produce at least 35,000 t and perhaps as much as 50,000 t annually (Guerrero 1985; BFAR 1986). The industry also has achieved a degree of maturity in that it is increasingly characterized by specialization of function (e.g., nursery pond specialists).

Beyond its growing importance for urban Philippine consumers as a relatively cheap source of protein comparable to round scads (*Decapterus* spp.), tilapias are beginning to assume a more important role as a source of additional income and protein for rural farmers and communities (Fermin 1985). This is a particularly important dimension in the country at present, which is suffering severely from the effects of economic stagnation and very much needs to identify means of increasing production and value-added activities, especially in rural areas. Problems of genetically deteriorating tilapia stocks and lack of regional integration in production and markets remain to be overcome, however.

Financial Analysis of Tilapia Technology and Culture Systems

The financial feasibility of tilapia production at the farm level is a sound indicator of the industry's potential for growth and development. A summary of economic studies conducted between 1980 and 1983 on different types of technology and culture systems practiced in various areas of the Philippines is presented in Table 1. Most of the surveys were conducted in Luzon (northern one-third of the country) where tilapia is more popularly grown and reared. Strict comparability of these surveys is not possible throughout but is sufficient to warrant comment.

The majority of the fish farmers surveyed had less than five years of experience in tilapia culture, implying the recent emergence of the industry. Tilapia production was the main source of income in Bicol and Mindanao. Almost all (92-99%) of the tilapia harvest in Laguna and Rizal provinces near Manila was sold by fish farmers whereas in Central Luzon a considerable proportion was consumed by the household. On the whole, the industry is more commercially than subsistence oriented.

Overall, tilapia culture has showed encouraging profitability during the first half of the 1980s. Net returns (total revenue minus total costs) were mostly positive. In some cases wherein negative net returns were realized, net cash income (total revenue minus cash costs) was still positive. Cases of negative net income arose when a relatively high positive value was imputed for family and operator's own labor. However, these values may overstate the losses because in rural areas where surplus labor exists, the opportunity cost of labor would be close to zero.

The poor financial and economic performance for the lake-based grow-out operations in Binangonan and Cardona, Laguna de Bay (Lazaga and Roa 1985) was caused by heavy losses due to rampant poaching, typhoon damage, inadequate natural food, degeneration of broodstock quality and lack of proper management skills in tilapia cage culture.

Table 1. Summary data from various recent economic studies of Philippine tilapia farms.

Culture system	Author Year of study	Location, # of respondents	Extent of respondent involvement	Average years in industry	Farm characteristics: Area	Average # of cages/ponds	Production	Nature of disposal		Total costs[1]		Returns[1, 2]
Hatcheries					(m^2)		(# fingerlings/farm/year)	(%) Sold	Allowance	Fixed	Variable	(Pesos/year) Net
Land-based ponds	Yater, L.R. and I.R. Smith (1985)	Laguna and Rizal Province (80)	full-time, 55%	2.9	All (3,900)	7	488,200	95	5	2,824	28,565	34,781
		Laguna, 69;			<1,250	4	81,700	92	8	491	4,801	4,437
		Pililia, 11			1,250-4,999	9	141,800	94	6	1,267	11,518	1,807
	9/81-10/82				5,000-9,999	20	919,200	96	4	4,381	64,346	39,647
					> 10,000	10	3,841,200	95	5	21,110	208,665	321,584
Land-based ponds	Broussard Jr., M.C. and C.G Reyes (1985)	Muñoz, Nueva Ecija, Central Luzon; a government hatchery; first year operation					(/ha)	(%) Sold	Given away			(Pesos/ha) Net
broodfish					600 and 1,300 m^2		890 kg and 400 fingerlings (115 days)	66	34	26,033		5,967
fingerlings	5/82-5/83				4,500 and 1,300 m^2		658,000 fingerlings (265 days)			55,739		3,099
advanced fingerlings					1,300 and 600 m^2		250,000 fingerlings (115 days)			42,143		7,857
Lake-based cages	Aragon, C.T., M.M. de Lim and G.L. Tioseco (1985)	See Aragon, C.T. et al. below. Study includes combined hatchery and grow-out operations for cage operations in San Pablo City.										
Grow-Out					(ha)		(kg/ha)			Non-cash	Cash	(Pesos/ha) Net	Net cash
Land-based ponds	Caddarao, P.S., R.E. Diapo, I.G. Tadas and E.C. de Torres (1985)	Selected provinces (176)	n/a	1-5 years – 78% 6-10 years – 11% 11-20 years – 11%	0.99		1,473			5,561	5,474	4,880	10,441
		Tarlac, 34;			0.98		1,341			4,548	5,194	6,609	11,157
		Pangasinan, 30;			0.76		1,350			5,582	6,570	5,630	11,212
		Pampanga, 25;			2.13		1,587			5,539	3,755	7,896	13,435
	1/82-12/82	Bulacan, 24;			1.43		1,684			7,140	7,074	2,288	9,428
		Isabela, 7;			1.10		767			3,464	3,987	3,046	6,5[illegible]
		Camarines Sur, 18;			0.19		1,338			3,098	4,717	6,507	9,6[illegible]
		Negros Occidental, 16;			0.77		1,032			2,994	2,810	2,583	5,582
		Nueva Ecija, 22			0.30		2,265			6,785	6,785	11,385	16,432

Continued

Table 1. Continued

Culture system	Author Year of study	Location, # of respondents	Extent of respondent involvement	Average years in industry	Farm characteristics Area	Average # of cages/ ponds	Production	Nature of disposal			Total costs[1]		Returns[1, 2]	
					(ha)		(kg/ha/year)	(%)*			(Pesos/ha/year)			
								Sold	Home	Given away	Non-cash	Cash	Net	Net cash
Land-based ponds	Sevilleja, R.C. (1985)	Central Luzon Provinces (100)	part-time, 92%	4	2.83	6	917	67	19	3	1,130	5,595	6,034	5,755
	1982	Pampanga, 30;		2	1.93	4	686	59	18	1	840	5,347	2,459	1,962
		Tarlac, 30;		3	2.99	8	1,565	52	31	1	897	4,737	8,099	7,896
		Nueva Ecija, 25;		4	4.62	7	714	79	13	5	649	4,709	4,286	3,098
		Bulacan, 15		8	1.40	6	1,466	89	6	4	2,654	8,184	13,732	15,781
					effective area (m^2)								Financial	Economic
Lake-based cages	Lazaga, J.F. and L.L. Roa (1985)	Rizal Province (21)	n/a											
		Binangonan, 6;			623								0.96	0.81
		Cardona, 15			874								0.92	0.80
	1980-1983													
					(m^2)		(kg/farm)				Non-cash	Cash	Net	Net cash
Lake-based pens	Laopao, M.L. and P.S. Caddanao (1984)	Rizal, 25;	part-time, 53%	2-3 years – 70%	3,913	3	1,997				7,312	5,558	(157)	7,255
		Laguna, 26		4-5 years – 18%	1,000	2	960				4,792	5,347	(104)	4,688
				6-10 years – 12%										
	1982													
					small (23)—<500		347				3,435	1,281	(500)	2,935
					medium (15)—501-1,500		921				4,643	2,669	1,126	5,769
					large (13)—>1,500		2,238				12,271	16,136	(2,424)	9,847
								(%)			(Pesos/farm/season)			
					(m^2)		(kg/crop)	Sold	Home	Given away	Non-cash	Cash	Net	Net cash
Lake-based cages	Aragon, C.T., M.M. de Lim and G.L. Tioseco (1985)	Laguna Provinces (90)												
		Los Baños, 29;	full-time, 54%	4	small—532 (₱10,000 capital investment)	2	370	98	1	1	4,361	1,697	(2,758)	1,570
	1982	San Pablo City, 63	full-time, 84%	3	small—420	2	2,561	98	1	1	3,273	5,004	22,469	25,140
					medium—848 (₱10,000-19,999)	3	5,622	99	1	< .05	5,892	10,351	51,219	56,369
					large, grow-out operation (≥ ₱20,000)	7	18,070	98	1	1	24,922	28,175	150,732	173,004
					large, plus hatchery-operation	15					73,416	27,348	230,484	285,694

Continued

Table 1. Continued

Culture system	Author Year of study	Location, # of respondents	Extent of respondent involvement	Average years in industry	Farm characteristics									
					Area	Average # of cages/ ponds	Production	Nature of disposal			Total costs[1]		Returns[1,2]	
					(m^2)		(kg/farm/crop)		(%)		(Pesos/farm/crop)			
								Sold	Home	Given away	Non-cash	Cash	Net	Net cash
Lake-based cages	Escover, E.M. and R.L. Claveria (1985)	Bicol Region (70)	part-time, 74%	2		5	401	87	6	70	1,664	1,890	(513)	793
		Lake Buhi, 50; Lake Bato, 20			small (31)—<99		253				1,303	1,014	(525)	666
					medium (19)—100-200		315				1,747	1,435	(639)	765
	1982				large (20)—>200		728				2,143	3,679	(374)	1,017
					(m^2)	size (m^2)	(kg/farm/crop)		(%)		(Pesos/farm/crop)			
								Sold	Home	Given away			Net	
Lake-based cages	Olivia, L.P. (1985)	Mindanao Region (113)	full-time, 33%	<5 years — 76 6-10 years — 16	1,548	179					28,115		35,234	
		Lake Buluan (Southern Phil. Dev't. Authority), 1;	full-time, 100%	>10 years — 8	55,000	50	550,000	100	0	0	2,487,125		3,012,875	
		Lake Sebu, 58;	full-time, 38%		1,638	250	3,191	92	6	2	7,387		6,376	
		Lake Lanao, 54	full-time, 26%		462	105	1,900	83	12	5	7,899		8,031	

[1] Exchange rates for US$1 = 1980 (₱7.51); 1981 (₱7.90); 1982 (₱8.54); 1983 (₱11.11).
[2] Net = revenue minus costs. Net cash = revenue minus cash costs.
[3] Does not total 100% because of other fish species harvested (*Channa striata, Carassius carassius, Cyprinus carpio* and *Clarias batrachus*).

Economic Profile of the Tilapia Industry

Supply

Data discussed in this section come from the statistics of the Philippine Fisheries Development Authority (PFDA) and Bureau of Agricultural Economics (BAEcon).

The increasing trend in tilapia production is evident from steady increases in unloadings in different markets of the country. Tilapia unloadings were highest in Navotas (the major fish landing in Metro Manila) with a total of 5,314 t from 1979 to 1986, representing an annual growth rate of 51% from a level of 110 t in 1979 to 1,924 t in 1986 (see Fig. 1 for monthly unloadings during this period). Increases in tilapia unloadings were also noted over the same time period in other regional markets.

PRICE AND VOLUME RELATIONSHIPS

With the exception of Zamboanga in the south, tilapia prices over the 1979-1986 period in all regions increased even when tilapia unloadings increased. This would imply that factors other than tilapia's own price have caused this increased demand.

SEASONAL VARIATIONS

The eight-year average monthly unloadings for the same period for all regions of the country showed no distinct peak or low months which would imply different production cycles among regions. However, it could be said that production is low during the first months of the year for most regions (Fig. 2).

Monthly wholesale prices at Navotas were more erratic relative to monthly retail prices (Fig. 3). This indicates that due to the market's relatively larger supply, it has become a buyer's market with buyers dictating the price. The other markets where smooth movements in the monthly wholesale prices relative to retail prices are seller's markets, with sellers commanding price regardless of the existing supply.

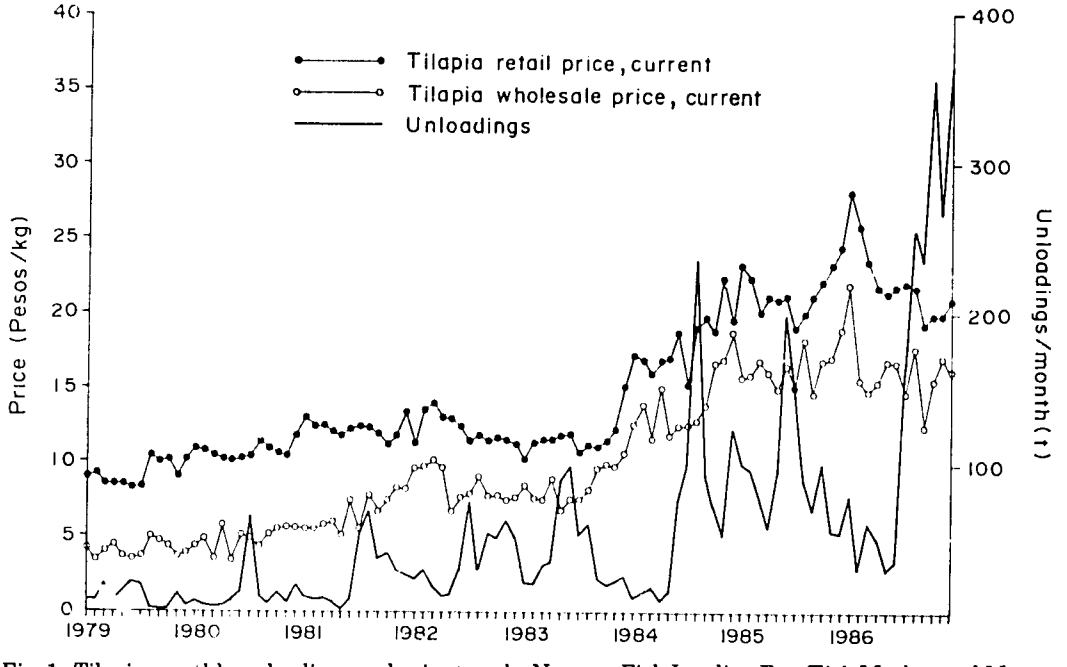

Fig. 1. Tilapia monthly unloadings and price trends, Navotas Fish Landing Port/Fish Market and Metro Manila, Philippines, 1979-1986. (Source: PFDA and BAEcon).

