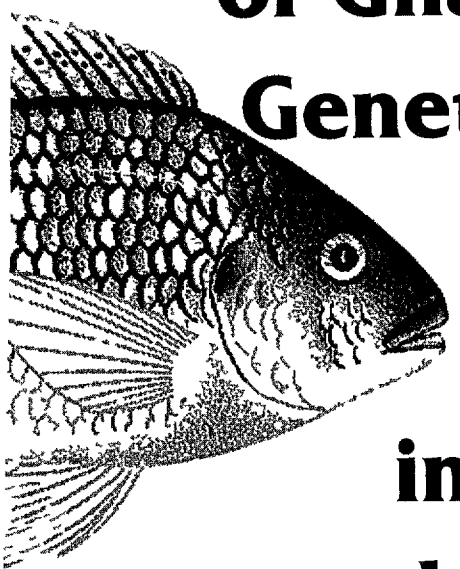
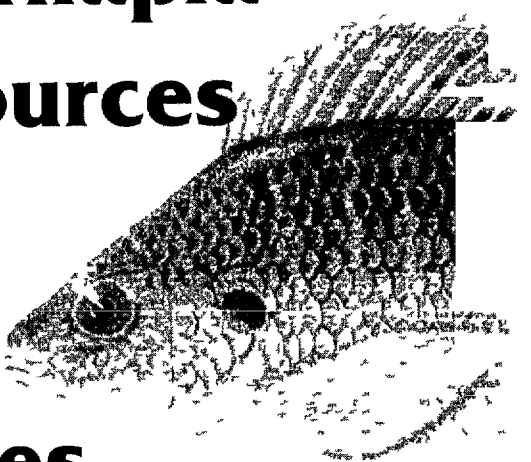
Characterization of Ghanaian Tilapia Genetic Resources for Use in Fisheries and Aquaculture





Edited by

Roger S V Pullin, Christine Marie V Casal
Eddie K Abban and Thomas M Falk

ICLARM
International Center for Living Aquatic Resources Management

(CSIR)
Institute of Aquatic Biology/ Council for Scientific and Industrial Research

Zoologisches Institut und Zoologisches Museum Universitat Hamburg

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Deutsche Gesellschaft fur Technische Zusammenarbeit

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Printed in Manila Philippines

Published by the International Center for Living Aquatic Resources Management (ICLARM) MCPO Box 2631 0718 Makati City Philippines Institute of Aquatic Biology/Council for Scientific and Industrial Research PO Box 38 Achimota Ghana Zoologisches Institut und Zoologisches Museum Universitat Hamburg Martin Luther King Platz 3 20146 Hamburg Federal Republic of Germany and Deutsche Gesellschaft fur Technische Zusammenarbeit Bonn and Eschborn Germany

Pullin R S V C M V Casal E K Abban and T M Falk Editors 1997 Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Copyediting Danilo B Abacahin and Marie Sol M Sadorra
Coordination and proofreading Ma Graciela R Balleras
Layout Ma Graciela R Balleras and Robbie N Cada
Graphics Robbie N Cada
Cover design Alan Siegfrid Esquillon
Black and white illustration of *Oreochromis niloticus* is from *Field guide to the freshwater fishes of Tanzania* 1992 Food and Agriculture Organization of the United Nations

ISSN 0115 4435
ISBN 971 8709 89 4

ICLARM Contribution No 1352

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Preface

The International Center for Living Aquatic Resources Management (ICLARM) Manila Philippines the Institute of Aquatic Biology (IAB) Accra Ghana and the Zoologisches Institut und Zoologisches Museum Universitat Hamburg (ZIM) Hamburg Federal Republic of Germany have been since 1991 partners in a project supported by the Bundesministerium fur Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ)/ Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) to develop techniques for characterizing tilapia genetic resources

This workshop was convened at the conclusion of the project in June 1996 Its purpose was to give the project team invited experts and participants from Ghana and other sub-Saharan Africa countries an opportunity to evaluate the project s major outputs to discuss common interests in tilapia biodiversity and genetic resources conservation and sustainable use and to consider further research and training needs

At the workshop methods were presented for the characterization of tilapia genetic resources with emphasis on biochemical and immunological techniques Techniques appropriate for laboratory and field use in tropical developing countries were discussed and invited experts shared their experiences A draft manual on these techniques was presented for suggestions and modifications The utilization of genetic data by policymakers scientists and extension organizations was also discussed These summary proceedings comprise extended abstracts of presentations transcripts of discussions and supplementary materials