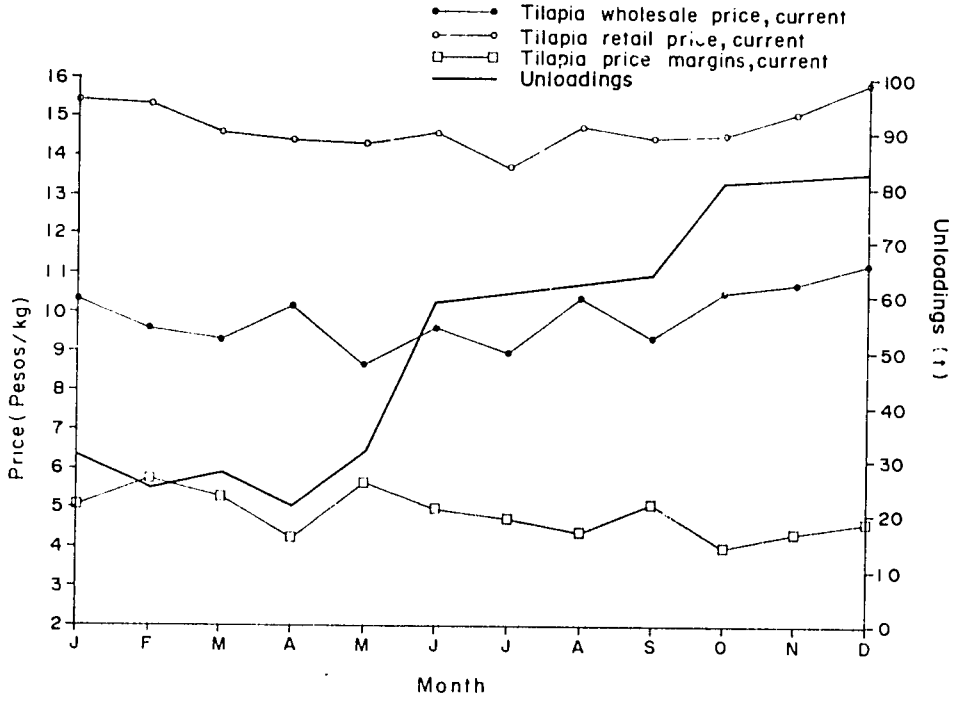

Fig. 2. Average seasonal/monthly unloadings and prices for tilapia, Navotas Fish Landing Port/Fish Market and Metro Manila, Philippines, 1979-1986. (Source: PFDA and BAEcon).

Prices

WHOLESALE AND RETAIL PRICES IN CURRENT TERMS

In general, tilapia prices in current terms (not adjusted for inflation) show upward movement for both wholesale and retail levels (see Fig. 3 for Navotas prices). Prices are higher in Luzon (Dagupan, Lucena, Navotas-Metro Manila) than in the Visayas (Iloilo) and Mindanao (Zamboanga). Annual average growth rates in tilapia prices at both wholesale and retail levels from 1979 to 1986 were highest at 28% in Navotas-Metro Manila and lowest at 11% in Zamboanga. Increases in current prices were more pronounced in 1984-1986.

WHOLESALE AND RETAIL PRICES IN REAL TERMS

In real terms, after adjusting for inflation, a 5% downward trend is exhibited for tilapia retail prices in Navotas over the past eight years (Fig. 4). If, as many believe, tilapia species mix has changed during this period to more predominant *O. niloticus* from *O. mossambicus*, prices have been maintained only because of the higher value to consumers of the former. Wholesale prices, in contrast, have increased slightly over the same period and the price margin between wholesale and retail prices has narrowed. Markets at Navotas-Metro Manila, Lucena and Iloilo showed pronounced monthly fluctuations, in contrast to Dagupan and Iloilo where monthly price fluctuations exhibited smooth decreasing movements.

COMPARATIVE PRICE ANALYSIS WITH ROUND SCAD AND MILKFISH

Prices of tilapia and round scad (*Decapterus* spp.) from 1979 to 1986 are competitively close in the Navotas-Metro Manila markets (Figs. 5 and 6). On the

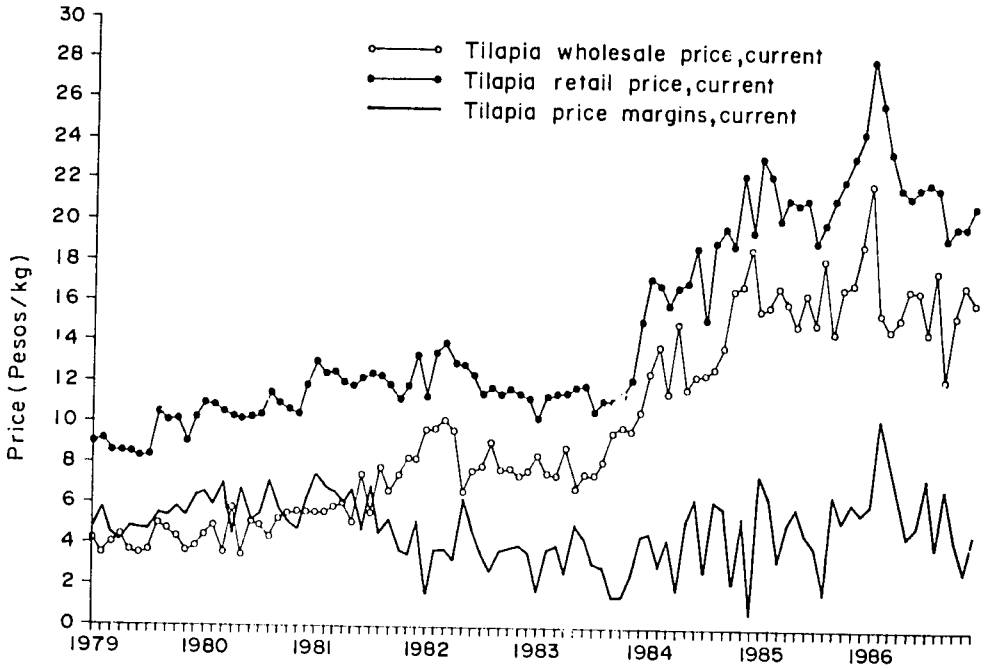

Fig. 3. Wholesale, retail and price margins for tilapia (current terms), Navotas and Metro Manila markets, Philippines, 1979-1986. (Source: PFDA and BAEcon).

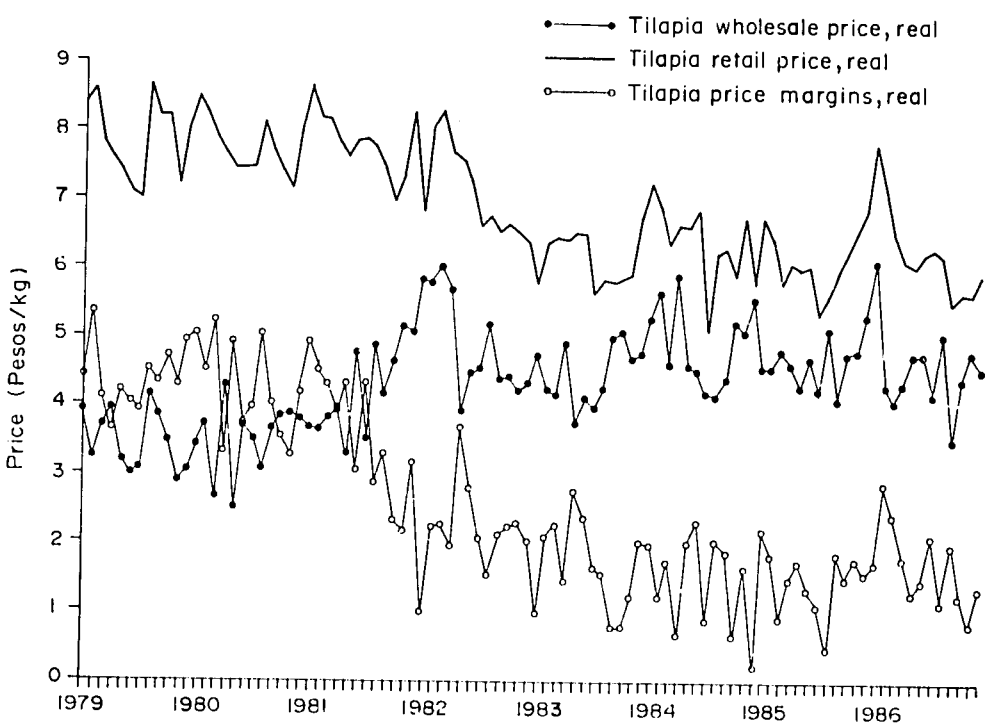

Fig. 4. Wholesale, retail and price margins for tilapia (real terms), Navotas and Metro Manila markets, Philippines, 1979-1986. (Source: PFDA and BAEcon).

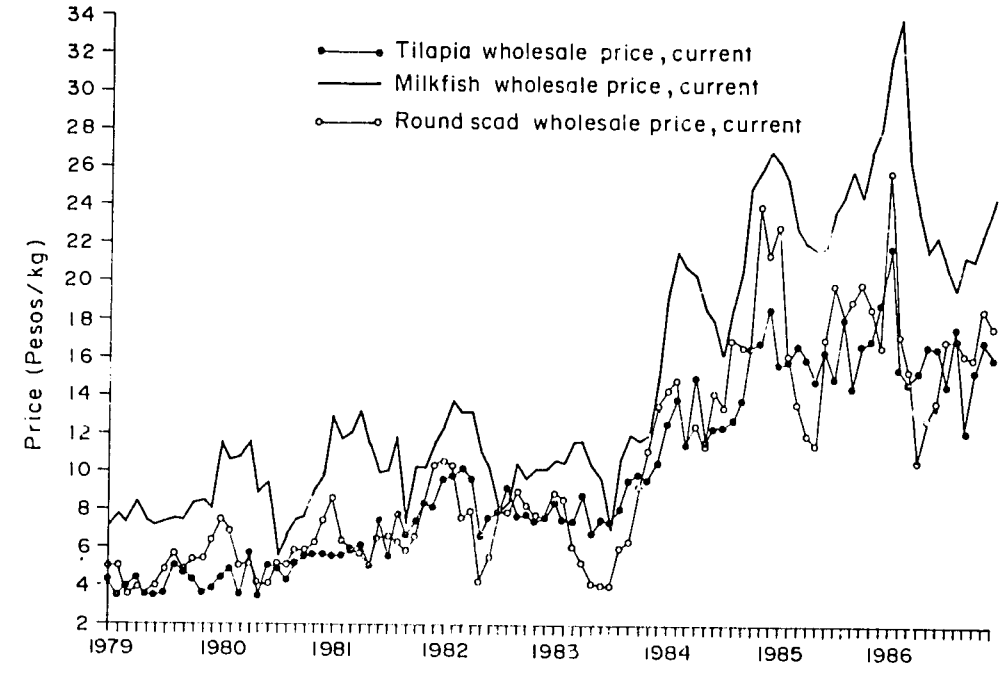

Fig. 5. Comparative wholesale price trends of tilapia, milkfish and round scad, Navotas and Metro Manila markets, Philippines, 1979-1986. (Source: PFDA and BAEcon).

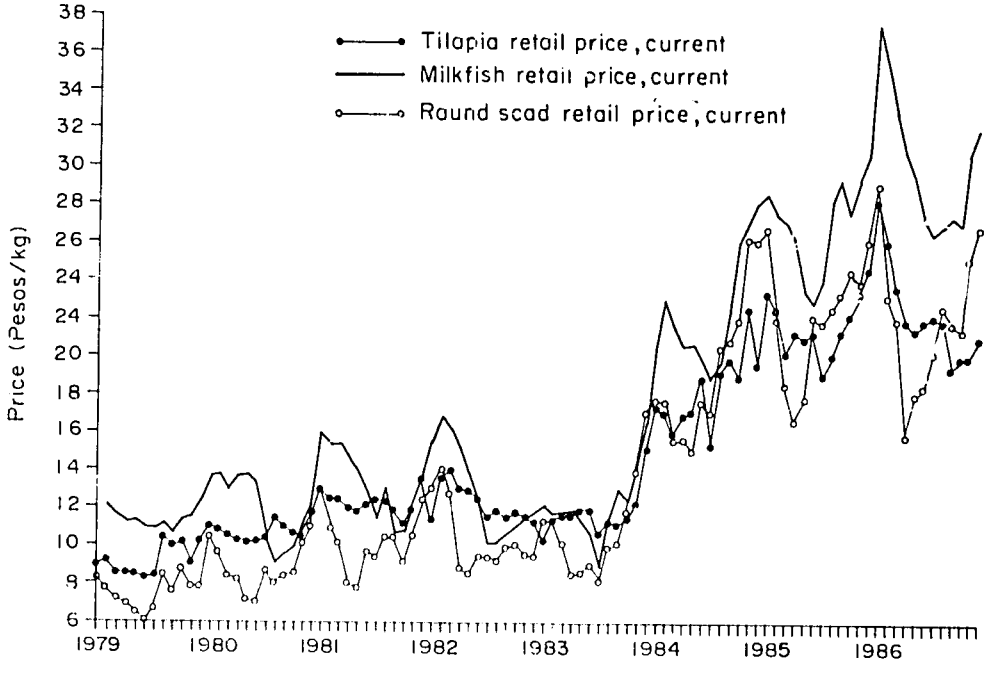

Fig. 6. Comparative retail price trends of tilapia, milkfish and round scad, Navotas and Metro Manila markets, Philippines, 1979-1986. (Source: PFDA and BAEcon).

average, wholesale price of tilapia in 1986 at P16.23/kg is lower by P0.39/kg than that of round scad while in retail levels, tilapia at P22.21/kg is P0.22/kg more than round scad. Prices elsewhere in the country for these two species were similarly close.

Prices of milkfish (the most commonly cited market substitute), however, are well above tilapia and round scad prices. The milkfish/tilapia differential averages P4.01/kg in Navotas-Metro Manila, P4.72/kg in Dagupan, P5.17/kg in Lucena, P6.42/kg in Iloilo and P3.51/kg in Zamboanga. Prices for tilapia and milkfish followed the same trend from 1979 to 1986 except for Zamboanga where pronounced milkfish price increases were noted starting 1985, thus widening its price gap with tilapia.

Price Margins

Price margins, the difference between two prices at different market levels, are rough representations of marketing costs. For tilapia, such costs involve storage, transportation and handling plus some profit for the middleman. Price margins at Navotas-Metro Manila initially decreased from P5.54/kg in 1979 to 1981 to about P3.21/kg in 1982 to 1983, followed by an increase to P5.10/kg in 1984 to 1986. Nevertheless, at the end of this eight-year period, price margins were still 2.2% higher than at the beginning. Price margins elsewhere in the country have exhibited mixed movements over this period and no clear pattern emerges, except perhaps in Zamboanga where the low levels and decreasing price margins pose a disincentive for producers/traders. This may explain why only a small quantity of tilapia is being marketed in the area.

Demand/Consumers Acceptance

NATIONAL AND REGIONAL PER CAPITA CONSUMPTION

National annual per capita consumption of tilapia grew 12% from 0.8 kg in 1974-1986 to 0.9 kg in 1977-1978 (Aviguetero et al. 1979a, 1982). The low consumption rates are in the southern part of the Philippines--all the Visayas and Mindanao except for Central Mindanao. Regretfully, consumption surveys were discontinued in the late 1970s, so more recent supply and price data are the best available indicators of continued growth of the industry.

COMPARATIVE ANALYSIS: PER CAPITA CONSUMPTION OF MILKFISH AND ROUND SCAD

National consumption rates of milkfish and tilapia were both higher during the late 1970s than they had been earlier. Nationwide, milkfish consumption per capita was 2.7 kg/year and round scad was 1.7 kg/year. Milkfish was definitely the choice fish species among these three for Metro Manila with a consumption rate of 5.65 kg/year, compared to those for tilapia and round scad which were 0.95 kg/year and 2.55 kg/year, respectively. As for tilapia, the regions in the north have higher consumption rates than those in the south.

NATIONAL PER CAPITA CONSUMPTION BY INCOME GROUP

National annual rates of tilapia consumption by income group reveal that the highest income group (> P1,500/month) has double the consumption levels (1 kg/year) of the lowest income groups (< P400/month) for the period 1970 to 1976 (Aviguetero et al. 1979b, 1980). The middle income groups (P400-799 and P800-1,499) have the same consumption level of 0.8 kg/year.

COMPARATIVE ANALYSIS WITH MILKFISH AND ROUND SCAD: NATIONAL PER CAPITA CONSUMPTION BY INCOME GROUPS

In contrast to tilapia consumption patterns, similarly annual per capita consumption for milkfish increased with income. The pattern for round scad shows

that the highest consumption rate per year was the middle income group at 1.8 kg, the lowest income group only 1.6 kg. However, in terms of levels of annual consumption rates for all income groups, milkfish is highest at 3.26 kg, round scad at 1.72 kg and tilapia with only 0.76 kg. These rates may have changed somewhat since these surveys were conducted ten years ago, but the relative importance of the three species is still the same.

It is important to keep in mind in the context of these data that per capita consumption of *all* seafoods declined from 40 kg/year in 1970 to around 25 kg/year in 1980 (Regalado 1984, cited in Gonzales 1985). This decline has occurred despite steady production increases, and apparently reflects the combined effects of declining Filipino purchasing power and increasing population and exports of aquatic products.

Discussion

While it is clear from production data over the past decade that tilapia has been increasing its small domestic market share relative to other species, it is possible to draw only tentative conclusions regarding its full market potential in the Philippines.

In part this problem is caused by uncertainty over future disposable incomes of consumers. Tilapia market potential also depends upon total supply of aquatic products. While one can be reasonably certain that total fish supply from the capture fishery (marine and inland waters) will not increase due to the national constraints of these resources and the increased cost (e.g., fuel) of exploiting them, to date much of the difference between this supply and consumer demand has been made up by aquaculture. Over the past three years, annual growth rates of the entire aquaculture sector as high as 25% are commonly cited.

Nevertheless, the most important factor for fisheries and aquaculture will be future consumer purchasing power. Since 1983, the purchasing power of the average Filipino has declined by almost 30% due to the very high inflation the country experienced in 1983-1985. Tilapia, while not a luxury product, is expensive enough to inhibit low income customers, the majority of all potential consumers. The minimum wage in the Philippines for nonagricultural workers (in Metro Manila) is currently only P51.70/day. An expanded market potential for tilapia will thus critically depend upon the future incomes of Filipino consumers, approximately two-thirds of whom (both rural and urban) currently fall below the country's official poverty line.

The various financial analyses of tilapia farms reported here indicate considerable room for expanded tilapia production even at the expense of prevailing profit levels. With the exception of Laguna de Bay cage culture systems, most tilapia culture operations are earning profits well above opportunity costs, thus indicating room for increased production even if market prices should fall somewhat as a result. Tilapia, therefore, offers considerable hope as a culture species which can be produced by a broad spectrum of rural producers for domestic markets.

To achieve this potential, it will be necessary to provide a supply of good quality fingerlings at affordable prices to the small farmers. The supply of higher quality (i.e., faster growing) fingerlings would be an alternative way to maintain farm level profits in the face of falling market prices. Indeed this would be the key to increasing production levels in a market with uncertain price dimensions. Technical support to small farmers and assistance with marketing will be necessary prerequisites to assure their continued participation in the industry. In this respect, the Philippines appears to be well ahead of most countries with the Bureau of Fisheries and Aquatic Resources (BFAR) hatchery in Muñoz and satellite hatcheries around the country capable of assuming a lead role in support of the country's tilapia industry.

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Pond Culture of Tilapia in Rwanda, a High Altitude Equatorial African Country

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Abstract

Tilapia culture in Rwanda is practiced in ponds at altitudes from 1,300 m to 2,500 m. Air and water temperature are lower than those in other tropical countries where tilapia culture is typically practiced. These conditions require careful pond water management practices for tilapia culture to be successful. Results from experiments and rural harvests indicate that *Oreochromis niloticus* is superior to *Tilapia rendalli* and *O. macrochir* in Rwandan conditions.

Reproductive tendencies of *O. niloticus* are different from those seen elsewhere: age at first reproduction is higher, time before resumption of reproduction after restocking is longer, and number of fingerlings produced per surface area is less. Natural productivity measured in local ponds ranged from 40 to 210 kg/ha/year and net productivity in poorly managed ponds receiving inputs was generally less than 500 kg/ha/year. However, in well managed ponds, *O. niloticus* can show average growth of over 1.0 g/day and net productivity of 3,000 kg/ha/year.

Introduction

Rwanda a mountainous, land-locked country in east-central Africa, has been the site of pond culture of tilapia since at least the 1930s. In the late 1950s, there were over 2,000 ponds in Rwanda, with an average production of 400 kg/ha/year (Aubray 1976). Most of these ponds were rural; but there were some governmental and institutional stations. Although institutional support for fish culture was curtailed in the early 1960s, there was continued interest in the rural sector. However attrition substantially reduced the number of active ponds.

In the late 1970's the Rwandan government began to encourage fish culture with the goal of supplementing the production of animal protein. However, there was some doubt as to the appropriateness of fish culture in Rwanda. These concerns arose largely from doubts as to the suitability of the culture of tilapia in the climatic conditions of Rwanda.

Climate

Although Rwanda is equatorial (approximately 2°S), its climate is tempered by altitude. The result is a temperature regime which is cooler than that found in most of the equatorial regions. The areas of the country where tilapia culture is practiced range from 1,300 m to 2,500 m. At these altitudes, minimum ambient air temperatures are often below 10°C while afternoon air temperatures rarely reach 35°C (Fig. 1).

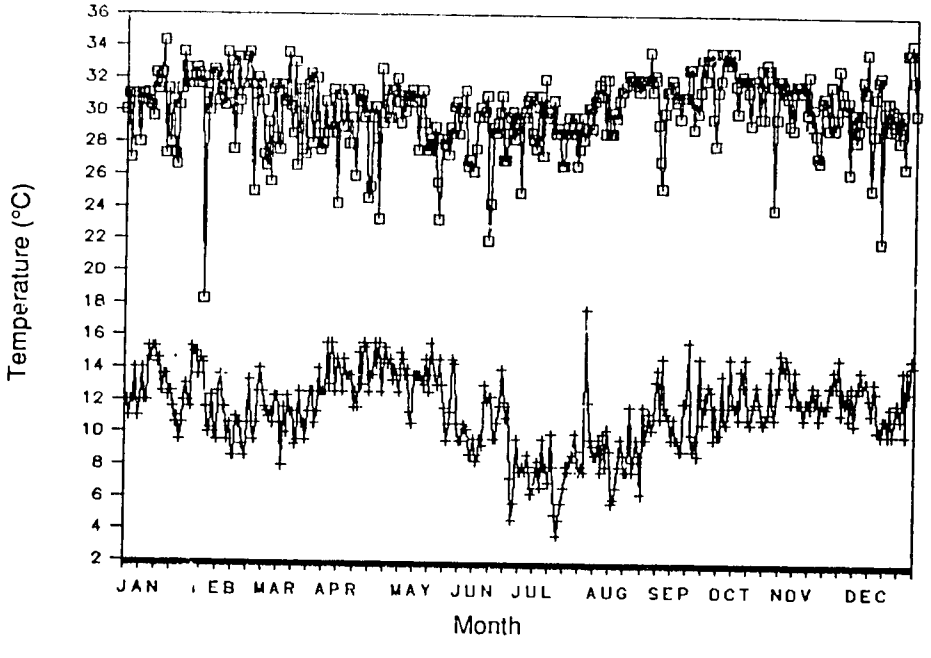

Fig. 1. Annual variation in daily minimum (+) and maximum (□) air temperature (in °C) at the National University of Rwanda fish culture station at Rwasave, near Butare, at an elevation of 1,700 m.

Since this temperature regime is inferior to that which is generally considered optimum for tilapia growth (e.g., Balarin 1979; Pruginin 1983), it is important to take advantage of the thermodynamic inertia of water through careful management of pond water. The heat retention capacity of standing water buffers the temperature fluctuations so that temperature variations in ponds are less than those of the air and the running water in feeder canals. In ponds with minimal water loss, minimum pond water temperatures are often 9-10°C higher than the minimum temperatures of nearby running water (Fig. 2). Ponds with good algal blooms may be an additional 3-4°C higher than those without blooms. The minimization of pond water losses becomes especially important at high altitude stations such as the National Fish Culture Program (PPN) station at Ndorwa (2,200 m) where minimum morning water temperatures in the canal fall to 10°C but are higher in the ponds (Table 1). Maximum (afternoon) water temperatures in the ponds are also usually 3-4°C higher than those in the canal.

Seasonal variations in temperature also influence tilapia growth and reproduction, although the magnitude of the effect is not well quantified. Rwanda has four seasons: a long dry season, which lasts approximately from mid-June to mid-September; a short rainy season, from mid-September to mid-December; a short dry season, from mid-December to mid-February; and a long rainy season from mid-February to mid-June. Pond water temperatures remain cooler in the dry periods; they drop lower at night and do not warm up during the day to levels experienced during the rainy periods.

Species cultured in Rwanda

Three species of tilapia have been cultured in Rwanda; of these, only one, *O.* .

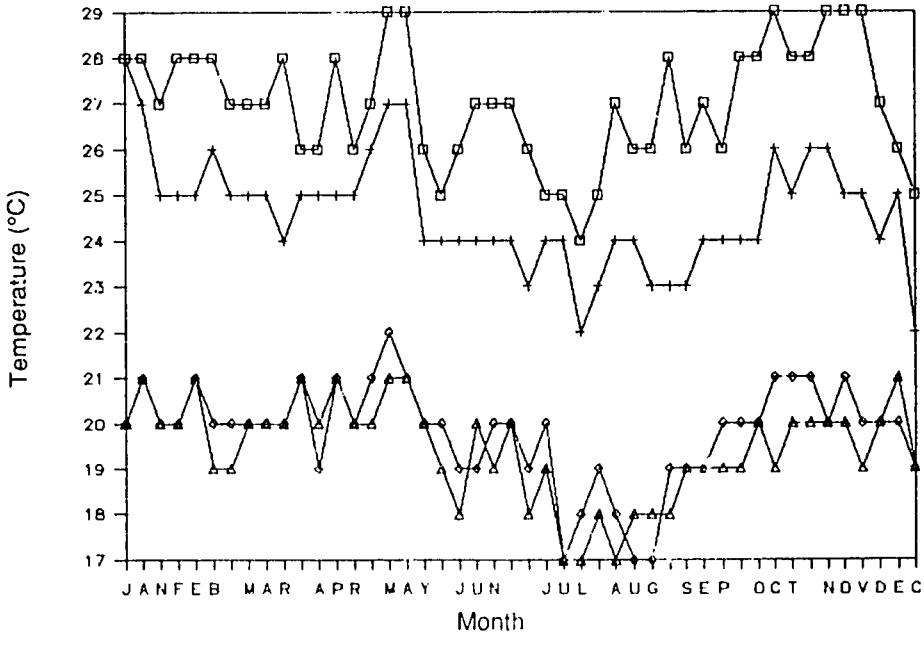

Fig. 2. Annual variation in weekly minimum and maximum water temperatures (°C) measured at depths of 25 cm and 50 cm at the National University of Rwanda fish culture station at Rwasave, near Butare. Symbols: min. at 50 cm (△); min. at 25 cm (◇); max. at 50 cm (+); max at 25 cm (□).

Table 1. Annual variation in monthly extreme (minimum and maximum) and mean morning and afternoon temperatures at the Ndorwa station of the National Fish Culture Program, Rwanda, located at an altitude of 2,200 m. Means are calculated from daily measurements in °C

	Morning				Afternoon			
	Minimum		Mean		Maximum		Mean	
	Canal	Pond	Canal	Pond	Canal	Pond	Canal	Pond
Jan	12	14	14	16	22	25	20	22
Feb	10	13	13	15	23	26	18	20
Mar								
Apr	15	18	16	19	22	26	20	24
May	11	16	16	18	24	26	21	24
Jun	12	16	16	19	23	27	20	22
Jul	13	16	16	19	24	27	20	22
Aug	10	15	13	17	20	24	18	21
Sep	12	17	15	18	21	24	20	23
Oct	13	16	15	19	23	26	21	24

niloticus is indigenous (Philippart and Ruwet 1982). The other two, *O. macrochir* and *T. rendalli*, were introduced. Pure native *O. niloticus* stocks were lost through hybridization with *O. macrochir*. Similar hybridization has been reported by Ruwet et al. (1975) and Moreau (1983). The loss of native *O. niloticus* stocks was unfortunate in view of the evidence of the superiority of this species in pond culture.

Maletoungou (1976) recommends *O. niloticus* after comparing it with *O. macrochir* and *T. rendalli*. His experience is that *O. macrochir* production is low and that mortality is high. *T. rendalli* growth is slower than *O. niloticus;* however reproduction is greater. He also notes that *O. niloticus* grows rapidly and is resistant to traditional methods of manipulation and transportation. George (1976) states that *O. niloticus* fry are hardy and tolerate long distance transportation, whereas Ruwet et al. (1976) note that *O. macrochir* and *T. rendalli* seem fragile. Bard et al. (1976) criticize *T. rendalli's* slow growth rate and emphasize that reproduction starts early and is abundant. They suggest that among the tilapias, *O. niloticus* is the most suitable for pond culture. Pullin (1983) states that an ongoing worldwide survey by the International Center for Living Aquatic Resources Management (ICLARM), Manila, Philippines, on the status of tilapia as a cultured food commodity is confirming the superiority of *O. niloticus* in many tropical situations. Schmidt and Vinke (1981) recommended *O. niloticus* for Rwanda, although they felt that *O. macrochir* and *T. rendalli* also may be useful for polyculture.

These considerations, as well as others, resulted in a proposal to reintroduce *O. niloticus* to Rwanda. After consideration of the logistics involved in transporting a satisfactory number of fish to Rwanda, it was decided that stocks from Auburn University, USA, would be used for the reintroduction. In 1984, three shipments, totalling approximately 450 fingerlings, were transported to Rwanda to be used as broodstock. The performance of the offspring has been examined in on-station trials, and fingerlings have been distributed for use in the rural sector.

Results

Comparison of species

There has been some question as to the appropriateness of tilapia culture in Rwanda. The body of data and observations, both experimental and anecdotal,

pertinent to pond culture of tilapia in Rwanda is growing rapidly but there are still unanswered questions and certain data are inconclusive.

Survival and growth of *O. niloticus* and *O. macrochir* were compared in a 13-month growth experiment conducted at the Gitarama station of the PPN. *O. niloticus* showed higher productivity (1,001 kg/ha/year *vs.* 780 kg/ha/year) and higher survival (82% *vs.* 48%) than *O. macrochir*. Adult fish accounted for a higher percentage of the total harvest weight for *O. niloticus* than for *O. macrochir* (74% *vs.* 53%).

We have also noted in Rwanda a marked difference between the survival rates of *O. niloticus* and *O. macrochir* fingerlings exposed to the same handling conditions. *O. niloticus* and *O. macrochir* fingerlings have been fished from adjoining ponds on the same day, and whereas mortality of *O. niloticus* fingerlings is generally less than 10%, that of *O. macrochir* fingerlings may approach 50%.

The performance of *T. rendalli* has been compared to that of *O. niloticus* at the Rwasave station, as well as at the Gikongoro station of the PPN. At Rwasave, *T. rendalli* production was 515 kg/ha/year, whereas *O. niloticus* production was 1,553 kg/ha/year. *T. rendalli* production was similar at Gikongoro; 597 kg/ha/year. The size attained by *T. rendalli* is less than that of *O. niloticus*. In growout ponds, *T. rendalli* adults seem to reach a weight plateau of about 100 g.

An additional factor influencing the choice of *O. niloticus* over *T. rendalli* is the apparent sensitivity of *T. rendalli* to *Aeromonas hydrophila*. At the Rwasave station, there have been two serious outbreaks of this bacterial infection in *T. rendalli*, which were evidently stressed by abnormally low temperatures. *O. niloticus* in neighboring ponds were unaffected. Both *T. rendalli* and *O. macrochir* have suffered similar infections and high mortalities (> 50%) at the PPN stations of Kigembe and Gikongoro, although the infection was not conclusively identified.

Reproduction of O. niloticus

Reproductive patterns of *O. niloticus* in Rwandan ponds are strikingly different from those observed in other countries. For example, the age at first reproduction is higher under Rwandan conditions. In grow-out ponds at medium altitudes *O. niloticus* rarely reproduce until they are 6-9 months old. The first spawns are minimal and only after another 2-3 months is there significant spawning. At higher altitudes, in cooler waters, reproduction is further retarded. At Ndorwa, 4-5 month old *O. niloticus* (average weight 69 g) were stocked at 1/m^2 and after 6 months had not begun to produce fingerlings, even though they had reached an average weight of 231 g.

After starting to reproduce, mature broodstock, when moved to a different pond, require a longer adjustment period before resuming reproduction. In the Congo (Brazzaville), *O. niloticus* fingerlings can be observed 45 days after the stocking of mature broodstock. In Côte d'Ivoire, the first fingerlings can be removed from broodponds 45 days after ponds are stocked with 100 g fish (Holl 1983). In Rwanda, at the Rwasave station and at the PPN station at Runyinya, when broodstock which are already reproducing are moved to different ponds, the first fingerlings are not seined until 90 days after stocking. Fingerling production does not reach its peak for an additional 2-3 months. The adjustment period in Rwanda is 2 or 3 times longer than that noted in other countries.

The number of fingerlings produced per unit area is substantially less in Rwanda than that reported elsewhere. In Côte d'Ivoire, broodstock given a supplemental feed of 23% protein at a daily rate of 6% of the initial body weight produced 210,000 fingerlings/ha/month. At Rwasave, fingerling production averaged 82,500 fingerlings/ha/month in a pond with a good algal bloom receiving supplemental feeding of wheat bran and rice bran at a rate of 5% of the body weight of the broodstock. The station averages

20,000 to 30,000 fingerlings/ha/month. At Runyinya, the average production is within the same range.

The average production per female is comparable in Rwanda to that observed elsewhere. The number of fingerlings per female at Loka, Côte d'Ivoire, averaged 41 (Holl 1983). At Runyinya the range is from 23 to 87 with 5-8 times fewer females per unit area than stocked at Loka.

The seasonal effect on reproduction is evident. Fingerling numbers dwindle following the colder months of the dry season, however, reproductive activity does not stop completely.

Fingerling production in the rural sector is often inadequate to provide for stocking needs, therefore fingerling production centers have been established (Veverica et al., in prep.). Research is ongoing to determine the most effective system of fingerling production. Stocking densities and sex ratios are being examined, as well as the period between stocking and draining. The delay between stocking of broodstock and their first reproduction precludes a system with frequent drainings, such as is practiced in the Congo. Even draining ponds after 5 months as practiced in Côte d'Ivoire would result in ponds being unproductive half the time.

Another factor being examined is the time interval between seinings. In Côte d'Ivoire, the interval time was 15 days. In Rwanda, several intervals (2 weeks, 1 month and 2 months) have been tried but the results are inconclusive. Nevertheless, it does appear that a 1-month seining interval is preferable to a 2-month interval. In some cases the number of fingerlings recovered after 2 months is equal or slightly superior to the number which would have been recovered after 1 month. In other cases, the number after 2 months is approximately double the number expected after 1 month. Cases in which the 2-month harvest is greatly superior to that which would have been expected with two 1-month seinings are rare. Seining once a month does not appear to have a deleterious effect on spawning females.

Another advantage to monthly seining is the periodic elimination of tadpoles and frogs, mostly *Xenopus* sp., which can cause severe problems when handling fingerlings. The total weight of frogs taken in a seine may exceed that of the fingerlings. In addition to the handling problems caused by the frogs themselves, their foamy secretion increases fingerling mortality due to suffocation.

Conclusion

The definitive answer to the question of the appropriateness of tilapia culture in Rwanda is still forthcoming. Many questions remain relative to the biology of tilapia in this climate and the optimum management techniques for the culture of tilapia in the conditions of Rwanda. But there is little doubt that in certain conditions, with an appropriate management scheme, tilapia culture is suitable to Rwanda.

As cited above, average production in the late 1950s was estimated at 400 kg/ha/year. Average production in the early 1980s similarly was found to be between 300 and 500 kg/ha/year. Both these results are some improvement over the natural productivity which was found to be from 40 to 210 kg/ha. Generally, production in ponds which are poorly constructed, poorly managed and receiving minimum inputs is less than 500 kg/ha/year. However, ponds which are carefully constructed and well managed are much more productive. Generally, ponds at Rwasave and at the PPN stations produce 1,500-2,000 kg/ha/year, with *O. niloticus* showing individual growth rates of 0.5-0.7 g/day. At the Rwasave station, *O. niloticus* grown in ponds receiving inputs of 500 kg/ha/week of chicken manure showed growth rates of 1.3 g/day and productivity exceeding 3,000 kg/ha/year. Although such inputs are not within the procurement possibilities of Rwandan farmers, the results show that such growth and production are possible in the climatic conditions of Rwanda. Thus, the

body of data and observations which is accumulating indicates that there is certainly a future for the pond culture of tilapia in this high altitude, equatorial African country.

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Development of Appropriate Pond Management Techniques for Use by Rural Rwandan Farmers

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Abstract

With fishponds at elevations of 1,300-2,500 m, Rwanda has a unique environment for tropical fish culture. Average pond temperatures are 21°C in the morning and 26°C in the afternoon. Introductory efforts at promoting aquaculture met with farmer support but their approach failed to compensate for cooler temperatures. Results were minimal with production of only 200-500 kg/ha/year, albeit, thousands of small hand-dug ponds were built.

With an ever-increasing population, competition developed for land and agricultural inputs. Pressure to increase farm outputs, especially those with high nutritional and cash value, focused attention upon increasing yields from existing fishponds.

With no commercial animal feeds, few agricultural byproducts, and little chemical fertilizer, fish production must depend upon limited quantities of organic fertilizer. Within this scheme, tilapia were viewed as the appropriate fish, although *Clarias* and carps had been cultured. Of tilapias cultured, *Oreochromis niloticus* proved most suited to management inputs.

Initial work centered upon maximizing pond temperatures through water management. With appropriate pond depths and water regulation, morning pond temperatures can be 2-5°C warmer than surrounding natural waters. Subsequent efforts focused upon comparing nutrient inputs and stocking densities. A mixture of grasses and manure was applied at high and low rates with two stocking densities in ponds with and without supplemental feeding of bran. Management relying only upon fertilizer, with high application rates and low stocking density, produced 854 kg/ha/year, with management techniques well within the scope of rural farmers. Average production of 1,382 kg/ha/year was obtained from fed ponds with similar management, but with inputs that were not appropriate for most farmers.

Introduction

Pond culture of tilapia was introduced into Rwanda during the Belgian colonial period. Early efforts were modeled after ongoing aquaculture programs in the Belgian Congo. However, their classic lowland approach did not prove applicable to a country where elevations are between 1,000 and 4,500 m and where morning temperatures can be below 10°C and afternoon temperatures rarely reach 35°C (Hanson et al., this vol.). Consequently, fishpond production remained low, averaging 400 kg/ha/year, only slightly above natural productivity (Aubray 1976). The efforts were successful, however, in generating a high interest in aquaculture among Rwandan farmers.

With an expanding population, Africa's densest, Rwanda is aware of the potential problems of feeding this population as land becomes less and less available. With average farm plots of 0.8 ha, unproductive farm activities cannot be tolerated when there are always alternative land uses. If farmers are to continue to practice fish culture it must be financially viable when compared with comparable land crops. In 1983 the National Fish Culture Project was established to revitalize the existing aquaculture facilities with the goal of increasing productivity through improved and appropriate fishpond management. Based on ponds harvested in 1983/84 average fishpond production was 343 kg/ha/year (Table 1).

Appropriate Management System

The fish

Early attempts at aquaculture employed a variety of culture species: tilapia; *Haplochromis; Clarias;* common, mirror and grass carps. However, tilapia have long been the focus of attention due to the ease of seed production and more easily satisfied dietary requirements. Although *Oreochromis niloticus* is

Table 1. Summary of rural fishpond production in Rwanda from 1983 until 1986. Total number of ponds indicates those ponds included in the National Fish Culture Project's annual census. Pond harvests indicated are those supervised by the project's extension service.

	Year			
	1983	1984	1985	1986
Total number of ponds	945	1,569	1,229	1,795
Number of ponds harvested	0	165	242	415
Production (kg/ha/year)	0	343	662	1,265

indigenous to Rwanda (Philippart and Ruwet 1982), initial attempts focused upon two species that were introduced in the 1940s: *T. rendalli* and *Oreochromis macrochir* (de Vries 1971). It was felt that combined culture of these two species would be well suited to local conditions and would be desirable since they occupy nonoverlapping niches (Schmidt and Vincke 1981). Subsequent studies, however, have shown that both introduced species possess several unfavorable characteristics that make them less desirable (Hanson et al., this vol.).

Renewed activity centered upon *O. niloticus* as a fish having broad dietary characteristics and being well atuned to an area where inputs are variable. Unfortunately, local stock of *O. niloticus* had been contaminated through hybridization with *O. macrochir*. In 1984, broodfish of Egyptian origin were imported into Rwanda from Auburn University, USA. Awaiting an adequate supply of seed for distribution to farmers, attention was directed towards designing an appropriate pond management system by first identifying the major constraints and then establishing approaches for farmers to overcome these constraints.

The environment

Since it is often accepted that tilapia do not grow well in cool climates (Chervinski 1982), preliminary studies included establishing a temperature scheme representing different regions of the country. Through a number of reporting stations measuring morning and afternoon temperatures in flowing and static water, it was established that average morning static water temperatures (at a depth of 10 cm) were 21°C, while the afternoon average was 26°C. As it was discovered that previous management systems encouraged flowing water through fishponds, static water was instituted instead, with the result that ponds maintained temperatures 2-5°C warmer than did the flow-through ponds (Fig. 1).

Eliminating flow-through also prevented a loss of valuable nutrients with the water leaving the pond and increased pond fertility. Since many of the ponds were old and poorly built, seepage accounted for significant water losses. To avoid cold water entering continuously to replace that lost through seepage and evaporation, farmers were presented the

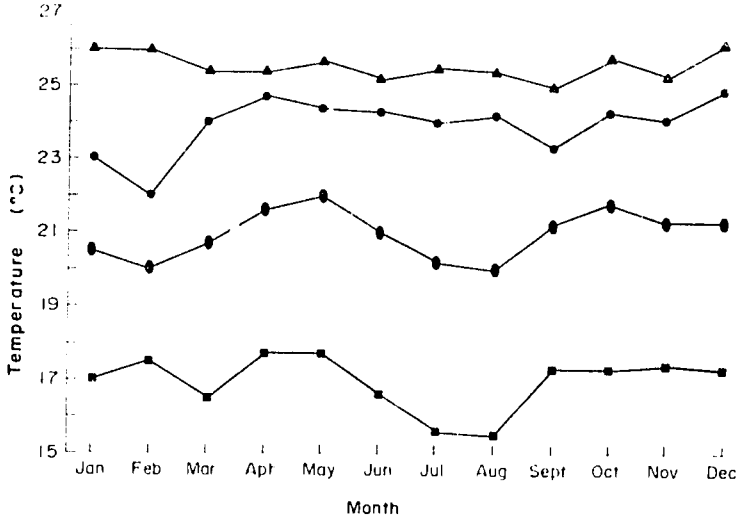

Fig. 1. Average morning and afternoon water temperatures in ponds (measured at the surface) and in the canal at the Gikongoro station, Rwanda. The monthly means are calculated from daily measurements. Symbols: canal, morning ■; pond, morning ◑; canal, afternoon ●; pond, afternoon ▲.

"on/off" system, whereby the pond inlet is kept closed until combined water losses equal a 10 cm reduction in pond depth, at which time the inlet is opened and the pond refilled.

To make ponds more effective thermal sinks, newly constructed and renovated ponds are designed to have shallower depths. Pond depth is presented as a function of the minimum rather than the maximum depth. Recommended minimum depths are 40-50 cm. Pond bottoms are built with a 1-2% slope such that maximum depth does not exceed one meter. Observations at government stations revealed that fish began feeding sooner in ponds with more shallow areas, which warm earlier in the morning. However, efforts were also taken to avoid ponds that were extremely shallow and which consequently offered little heat storage and encouraged growth of macrophytes and bird predation. With proper water management, combined with a fuller understanding of the fundamentals of raising fish, rural farmers increased fishpond production in 1985 to an average of 662 kg/ha/year (Table 1), almost a 100% increase over 1984.

Another important environmental factor is the soft acid water found throughout a large part of the country. Natural waterways frequently have pH of 6.0-6.5 with a total hardness and alkalinities of 15-20 ppm. Unquestionably these waters do not provide an ideal culture medium. Although raw limestone is available, its prohibitive cost precludes liming fishponds. Chemical fertilizers are also prohibitively expensive and beyond the scope of rural fish culture.

Inputs available

Subsistence agriculture practiced in a country with a high population density and with limited large-scale agro-business creates a situation where there are few inputs available for animal husbandry. All inputs have alternative uses. Using organic fertilizers on land crops is a common practice. These fertilizers, consisting chiefly of compost and manures, are the only inputs readily available for aquaculture. In areas of the country where there is grain production, organic fertilizers can be supplemented by brans (e.g., rice or wheat) with the potential of doubling production. Depending upon pond fertility and feeding rate, bran can have a conversion of 5-10 kg of bran per kg of fish flesh. If any transport costs are incurred in obtaining the bran, it is not cost effective and is not considered as an essential element in a pond management system.

After surveying the rural sector, it was concluded that the only inputs generally available and affordable would be the organic fertilizers and that any management system would need to rely heavily upon them. To assess the role of organic fertilization on fishpond production, organic fertilizer was defined (for the purpose of replication) as a mixture of, by volume, 80% dried grasses and 20% manure.

Field observations and interviews indicated that a farmer could quite readily obtain a quantity of this grass/manure mixture roughly equal to an application rate of 0.25 m^3/ha of pond surface prior to stocking and subsequent monthly applications (i.e., 48 m^3/ha total application for a 9-month growing period). Although later studies showed that this quantity is indeed available to farmers, it was found to be insufficient to produce economically acceptable results. In field trials, *O. niloticus* fed 3% (biomass) rice bran with the above-mentioned organic fertilization (compost) rate had an average production of 1,010 kg/ha/year while better farmers utilizing compost on an "as much as available/whatever available" basis produced quantities exceeding 2,000 kg/ha/year. Thus, it was decided to proceed with further trials to evaluate higher application rates in the hope that the increase in production attributable to increased fertilization would encourage farmers to obtain the required quantities of compost.

Field trials maximizing fishpond production concentrated upon input levels, using the previous 0.25 m^3/ha of compost

and 3% bran as a starting point. At the same time it was decided to examine the role of stocking rate on production and average size of fish within given management systems. Previous efforts had proposed a stocking density of 2 fish/m^2 (Schmidt and Vincke 1981); and indeed many of the older ponds in the rural sector had been stocked at this density with *O. macrochir* and *T. rendalli* mixtures. However, post-stocking mortality was high and true densities, as indicated by the number of fish harvested, were frequently as low as 0.5/m^2. Furthermore, given that Rwandan markets do not easily accept fish smaller than 150 g (Veverica et al., this vol.), overstocking would be expected to encourage competition for limited nutrients with the result that a large portion of the harvest would be of submarket size. Consequently, to answer the question of the most appropriate stocking density for the various management systems, this factor as well was varied in different treatments as indicated in Table 2. Table 3 indicates the results of these trials.

It can be seen that even with a quadrupled compost application, compost alone does not compensate for reductions in supplemental feeding. Treatments using compost alone had lower productivity than those using less compost but supplemented with feeding for both stocking densities. In addition, the average size of fish harvested was small at both densities resulting in a lower value of harvest.

High compost applications without feeding do give yields that approach the break-even point for Rwandan fish culture as established by Schmidt and Vincke (1981). It can be noted that although total productivity was greater at higher stocking densities with similar management, the per cent of marketable fish was greater with the lower density. However, how this translates into the cash value of the crop varies with the intensity of management; lower stocking rates being more profitable at lower management levels. At present market values, the lower density would have a value of $11.25 per are ($1,125/ha) with high management and $2.58 per are ($258/ha) with low; while the higher density would have a value of $14.34 per are ($1,434/ha) at high management and $1.41 per are ($141/ha) at low. These figures could be compared to values of $18.84 per are ($1,334/ha) for low density and $87.69 ($3,769/ha) for high, if all the fish harvested were of market size.

Management practices

If the yields presented in Tables 1 and 3 are compared to those cited in Table 4, it is evident that the management systems employed demonstrate a noticeable improvement over previous efforts under similar conditions. Moreover, although considerable attention has been focused upon *O. niloticus*, the species used for the

Table 2. Treatments used in field trials to evaluate possible management systems for tilapia *(Oreochromis niloticus)* culture by rural Rwandan fish farmers.

Treatment	Stocking density (No. fish/m^2)	Compost application (m^3/ha)	Feeding
I	0.67	0.25	3% bran
II	0.67	1.00	3% bran
III	0.67	1.00	none
IV	1.33	1.00	3% bran
V	1.33	1.00	none

Table 3. Results of field trials for tilapia *(Oreochromis niloticus)* culture in Rwanda on an individual pond basis, each treatment having three ponds for replications.

Treatment	Net production (kg/ha/year)	Average weight of females (g)	Average weight of males (g)	Overall average weight (g)
I	940	67.8	116.6	92.4
	980	72.6	116.5	98.6
	1,142	76.0	128.0	99.9
II	1,323	98.0	148.5	125.1
	1,679	110.4	197.8	156.5
	1,144	92.4	142.2	117.0
III	1,016	79.6	129.1	106.9
	568	48.8	74.8	58.6
	981	68.8	118.1	98.1
IV	1,535	65.9	122.7	93.6
	2,101	73.0	121.9	100.7
	2,090	72.3	117.8	95.6
V	1,893	57.8	88.6	69.7
	1,840	65.6	104.1	84.1
	1,001	41.0	75.1	58.0

Table 4. Fishpond yields reported by various authors from previous efforts to develop a grassroots aquaculture program in Rwanda.

Author	Date	Production cited (kg/ha/year)
Philemotte	1955	976
de Vries	1971	574
Dunn	1974	500-4,000
Aubray	1976	400
Schmidt and Vincke	1981	243

above-mentioned trials, rural ponds employing improved management techniques with *O. macrochir* and/or *T. rendalli* also showed marked improvement. A sample of such ponds harvested during the last quarter of 1986 shows an average production of 1,397 kg/ha/year while ponds stocked with *O. niloticus* and harvested during the same period produced 1,389 kg/ha/year on the average. Table 1 shows that overall pond production in 1986 was 1,265 kg/ha/year, a 370% increase over the 1984 figure. As there has been a marked increase in fishpond productivity through improved management, the average 1986 figure should be viewed with consideration to the fact that during that same year, 12% of Rwandan rural ponds produced more than 2,000 kg/ha/year.

The quantity of inputs available continues to be the chief limiting factor to further increases in productivity. Composting remains the principal means of nutrient input. In more densely populated areas there is a definite ceiling to the quantities of organic materials available for composting. Additional inputs in the form of supplemental feeds, therefore, are required for higher production. Agricultural and/or industrial byproducts are of practical value in only localized areas of the country due to high transport costs. Most rural farmers supplement composting by feeding a variety of green leaves such as manioc and arrowroot. However, efforts to quantify production increases through feeding leaves have been blocked by a lack of sufficient quantities necessary for a comprehensive field trial study.

Stocking densities recommended for rural fish farmers remain comparatively low. Initial stocking is 1 fish per 1.5 m^2 (0.67/m^2) as employed in the trials. If results of their first and second harvests are favorable, good adopters will be encouraged to increase the density to 1/m^2. As small fish are not readily marketable, it is important that rural ponds produce a more-or-less uniform size fish of 100-200 g if the crop is to have maximum value. With restricted input levels, high densities will not provide the desirable crop.

Frequently, rural ponds are in production for 9-12 months. This is a result of numerous climatological, biological, and social factors. The trials previously mentioned took place over a 6-month period (i.e., 167-171 days). With average growth noted during the trials as 0.5 g/day (varying considerably with the treatment) a pond should be in production for 8-9 months for market-size fish to be produced. Increased growth of over 1 g/day has been noted (Hanson et al., this vol.), but the level of inputs required are beyond the means of the average farmer. Although productivity of a pond may be at maximum after 8-9 months, there may not be enough fingerlings at that time to restock. It has been noted that reproduction starts only after fish are 6 to 9 months old, and that there may not be significant numbers of fingerlings in a pond until reproduction has been taking place for 2 to 3 months (Hanson et al., this vol.). Hence, farmers often have to choose between a management system with maximum production but little or no seed for restocking, or a system with sufficient seed but reduced food fish production.

Conclusions

Through improved management, fishponds have proven to be a viable on-the-farm operation for Rwandan farmers. Tilapia culture in cool, high altitude regions of Rwanda has proved to be economically feasible. Although production is noticeably less than in lowland areas, it is mostly attributable to limited nutrient inputs. While effective water management can reduce the impact of cooler temperatures within certain limits, temperature will undoubtedly serve as the ultimate limiting factor if nutrient shortages can be overcome.

Present efforts continue to focus on methods of further increasing production despite severely restricted inputs. As management in terms of inputs and outputs is solidified, attention must turn to stocking density and size at stocking as they relate to production period and the market value of the crop. As corresponding improvements are made in the management of land crops, fish production, or the value of the fish crop, must continue to increase if it is to continue to attract the attention of farmers.

Acknowledgements

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Economics of Tilapia Pen Culture Using Various Feeds in Thale Noi, Songkhla Lake, Thailand

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Abstract

The objective of this study was to evaluate the production of Nile tilapia *(Oreochromis niloticus)* in pen culture using various feeds. The study was conducted in six 26.33 m^2 subdivided circular cages with stocking density of 9 fish/m^2. Tilapia grew from 10.63 g to 300 g in 14 months when fed with chicken pellets, from 9.13 g to 93.03 g with no supplementary feed and from 21.74 g to 86.99 g when fed with fresh aquatic weed *(Ceratophyllum demersum)*. The results of economic evaluation showed that chicken pellets gave the best production (1.74 kg/m^2) but production costs were higher than the other two treatments. None of the three treatments was profitable. With regard to the type of feed for tilapia pen culture in Thale Noi either no supplementary feed or feeding tilapia with fresh weed *(Ceratophyllum demersum)* or other suitable weed may be preferred to reduce losses.

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Introduction

This study is part of the project entitled "Utilization of Aquatic Plants in Lake Songkhla (Thale Noi)", the largest lake in Thailand. The aim of study was to investigate the utilization of natural resources in the lake. Songkhla Lake can be divided into three parts according to the salinity of the water (Fig. 1): 1) the northern most part is a freshwater area covering 70% of the lake surface; 2) the southern part of the lake is a seawater area covering about 25% of the lake surface; and 3) the middle area is a brackish-water area covering about 5% of the lake surface. Thale Noi, in the northernmost part of the lake is a freshwater lagoon of approximately 25 km^2. There is potential in this area for fish culture, especially tilapia which gave the best survival rate and highest production in an earlier study in Thale Noi (Tansakul 1985). Tilapia have a high tolerance to adverse environmental conditions (Guerrero 1982), especially acidic water as is found in Thale Noi (Tansakul 1985).

The present study was undertaken to investigate the production and profitability of tilapia fed on 1) chicken pellet; 2) aquatic weed *(Ceratophyllum demersum);* and 3) with no supplementary feeding.

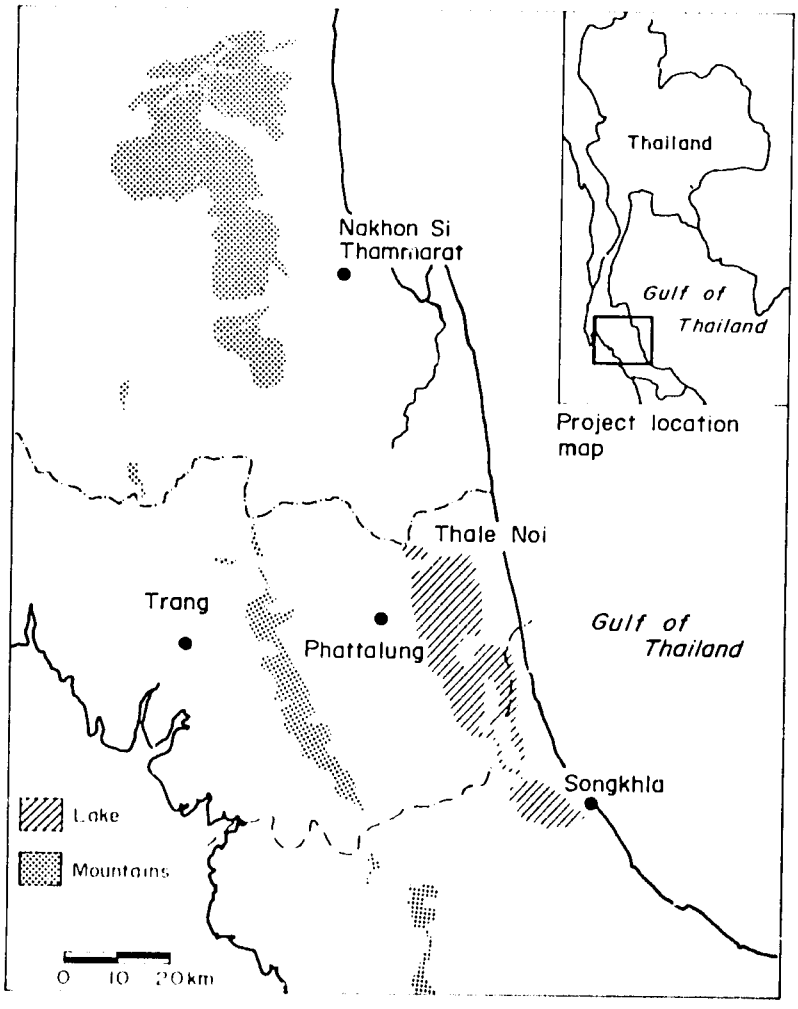

Fig. 1. Location of the study area.

Materials and Methods

For replication purposes, three 10-m diameter wooden pens were divided into three equal sections, each section having an area of 26.33 m^2. Nile tilapia *(Oreochromis niloticus)* were stocked at a density of 9 fish/m^2 into each compartment. The experiment was separated into three feeding treatments.

1) Treatment A - fed on chicken pellets

2) Treatment B - fed on fresh aquatic weed *(Ceratophyllum demersum)*

3) Treatment C - no supplementary feeding.

Fish were fed at 5% body weight/day, once a day. The experiment was carried out for 14 months from July 1983 to September 1984. Each month fish were captured and checked for growth rate, mortality rate, food conversion rate and the feeding rate was adjusted accordingly.

Water quality parameters inside the fish pens were taken at monthly intervals and at the same time of the day (0900 hours). Dissolved oxygen (DO), turbidity, temperature, conductivity and pH were measured with a Horiba Water Checker (Horiba Co., Ltd., Japan). Alkalinity and depth were measured following standard methods (APHA-AWWA-WPCF, 1981).

Results

Tilapia growth

The results of this study are shown in Table 1 and Fig. 2. Tilapia grew from 10.6 g to 300 g in 14 months when fed on chicken pellets, from 21.7 g to 87.0 g when fed on *C. demersum* and from 9.3 g to 97.0 g when no supplementary feed was added. There was no significant difference (ANOVA) in survival rates among the three treatments which ranged from 73 to 82%. With regard to fish production, Treatment A gave the best production of 1.74 kg/m^2.

The water quality measurements obtained during the study are shown in Table 2. Water quality parameters among the pens, and on a seasonal basis were not significantly different (ANOVA) (Cochran and Cox 1957). The water quality in all of the pens was poor. For example, between November 1983 and August 1984, both pH and DO values were low ranging from 5 to 6 and 1 to 5 ppm, respectively. The water depth in all of the pens was low ranging from 70 cm in August 1984 to 180 cm in December 1983.

Economic considerations

The production costs of each treatment have been summarized in Table 3. As the same experimental design was used for each treatment, input costs were also considered to be the same. Differences in production costs were, therefore, due to feeding costs, which varied according to the type of feed used. The comparative costs of each culture system were calculated according to the method of Omar (1984) and are tabulated in Table 3. The analysis showed that although the production of fish fed on chicken pellet was the highest, the net profit (-135.88 baht/m^2) obtained from this treatment was much lower than the net profit obtained from Treatments B and C. The profitability of the two latter treatments appeared to be similar, i.e., net profit of -10.03 baht/m^2 and -10.24 baht/m^2 for Treatments B and C, respectively.

Thus fish culture with no supplementary feed was the most economical system as input costs are low and the value of production is high, compared with the other two feeding systems. However, none was profitable over the study period.

Discussion and Conclusions

The results of this study correspond well with other recent studies, which indicate that tilapia are omnivores and show excellent growth rates on low protein diet, whether obtained from natural aquatic life or from supplementary feed (Kuo and Neal 1982). Nile tilapia have also shown an ability to select food which

Table 1. Growth rate of Nile tilapia *Oreochromis niloticus* in 26.33 sq m^2 pen culture with different types of feeds from July 1983 to September 1984.

Treatment	A	B	C
Stocking density (no./m^2)	9	9	9
Type of feed**	c.p.	c.d.	—
Feeding rate (% body weight/day)	5	5	no feed
Initial weight (g/fish)	10.63	21.74	9.33
Final weight (g/fish)	300.70	86.99	97.03
Survival rate (%)	81.57	78.34	72.86
Food conversion rate (g feed/g fish)***	15.10	15.20	–
Net production (kg/m^2)	1.74	0.58	0.50

**c.p. = chicken pellet.
c.d. = *Ceratophyllum demersum*.
***High values because much feed drifted away from the cages.

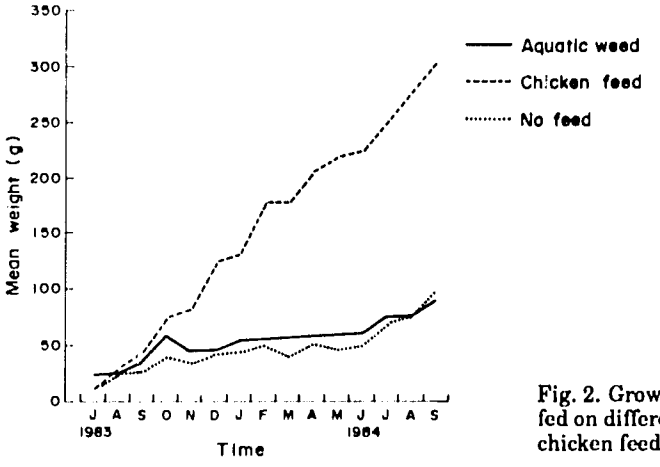

Fig. 2. Growth rates of Nile tilapia *(Oreochromis niloticus)* fed on different types of feed. Note that rapid dispersion of chicken feed in the water caused high losses.

Table 2. Mean values of water quality in fish pens, at 0900 hours (from July 1983 to September 1984).

Month	Alkalinity (ppm)	Conductivity (mS/cm)	D.O. (ppm)	Temperature (°C)	pH	Turbidity (ppm)	Depth (cm)
1983							
Jul	22.53 ± 1.59	0.20 ± 0	6.97 ± 0.58	31.40 ± 0.17	5.87 ± 0.12	56.33 ± 5.78	74.67 ± 2.60
Aug	25.70 ± 1.10	0.10 ± 0	9.03 ± 0.99	29.57 ± 0.18	9.10 ± 0.12	29.67 ± 2.67	70.30 ± 1.20
Sep	26.67 ± 1.01	0.36 ± 0.03	6.67 ± 0.58	29.03 ± 0.09	7.97 ± 0.12	17.33 ± 3.38	74.33 ± 2.33
Oct	27.77 ± 0.43	0.30 ± 0.12	4.20 ± 0.45	28.03 ± 0.29	7.90 ± 0.17	23.33 ± 5.84	86.67 ± 1.67
Nov	11.10 ± 2.08	0.53 ± 0.03	2.00 ± 0.42	27.67 ± 0.09	6.47 ± 0.03	34.33 ± 4.10	136.33 ± 0.33
1984							
Jan	8.82 ± 0.52	0.90 ± 0	2.43 ± 0.62	28.17 ± 0.33	5.53 ± 0.58	36.67 ± 12.02	136.33 ± 3.33
Feb	12.67 ± 0.17	0.73 ± 0.12	2.23 ± 0.23	27.87 ± 0.09	6.30 ± 0.06	13.33 ± 3.18	115.00 ± 1.00
Apr	16.67 ± 0.88	0.73 ± 0.33	3.07 ± 0.58	29.47 ± 0.03	5.73 ± 0.17	25.33 ± 2.19	75.00 ± 0.58
May	22.53 ± 0.44	0.70 ± 0	2.40 ± 0.06	29.43 ± 0.03	6.17 ± 0.03	9.67 ± 2.96	77.00 ± 1.53
Jun	16.67 ± 0.93	0.70 ± 0	2.63 ± 2.31	28.87 ± 0.03	6.53 ± 0.03	38.00 ± 10.12	68.00 ± 2.00
Jul	18.70 ± 0.76	0.70 ± 0	2.70 ± 0.21	29.50 ± 0.05	6.47 ± 0.03	64.33 ± 5.33	80.33 ± 1.20
Aug	14.87 ± 0.30	0.70 ± 0	5.53 ± 0.28	28.20 ± 0.10	6.73 ± 0.17	45.33 ± 11.47	57.67 ± 1.45
Sep	19.17 ± 0.44	0.70 ± 0	5.17 ± 0.99	28.80 ± 0.06	7.07 ± 0.07	47.67 ± 3.67	75.00 ± 2.65

Table 3. Cost evaluation of Nile tilapia *(Oreochromis niloticus)* in pen culture fed on different types of feeds (US$1.00 = 26.50 Baht). All costs except for feed costs apportioned equally among treatments. (Production period = 14 months).

Annual production costs (Baht/m^2)	Chicken pellet	Feed *Ceratophyllum demersum*	No feed
Fixed costs			
Salary of one permanent staff	9.67	9.67	9.67
Wage and labor for harvesting	0.64	0.64	0.64
Subtotal	10.31	10.31	10.31
Variable costs			
Seeds	4.71	4.71	4.71
Feeds	170.00	0.91	—
Transportation costs	1.53	1.53	1.53
Miscellaneous expense	1.23	1.23	1.23
Subtotal	177.47	8.38	7.47
Total	187.78	18.69	17.78
Production (kg/m^2)	1.73	0.58	0.50
Gross revenue (Baht/m^2)*	51.90	8.67	7.54
Feed costs (Baht/kg fish)	98.26	1.58	0
Labor costs (Baht/kg fish)	0.37	1.11	1.27
Net profit (Baht/m^2)	−135.88	−10.03	−10.24

*Market price of fish fed on *C. demersum* and no feed at 15 Baht/kg and fish fed on chicken pellet at 30 Baht/m^2

will maximize growth (Bowen 1982). Although Treatment A (chicken pellets) showed high production/m2, investment costs were also much higher compared with Treatments B and C (Table 3). This is due to the high cost of feed. Dela Cruz (1980) and Balarin (1982) also found that feed was the most expensive component in tilapia culture systems.

In conclusion, high growth rates and food conversion rates are not the only factors to be considered when evaluating the potential of a particular feed for fish culture. The financial aspects of each treatment must also be examined.

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Extension Programs in Support of the Tilapia Industry in the Philippines

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Abstract

This paper presents the status of freshwater aquaculture extension in the Philippines with emphasis on Nile tilapia *(Oreochromis niloticus)*. The extension and training programs of the Philippine Bureau of Fisheries and Aquatic Resources (BFAR) are explained and discussed. The importance and value of the practical application of extension are underscored. BFAR demonstration projects such as growout fishponds, hatchery fishponds, backyard fishponds and integrated rice-fish and gabi (taro)-fish farming are noted. The paper concludes with a recommendation for intensified local participation in information dissemination, establishment of pilot demonstration projects and closer coordination among food production agencies, research institutions and the private sector to advance tilapia farming.

Introduction

The Philippines has a predominantly agricultural society composed of small farmers, agricultural laborers, fishermen, fish farmers and other related industry workers. Aquaculture is recognized as a major economic growth sector by the Department of Agriculture, which includes the Bureau of Fisheries and Aquatic Resources (BFAR).

In 1985, the fisheries sector contributed 4.7% at current prices to the Gross National Product or 5.0% based on constant prices. Directly, the industry employs about one million individuals engaged in fishponds, fishpens, fish cages, and municipal and commercial fishing.

In general, fish ranks second to rice in order of importance as a staple food. The average annual per capita food fish consumption as determined by the Food and Nutrition Research Institute as of 1982 was 40 kg.

The principal objectives of the National Development Plan (1987-1992) are: the alleviation of poverty, the generation of more productive employment, the promotion of equity and social justice and the attainment of sustainable growth and development (NEDA 1986).

BFAR's role is to implement the Short Term Recovery Plan for the Fishery Sector which is geared to create a favorable economic environment for fishermen/fish farmers and to provide them with access to land, new technology, adequate infrastructure, a market information service and to provide fisheries households and other elements of the rural society, particularly small-scale fishermen/fish farmers, with greater opportunities in life. A program on aquaculture development is included.

Aquaculture has been practiced in the Philippines for centuries, based on brackishwater pond culture of milkfish and in recent years freshwater aquaculture. Tilapia farming began in 1950 with the introduction of *Oreochromis mossambicus* from Thailand. This species created technical and managerial problems for fish farmers. However, the introduction of the faster growing *Oreochromis niloticus* or Nile tilapia in the early 1970s accelerated the growth and development of freshwater farming in the country. Nile tilapia has grown to be an important food fish for domestic consumption and has export potential. It is now widely accepted in many areas of the country.

The production of tilapia has been consistently increasing. In 1980 annual production was 10,014 t; by 1986, production was 47,000 t. This yield came from aquaculture and municipal inland waters through capture as documented in BFAR statistics. The economics on the country's tilapia industry have been recently discussed (Smith et al. 1985).

Support Program for Freshwater Aquaculture

One major support program of BFAR in the expansion and development of tilapia aquaculture is the Freshwater Fisheries Development Project (FFDP) which began in 1979; the project established the Freshwater Fish Hatchery and Extension Training Center (FFH-ETC) and stimulated the development of freshwater aquaculture in Central Luzon (Fig. 1). The Center was assisted by the

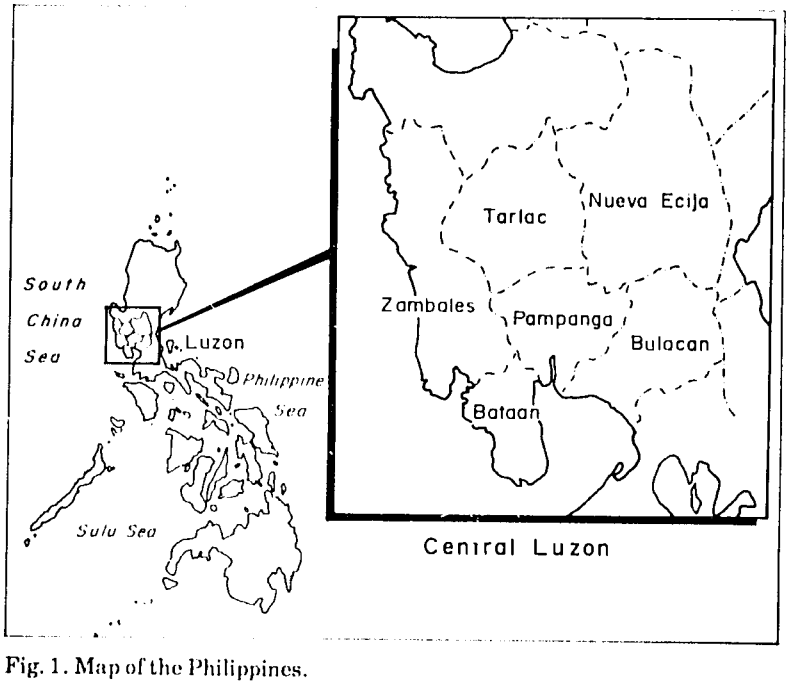

Fig. 1. Map of the Philippines.

United States Agency for International Development (USAID) for a 5-year period. The FFH-ETC, now called the National Freshwater Fisheries Center (NFFC), is located at Central Luzon State University, Nueva Ecija, adjacent to the Freshwater Aquaculture Center, a basic research center on inland fisheries. The NFFC has a national role in freshwater aquaculture development, with the primary aim of increasing tilapia production and distribution of good quality tilapia and carp fingerlings and breeders for hatcheries and growout ponds. The Department of Agriculture's 33 freshwater demonstration farms presented in Table 1 are strategically situated in all regions. They serve as satellite farms to receive fingerlings and breeders of Nile tilapia and other promising strains mass-produced from the NFFC.

The objectives of FFDP are to augment the income of fish farmers and to increase protein consumption of the people through freshwater aquaculture development.

In order to realize these objectives, pilot demonstration projects were established at the FFH-ETC on various aquaculture technologies.

The results of four demonstration extension projects are presented in Table 2. Varied types of farm operations are considered: backyard fishpond, hatchery, gabi-fish culture and rice-fish culture. The projects were carried out by farmer cooperators.

As the table clearly shows, such projects can provide a farmer with ample income as well as fish protein for his family and at the same time elevate the standard of living in the community.

Extension Delivery System of the NFFC

One of the essential elements in the extension program is the capability of the government freshwater fish farm to provide the necessary tilapia fingerlings. The demand for good quality fingerlings and breeders is great and maintenance of the breeding stock necessitates a proper hatchery management program (Broussard et al. 1984).

The NFFC started to assist the freshwater fisheries industry, especially in Nile tilapia farming, in 1981 through the concerted efforts of the three functional units of the center: the hatchery, and the extension and training groups.

The hatchery group provides the necessary fingerlings and breeders for individual fishfarmers with backyard fishponds, hatcheries, growout fishponds, cages, fish pens and integrated projects as well for communal stocking in livelihood projects. Nile tilapia fingerlings/breeders are distributed at nominal prices to farmers and free of charge to communal and government projects.

The Extension and Training group promotes the transfer of aquaculture technology generated by the Center (BFAR 1984). Training programs are also offered by the center to update the knowledge and skills of extension officers/technicians and fish farmers on the latest trends in aquaculture techniques. The trainees are given hands-on experiences in tilapia hatchery procedures, fishpond management, harvesting techniques and practical fish culture.

Distribution of Nile tilapia fingerlings/breeders up to 1986 is presented in Table 3. Over 16 million fish were dispersed. Central Luzon was the top recipient of fingerlings (60%), followed by Southern Luzon. Small-scale fish farmers with backyard fishponds, hatcheries, growout fishponds and integrated fish projects from Central Luzon were the main recipients. This clearly shows that Central Luzon benefited most from the services and assistance rendered by NFFC.

Pilot Demonstration Farm Concept

A demonstration pilot farm is one of the most effective methods used by the NFFC Extension group. The main purpose

Table 1. Regional freshwater fish farms of the Philippine Bureau of Fisheries and Aquatic Resources.

	Location	Region	Developed area (ha)
1.	San Isidro Fish Farm San Isidro, Abra	I	8.00
2.	Batac Fish Farm Billoca, Batac, Ilocos Norte	I	10.34
3.	Laoag Fish Nursery Laoag City, Ilocos Norte	I	1.75
4.	La Trinidad Fish Nursery La Trinidad, Benguet	I	3.00
5.	Paoay Lake Fish Nursery Paoay, Ilocos Norte	I	1.25
6.	Vigan Fish Nursery Vigan, Ilocos Sur	I	0.32
7.	Natividad Freshwater Fish Farm Natividad, Pangasinan	I	3.30
8.	Sta. Rita Fish Farm Sta. Rita, Agoo, La Union	I	0.80
9.	Lal-lo Freshwater Fish Farm Lal-lo, Cagayan	II	0.18
10.	San Mateo Fish Farm San Mateo, Isabela	II	3.00
11.	San Pablo Fish Farm San Pablo, Isabela	II	2.50
12.	Polloc Freshwater Fish Farm Roxas, Solano, Nueva Viscaya	II	2.50
13.	Ambuaya Lake Freshwater Fish Farm Kiangan, Ifugao	II	2.50
14.	Camalaniugan Fish Farm Camalaniugan, Cagayan	II	1.80
15.	Banaue Fish Farm Banaue, Ifugao	II	0.18
16.	Magsaysay Memorial Fish Nursery Looc, Castillejos, Zambales	III	1.07
17.	Bitas Freshwater Fish Nursery (MARATAF) Bitas, Arayat, Pampanga	III	0.50
18.	Los Baños Freshwater Fish Farm & Research Center Los Baños, Laguna	IV	0.50

Continued

Table 1. Continued

	Location	Region	Developed area (ha)
19.	Bay Freshwater Demonstration Fish Farm & Nursery Bay, Laguna	IV	7.00
20.	Butong Experimental Freshwater Fish Farm and Research Station Butong, Taal, Batangas	IV	1.00
21.	Sta. Cruz Freshwater Demonstration Fish Farm and Nursery Sta. Cruz, Laguna		
22.	Tanay Research Laboratory Tanay, Rizal	IV	1.80
23.	Buhi Freshwater Demonstration Fish Farm & Nursery Buhi, Camarines Sur	V	3.17
24.	Bato Fish Impounding Pen and Hatchery Bato, Camarines Sur	V	3.10
25.	Iloilo Freshwater Demonstration Fish Farm Dumangas, Iloilo	VI	
26.	San Francisco Freshwater Fish Farm San Francisco, Cebu	VII	0.50
27.	Leyte Freshwater Fish Hatchery Babatngon, Leyte	VIII	5.20
28.	Calarian Freshwater Fish Farm Calarian, Zamboanga City	IX	40.00
29.	Kitcharao Freshwater Fish Farm Kitcharao, Agusan del Norte	X	4.30
30.	Nabunturan Freshwater Demonstration Fish Farm Nabunturan, Davao del Norte	XI	3.00
31.	Marantao Freshwater Demonstration Fish Farm Kialdan, Lanao del Sur	XII	3.00
32.	Tacurong Freshwater Demonstration Fish Farm and Nursery Tacurong, Sultan Kudarat	XII	2.30
33.	Kabacan Freshwater Demonstration Fish Farm and Nursery Kabacan, North Cotabato	XII	
	Total		134.51

Source: BFAR (1986a, 1986b).

Table 2. Results of tilapia extension demonstration projects at BFAR-NFFC, Muñoz, Nueva Ecija, Philippines.

Cooperator:	Capt. Quinciano Padua, Jr.	Bienvenido Taroma	Manuel Vidad	Primo Berdulaga
Location: N.E.	Guiling, Rosales, Pangasinan	Anao, Tarlac	San Felipe, Muñoz, N.E.	Maligaya, Muñoz,
Pond Area (ha):	0.8514	0.7282	0.5	0.0986
Type of Operation:	Backyard fishpond	Hatchery	Gabi-fish culture	Rice-fish culture
Date Stocked:	3 April 1985	15 December 1984	13 November 1986	January 1984
Date Harvested:	9-22 August 1985	March-May 1985	11 March 1987	March 1984
Treatments:				
Fingerling Stocking rate (per ha)	20,000	200 kg	10,000	5,000
Fertilization rate (kg/ha/mo)				
Inorganic	100	100		
Organic	3,000	3,000	3,000	2,000
Inputs (quantity):				
No. fingerlings	17,542	-	5,000	800
No. breeders	-	3,234	-	-
No. gabi corms	-	-	10,000	-
Palay seeds (kg)	-	-	-	15
Chicken manure (bags)	292	310	243	-
Mono-ammonium phosphate (bags)	8	7.5	-	0.5
Urea (kg)	-	-	-	14
Pesticide (liters)	-	-	-	0.25
Feeds (kg)	-	-	-	20
Expenses (P):				
Land preparation	-	-	80.00	120.00
Fingerlings	Free	-	750.00	Free
Breeders	-	Free	-	-
Gabi corms	-	-	3,500.00	-
Palay seeds	-	-	-	37.50
Chicken manure	3,796.00	4,030.00	3,159.00	-
Mono-ammonium phosphate	2,000.00	1,875.00	-	81.00
Urea	-	-	-	45.92
Pesticides	-	-	-	182.00
Feeds	-	-	-	14.00
Labor (seining/harvesting/ transplanting)	-	800.00	100.00	100.00
Total	5,796.00	6,705.00	7,589.00	580.42
Output (quantity):				
Fish (kgs)	1,000	-	593	23.5
Fingerlings (pcs)	-	299,000	-	-
Gabi Corms (pcs)	-	-	100,000	-
Palay (kg)	-	-	-	483
Revenue (cost):				
Fish	23,000.00	-	13,639.00	538.20
Fingerlings	-	44,850.00	-	-
Gabi corms	-	-	40,000.00	-
Palay	-	-	-	1,110.90
Gross income:	23,000.00	44,850.00	53,639.00	1,649.10
Net income:	17,204.00	38,145.00	46,050.00	1,068.00

Table 3. Distribution of Nile tilapia *(Oreochromis niloticus)* by NFFC, Muñoz, Nueva Ecija, Philippines, 1981-1986.

Region	No. fish dispersed	%	No. recipients	%
III (Central Luzon)	9,852,889	60.2	2,063	73.1
IV (Southern Tagalog)	2,853,202	17.4	297	10.5
II (Cagayan)	1,715,702	10.5	229	8.2
I (Ilocos)	1,690,234	10.3	202	7.2
V (Bicol)	221,165	1.4	19	0.6
Other Regions	29,275	0.2	12	0.4
Abroad	312		2	-
Total	16,362,779	100	2,824	100

is to show to the farmers that new technologies in fish culture are viable and appropriate in local conditions.

The concept of the cooperator demonstration farm is the transfer of technology right in the farmers field. The center extension staff and the farmer cooperator sign a 2-year agreement regarding the extension services that will be provided by the extension specialists and also the role of the farmer cooperator. This arrangement has proven effective, giving practical learning experience to both parties. During the visits, problems are noted and given preferential attention and immediate solution. Also meetings among the household families and their neighbors are held to get the whole barangay/community aquainted with the project. Open discussions are also encouraged for better degression of their perceived needs.

Some ongoing demonstration projects being undertaken by the Center are presented in Table 4. These demonstration projects are strategically located in Central Luzon provinces where tilapia farming is still beginning to expand. The adoption and modification of the Bureau's Training Visit System by the Center's Staff also increased the effectivity of extension effort.

The extension services rendered by the NFFC during 1983-1986 reached 2,880 cooperators in various types of freshwater aquaculture. Table 5 shows the detailed breakdown of these cooperators.

Practical Training for Technicians and Farmers

The Center is staffed with trained specialists who share their expertise with both government technicians and private sector. The training programs offered by the Center facilitate the transfer of tilapia technology and update the knowledge and skills on freshwater aquaculture technology. The trainors provide the trainee with theoretical and practical exercises on tilapia hatchery management, fish health management, fishpond management, fishpond engineering and other applied subject-matter on fish culture. Field days conducted at the

Table 4. Ongoing demonstration projects of the National Freshwater Fisheries Center (NFFC), Muñoz, Nueva Ecija, Philippines.

Name of cooperator	Location of project	Type of operation	Area (ha)	Water source	No. fish stocked	Ave. wt. (g)	Variety of rice
1. Severino Bayna	Maragol, Muñor, N.E.	Rice-fish	0.73	Irrigation	3,640	12	IR-42
2. Gabriel Valdez	Maragol, Muñoz, N.E.	Rice-fish	0.98	Irrigation	4,000	6	IR-64
3. Clemente Flores	San Isidro, Tarlac	Grow-out	0.57	Rainfed	17,200	6	
4. Juhan Gonzalvo	Calumpang, Pangimay, Butacan	Grow-out	2.00	River	20,000	.9	
5. Manuel Manalo	San Agustin, Sta. Ana, Pampanga	Hatchery	0.17	Irrigation	985	20	
			0.41		2,363	26.5	
6. Regalado Razon	San Miguel, Luban	Hatchery	1.01	Irrigation	1,446	20	
					4,341		
7. Rogelio Guevarra	Nagualing, Pilar, Bataan	Grow-out	0.20	Irrigation	4,000	6	
8. Edilberto Robles	Tuyo, Balanga, Bataan	Grow-out	1.00	Spring & Irrigation	20,000		

Table 5. Extension services rendered by NFFC from 1983 to 1986.

Type of operation	Number of cooperators 1983	1984	1985	1986
In field				
Fishpond	481	290	188	243
Rice-fish	115	125	21	5
Hatchery	78	50	20	41
Duck-fish	-	-	1	-
Fishpen	12	23	4	1
Research	1	2	4	6
Livelihood	3	154	73	25
Gabi-fish	15	10	2	4
Fish cages	39	71	7	3
Backyard fishpond	-	-	-	-
Communal	3	16	3	15
Integrated	3	38	1	-
Office visit				
Regular (picked-up)	-	-	-	819
New walk-in (technical assistance)	-	-	-	124
Subtotal	750	518	324	1288
Grand total				2,880

Center create awareness and interest among participants. Table 6 summarizes the trainings/seminars conducted by the center since it started operation in 1981. Altogether there have been 1,399 participants.

Training of extension officers/technicians, fish farmers, local people in the community is necessary in the dissemination and adoption of tested technologies in their respective projects and to share the experience gained with their fellow workers and neighboring families. The training conducted is designed to a particular need of the clientele which can be applied when completed. The publication of the Philippine (BFAR) Freshwater Aquaculture Extension Training Manual is expected to guide fisheries extension officers in delivering extension services with more confidence and dedication.

Table 6. Summary of training/seminars conducted by NFFC, Muñoz, Nueva Ecija, Philippines from 1981-1986.

Year	No. sessions/ seminars conducted	No. participants
November 1981-December 1982	11	190
1983	13	179
1984	20	270
1985	18	337
1986	20	423
Total	82	1,399

Conclusion

The program trust of the NFFC was underscored by Tayamen (1986) in a recent Symposium and Workshop on Tilapia Farming in the Philippines. Fusion of services and resources demands a unified effort of the people involved in the tilapia industry. Coordination and cooperation must be improved to impact on the rural people, especially the small-scale fish farmers.

The growing number of agencies directly or indirectly interested in freshwater aquaculture of tilapia must establish a more effective linkage mechanism at the operational level. Among the areas needing better linkages are:

1) The participation of local government in the planning, formulation and execution of fisheries programs with any food agency needs to be institutionalized.
2) The research results and tested technologies must be continuously passed on to the extension disseminators for packaging and verification and finally to reach and assist the fish farmers/private sector.
3) The national extension program in fisheries must be formulated from the fish farmers level to the administrator and finally to the policymaker. It must translate planning into action.
4) Extension education and services through technology dissemination should be implemented by both government agencies and the tilapia industry.

Active and deep involvement from planning, execution and evaluation of fisheries extension programs require commitment from all groups. There is a greater necessity for organized (research, extension and training) institutional schemes that would integrated various approaches into a viable tilapia aquaculture development program.

The potential of tilapia aquaculture to increase farm profitability and as a source of low-cost protein food is, however, constrained by the lack of good quality fingerlings and breeders (PCARRD 1985).

A Tilapia Industry Development Program is proposed to help solve the problems of the industry through intensive research on genetic improvement of existing tilapia broodstock and effective dissemination of the developed technology to tilapia fish farmers. The program components are: 1) genetic improvement, 2) hatchery technology development, 3) extension and technology verification.

The proposal was prepared jointly by the Bureau of Fisheries and Aquatic Resources (BFAR) and the International Center for Living Aquatic Resources Management (ICLARM).

The proposed project has been submitted to the National Economic and Development Authority (NEDA) through the Department of Agriculture for evaluation and approval.

The project calls for a facility that will serve as a reference center and depository of good quality and promising tilapia species/strains with the ultimate objective of generating employment opportunities and uplifting the economic conditions of the small-scale rural fishfarmers and finally increasing tilapia production and consumption in the Philippines.

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B. POSTER PAPERS
CULTURE SYSTEMS, MANAGEMENT AND PRODUCTION

Toxicity of Synthetic Pyrethroids - Cypermethrin and Lambdacyhalothrin - Insecticides to *Oreochromis niloticus*

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Abstract

Synthetic pyrethroids, a new group of insecticides for rice with chemical structures patterned after a potent insecticide, called pyrethrin, extracted from chrysanthemum flowers, offer some prospects for safe rice-fish culture. We studied the toxicity of pyrethroid insecticides to Nile tilapia *(Oreochromis niloticus)* under field conditions. Their effects on fish growth, production and survival in integrated rice-fish culture are discussed.

The first application of insecticides was done 15 and 30 days after rice transplanting (DAT) and the second application was done 45, 60 and 75 DAT. Two trials were conducted during the wet and dry seasons. Rates (g a.i./ha) of insecticides during the first and second applications were: cypermethrin - 25 and 50, respectively, for two trials, and 50 and 100, respectively, for dry season trial; lambdacyhalothrin - 6.25 and 12.5, respectively, and 12.5 and 25, respectively, for two trials. Fish were stocked 7 days after and before the first application (15 DAT) during the wet and dry season trials, respectively.

Results from two trials showed that daily gains in weight of fish, generally ranging from 0.3 to 0.43 g/day, appeared unaffected by the varying rates of cypermethrin and lambdacyhalothrin. Fish stocked in pyrethroid treatments prior to the first application of the insecticides showed 100% survival. No chronic effects such as reduced fish growth, total biomass production and survival were observed in any pyrethroid treatment.

"Hosha" - a Fishery System Practiced in Lake Manzala, Egypt

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Abstract

"Hosha" is a local Egyptian name for a unique form of fishing technique generally operated both as a harvesting mechanism and a fish farm. This system occupies about 13,760 hectares (14%) of Lake Manzala. The annual fish yield from hosha varies according to locality between 80.9 and 809 kg/ha, and in general reflects the open fishing yield in the same area. Species composition varies from one region to another according to salinity, tilapias being the bulk of the catch.

The hosha system is generally considered harmful to the open water fisheries where the bulk of the yield consists of low value, small fish. But, on the other hand, some biologists have judged hosha to be beneficial, based on the fact that its operations, involving periodic pumping and drying of sediments, are believed to stimulate aquatic productivity by recycling of nutrients (algal blooms have been observed in newly flooded hosha). Moreover, preliminary economic analysis suggests that the harvesting efficiency of hosha compares favorably with open water fishing methods, especially in sparsely stocked areas. Also, the impact of hosha on the open water stock is greatest in densely stocked areas, where it serves the function of thinning and reducing the dense juvenile populations.

Net Pen Rearing of *Oreochromis* spp. in the Lagoons of Bénin

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Abstract

Lake Nokoué is a 15,000-ha lagoon situated at the mouth of the Oueme River in southern Bénin. Following an ecological alteration, fish catches in the lake have fallen from 12,000 t in the late fifties to a mere 8,000 t or so today. With an important financial support from the E.E.C., a national scheme attempts to contribute to the improvement of the situation by adapting and extending to the traditional fishermen an intensive system of raising *Oreochromis* spp. in net pens placed in the open water of the lake.

Among the three varieties of *Oreochromis* tested (*O. niloticus, O. mossambicus* and the hybrid *O. mossambicus* F. x *O. niloticus* M.), only the hybrid seems to adapt to production system in the brackishwater of Lake Nokoué and concludes with a speculative thè main problem encountered being periodicel fish losses related to bacterial disease. Nonetheless, high yields up to 60 t/ha/year were obtained from both experimental structures and pens run by local fishermen. Acceptance of the new system by the local fishing population has been excellent to an unexpected degree in the difficult context of African small-scale fish farming.

This paper sums up the results and the problems encountered by introducing a pen production system in the brackishwater of Lake Nokoué and concludes with a speculative analysis of the perspectives of comparable methods in other West African lagoons.

Golden Fish Culture Under Indian Conditions

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Abstract

A golden variety of *Oreochromis* sp. has been developed by and is being grown at Vorion Chemicals and Distilleries fish farm, Vedanarayanapuram, southern India.

The production method, which involves sex reversal, is described. There are about ninety 0.2-ha growout ponds, which produce annually 20 t/ha in two crops. Demand for the fish is growing.

Growth and Production of *Oreochromis niloticus* of Three Strains in Cage Culture With and Without Caudal Fin Cutting

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Abstract

An investigation regarding growth and production of *Oreochromis niloticus* was conducted at Lake Ciburuy, Bandung, West Java, from September to December 1983. Three strains of Nile tilapia *(Oreochromis niloticus)* -- the common, the red, and the white -- were observed separately by sex and caudal fin cutting treatments, in 2 x 1 x 1 m cages of polyethylene net. Commercial pellets containing 25-26% protein were fed three times a day using a feed-sac.

The results revealed that growth and production of fish under caudal fin cutting treatment were higher than those of uncut fish. There was no significant difference among the three strains in growth and production, while between sexes, the males grew faster than the females.

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Culture Trials of Red Tilapia in Polyethylene Tanks

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Abstract

Red tilapia fry were reared to maturity in 1.50 x 0.75 x 0.75 m tanks made of polyethylene with wooden frames. Forty-five red tilapia (mean weight, 1.8 g) were in a 560-l tank. Temperature, DO and pH levels were periodically monitored and fish sampling was done bimonthly. Feeding was administered twice a day at 10% of their body weight/day. At the end of the fourth month, survival rates ranged from 90 to 95% and the growth rate did not differ significantly from those reared in earth ponds. On the basis of the trial results, polyethelene tanks could be considered practical and economical. They are portable fish production units which may be integrated with backyard gardening. Species and culture unit construction, recommendations and suggestions for further investigation are discussed.

Feasibility Study on a Wastewater Treatment - Fish Farming System for Cola Wastes

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Abstract

An algae-fish-waste treatment pond showed potential for COD removal up to about 88%, treatment of high pH effluent to levels acceptable to both fish and the National Pollution Control Commission standards (pH 7.0-9.0), and as a medium for raising tilapia. The algae, however, gave low production. Consequently, photosynthetic oxygen input alone was insufficient to satisfy the COD load of the effluent. Paddle wheels and/or aerators, therefore, cannot be dispensed with if aerobic conditions are to be maintained. The low algae number (less 100,000 cells/ml) was reflected in the slow growth increment of the test fishes and their low fat content (1%) indicative ofn starvation. The study concluded that the following are suitable pond design/management criteria: 7-10 days detention time, a depth of 30-50 cm, supplemental oxygen input through the use of aerators and supplemental feeding of tilapia.

Tilapia Intensive Breeding Unit

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Abstract

The paper presents construction details of a 4.00 m diameter x 1.25 m high circular cement intensive breeding pool for tilapia. Problems concerning management of the earth pond systems used as a source of tilapia fry are discussed. The advantages of the intensive breeding pool and comparisons between this unit and the more traditional earth pond system are discussed. As a result of the ease of management of the intensive breeding unit it has been shown possible to sex-reverse fry from the system. The possible consequences that a source of all-male tilapia fry may have for the farming communities in northeastern Thailand are also discussed.

GENETICS AND REPRODUCTION

Inheritance of Body Color in Taiwanese Red Tilapia

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Abstract

The inheritance of body color was studied in Taiwanese red tilapia. Randomly selected red tilapia broodfish were stocked in aquaria and in small concrete ponds with a male to female ratio of 1:3. Eggs or newly hatched larvae were collected from each individual female every two weeks or whenever mouthbrooding was observed. Eggs removed from each mouthbrooder were artificially incubated. The fry in each brood were separated for red and black color and the numbers of fry for each color were counted. A phenotypic ratio of 3:1 for red and black color of red Taiwanese tilapia was found, suggesting that the color is controlled by a single gene and red is dominant over black.

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Salinity Range Related to Sperm Motility and Propagation Response in Some Tilapiines

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Abstract

This paper investigates the feasible salinity range for reproduction of *Oreochromis niloticus*, *O. aureus* and *O. mossambicus*. The results indicate that *O. mossambicus* can breed throughout the salinity range 0 to 33 ppt. *O. niloticus* and *O. aureus* can spawn in seawater but hatching is found only from 0 to 20 ppt. The sperm motility patterns of *O. aureus* and *O. niloticus* suggest they are stenohaline species, unlike euryhaline *O. mossambicus*. For seawater breeding programs and genetic studies, the prospect of producing new hybrids by crossing less salt-tolerant species with *O. mossambicus* is worthy of further investigation.

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NUTRITION

Food Habits of Florida Red Tilapia Fry in Manured Seawater Pools

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Abstract

In experiments conducted on Lee Stocking Island, Exuma Cays, Fahamas, Florida red tilapia (a hatchery strain derived from female *Oreochromis urolepis hornorum* x male *Oreochromis mossambicus*) fry were grown in seawater pools (38 ppt) which were intensively enriched with chicken manure. The manuring rate was 105 kg/ha/day of dried manure applied as a slurry. Diet was monitored for 20 days. In one manured pool (#5) the diet consisted largely of macroalgae, while in the other pool (#8) phytoplankton and particulate organic matter comprised the bulk of the diet. Although zooplankters were utilized differently in each pool, their dietary importance decreased as the tilapia grew in both pools. Specific daily growth rates were 8.8% in pool 5 and 10.0% in pool 8. In a parallel series of pools, daily growth rates of fry that were fed pelletized feed ranged from 9.0 to 9.9%.

PHYSIOLOGY

A Preliminary Investigation of Some Blood Properties of *Oreochromis mossambicus* and Red Tilapia

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Abstract

Hematocrit values, hemoglobin concentrations, erythrocyte counts and erythrocyte sedimentation rates of *Oreochromis mossambicus* and red tilapia were studied. *O. mossambicus* males appear to have higher hemoglobin content, erythrocyte count and hematocrit values (6.77 ± 1.00 g/100 ml, 1.62 ± 0.23 million/mm^3, 13.00 ± 3.87%, respectively) than the females (5.18 ± 1.32 g/100 ml, 1.29 ± 0.30 million/mm^3, 8.21 ± 5.19%, respectively), $P < 0.05$. The females, however, showed higher (2.56 ± 0.81 mm/hour) erythrocyte sedimentation rates than the males (1.71 ± 0.62 mm/hour), $P < 0.05$. Red tilapia females appear to have higher hemoglobin concentrations (6.33 ± 0.85 g/100 ml) than males (5.58 ± 0.74 g/100 ml), $P < 0.05$. Hematocrit values, erythrocyte counts and erythrocyte sedimentation rates did not show any significant differences between sexes.

Ultrastructure Study of Organogenesis in *Oreochromis niloticus*. I. Hypophysis

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Abstract

Using the mature hypophysis as basis for comparison changes in the cytoarchitecture of the various hypophyseal cells during development of *Oreochromis niloticus* were analyzed ultrastructurally. The adenohypophyseal bud at hatching is antero-ventral to the diencephalon and is composed of small, undifferentiated, finely granulated cells with euchromatic nuclei and thin cytoplasmic rim. The neurohypophysis has invaded the caudal zone of cells.

At 16 days posthatching, the neurohypophysis penetrates deeper into the adenohypophysis. With PAS-PbH-OG stain, acidophils and putative corticotrophs, melanocyte stimulating hormone cells and thyrotrophs are observed. Cells are larger and more abundant but there are no marked differences between them. The cytoplasm still lacks the granules characteristic of the active adult pituitary cells.

At 3-4 weeks posthatching, the rostral pars distalis, proximal pars distalis and pars intermedia are easily recognizable. Prolactin cells and somatotrophs are markedly more eosinophilic. Ventral basophils (putative gonadotrophs) are first observed, about the same time that the hypophysis reacts to FITC-labelled anti-ovine LH.

Bacilliform inclusions, salient features in adult thyrotrophs, are first observed at day 30.

By day 40, the hypophysis, now found postero-ventral to the diencephalon, contains fully differentiated cells.

Changes in the Growth Rate of *Oreochromis mossambicus* Following Treatment with the Hormones, Triiodothyronine and Testosterone

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Abstract

Studies were undertaken to assess the growth promoting activity of testosterone (MT) and triiodothyronine (T_3) in seawater-adapted tilapia. To determine the optimal dose of these hormones a dose-specific-growth response was characterized for both thyroid hormone and testosterone. T_3 dose levels were 1, 5, 10 and 25 mg/kg of feed. MT doses employed were 0.1, 1, 5 and 10 mg/kg of feed. Hormones were dissolved in 50 ml of ethanol and the solution sprayed on the surface of feed pellets. The control diet was sprayed with ethanol only. Diets were fed to treatment groups of four hundred fish, at 4% of body weight, twice daily (total 8%) for 10 months. Biweekly random samples of ten fish per treatment were killed.

Growth was inversely related to dosage in the thyroid treatment. The smallest dose (1 mg) resulted in the highest response, whereas the largest dose (25 mg) inhibited growth significantly. Growth and dosage were positively correlated in the MT treatment. All treatments resulted in increased growth rates with the highest dose (10 mg) being most effective. Fish from this treatment were more than twice the size of controls at the end of the treatment period. There was no significant difference in condition factor throughout treatment groups. Proximate analysis studies indicated that the hormone treatments produced no significant change in tissue water, protein or lipid content. Sex ratio in the T_3 treatment was similar to the control group whereas in the MT treatment the altered sex ratio was dose dependent. The maximum dosage (10 mg) produced 94% males, while the minimum dosage (0.1 mg) did not significantly alter the sex ratio.

BIOLOGY

Density-Dependent Behavioral Shift in *Oreochromis niloticus*

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Abstract

The aggressive behavior of *Oreochromis niloticus* is density dependent. Earlier experiments (in tanks of 200 liters) had shown that the frequency of aggressive acts was significantly increased in groups comprised of 4 males and lowered in groups comprised of 16 males.

The aim of the present experiment was to investigate if territorial and nonterritorial males (experimental males originated from groups constituted either by 4 or 16 males, whereas control males were taken out of community tanks) show differences in several motivational systems. The behavioral test consisted in the measurement of aggressive, sexual, aggregating and hiding behavior.

Important differences were revealed not only between territorial and nonterritorial males but also between males of the same hierarchical rank which had previously belonged to groups of different densities. When tested individually, males of high density groups exhibited less aggressive and sexual behavior and schooled much more.

From this, we conclude that the aggressive and sexual behavior of *Oreochromis niloticus* males derived from low density groups shifts towards schooling behavior under high density conditions. These results also explain why, in high production systems, aggressive and sexual behavior disappear almost completely in this species.

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ECONOMICS AND SOCIOECONOMICS

Small-Scale Cage Culture of Tilapia *(Oreochromis niloticus)* in Communal Ponds in Northeastern Thailand

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Abstract

Communal ponds in northeastern Thailand are primarily constructed for rainwater storage, and therefore large and deep. Fish production in these ponds usually is low. Management is hampered due to the size of the ponds and the number of villagers involved. Hapa culture is an appropriate method to increase fish production of village ponds, by privatizing parts of the common resource.

With the local infrastructure rapidly improving and a high demand for live freshwater fish, the intensive culture of tilapia, *Oreochromis niloticus*, has become economically feasible. At Srisaket Agricultural College, a series of rearing trials is being undertaken to develop a small-scale hapa culture system. Hapas are made of cheap blue nylon mosquito netting (6 ply per cm), measuring 3.7 x 1.8 x 1.8 m (water volume: 8 m^3).

At a recommended stocking density of 150 tilapia per hapa of average 50 g, about 25 kg of tilapia may be harvested in 100 days. A commercial catfish pellet (30% crude protein) is fed *ad libitum*, three times a day. The returns on feeding and fingerling costs (benefit/cost ratio) are higher than 67%. The monthly internal rate of return (based on three rearing cycles per year) was estimated at about 16%.

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Establishment of a Fish Culture Extension Service in Africa's Most Densely Populated Country

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Abstract

Rwandan farmers have practiced tilapia culture since the 1950s. Although yields rarely exceeded natural productivity, farmer interest remained high. Previous authors have stated that ponds producing 1,200 kg/ha/year would be cost-effective.

Farmers surveyed said that the lack of fingerlings and of technical counsel were their greatest constraints. Additional socioeconomic and cultural constraints identified included the lack of inputs and long distances from home to pond.

An extension service was designed to provide close farmer extension agent contact, with a minimal amount of infrastructure, to improve management of existing ponds. Agents extended integrated tilapia culture and stressed its complementarity with other valley uses. Government hatcheries were renovated, and a transport system established to supply high quality fingerlings to the rural sector.

An increase in average net productivity from 300 kg/ha/year to 1,100 kg/ha/year was observed as improved management techniques were adopted in varying degrees. Net productivities surpassing 1,500 kg/ha/year were obtained by 29% of farmers harvesting in 1986.

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GEOGRAPHIC INDEX

Includes rivers, water bodies and research institutions

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