We hope the meeting and this publication help in boosting institutional partnerships in fish genetic resources research facilitating training and information activities and developing proposals for funding these to strengthen national programs

We thank IAB Acting Director Dr Charles A Biney and the IAB staff for their hard work and support prior to and during the workshop

The Editors

Opening Addresses

DR CHARLES A BINEY
Acting Director
Institute of Aquatic Biology
PO Box 38 Achimota Accra
Ghana

Mr Chairman distinguished guests colleague scientists ladies and gentlemen I deem it a great honor to welcome you all to the opening ceremony of the Workshop of the Characterization of Ghanaian Tilapia Genetic Resources for Use in Fisheries and Aquaculture This workshop is convened by the International Center for Living Aquatic Resources Management (ICLARM) the University of Hamburg in Germany and the Institute of Aquatic Biology (IAB) supported by GTZ

IAB is one of the 18 institutes of the Council for Scientific and Industrial Research (CSIR) of Ghana From the institute s humble beginnings in 1965 when most of the activities were confined to the Volta Lake the Institute now has the mandate to conduct research into the resources of inland estuarine and coastal waters of Ghana towards their sustainable utilization In addition to its headquarters in Accra IAB has a field station in northern Ghana in Tamale and an Aquaculture Research and Development Center in Akosombo Current research thrusts of the institute include limnochemistry and pollution monitoring of indicators of global warming and monitoring of productivity of major rivers in Ghana hydrobotany and river basin management production and field application trials of biological agents for the control of vectors of water associated diseases and ecological studies on the vectors Finally IAB is greatly involved in fishery and aquaculture-centered research activities aimed at enhancing local fish production with conservation sustainable use and development of aquatic resources including fish as a constant objective

IAB collaborates with national and international institutions in its research activities Currently international institutions in collaboration with IAB include the World Health Organization (WHO) the Food and Agriculture Organization (FAO) the International Development and Research Agency (IDRC) and the German Technical Cooperation/ Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) the University of Bergen in Norway the University of Hamburg in Germany and the International Center for Living Aquatic Resources Management (ICLARM) in the Philippines

Through collaboration with the University of Hamburg this project was initiated in 1991 in conjunction with ICLARM and supported by GTZ I need not elaborate since details will be presented to you during this opening ceremony The results and achievements of this collaboration for the last six years will be presented and discussed during the next three days It is with great pleasure that I welcome you distinguished scientists to Ghana and particularly to the IAB of the CSIR to participate in this workshop

As Ghanaians we pride ourselves on our hospitality I therefore hope that you will not only confine yourselves to the workshop but also find time to visit other places of interest while enjoying our presently cool weather Welcome and thank you

DR ROGER S V PULLIN
Principal Scientist and Program Leader
Biodiversity and Genetic Resources Program
International Center for Living Aquatic
Resources Management
MCPO Box 2631 0718 Makati City
Philippines

Mr Chairman distinguished representatives from participating national regional and international agencies and institutions colleagues and friends on behalf of ICLARM and its Director General Dr Meryl J Williams it is my great pleasure to welcome you all to this workshop The ICLARM party is also most fortunate to include among its members one of ICLARM s Board of Trustees and the current Chairperson of its Program Committee Dr Benedict Satia

ICLARM is a small strategic research center It works almost exclusively through partnerships with institutions in developing and developed countries These partnerships are we feel a cost-effective and mutually beneficial means to undertake research training and information activities for aquaculture and fisheries development As Dr Martin Odei has mentioned the IAB-ZIM-ICLARM partnership has been one of the most enduring and productive examples In common with over 90% of ICLARM s collaborative work in sub-Saharan Africa all projects undertaken so far through this partnership have been funded by the Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) Germany This support is gratefully acknowledged

The world itself is very small considering its growing population s demands on natural resources Living aquatic resources are widely abused rather than assessed and used sustainably for the benefit of the present and future generations Freshwater fishes are the most threatened of all widely exploited vertebrates

ICLARM and its partners hope to contribute to the future conservation and sustainable use of fish genetic resources through projects like the one through which this workshop is being held We hope that the research results and methods to be discussed here will help those who seek to characterize and evaluate tilapia genetic resources We hope these will indicate similar approaches that could be taken for other fishes Thank you all for coming to participate in this task

DR CHRISTINA AMOAKO-NUAMA
Minister for Environment Science and Technology
Ghana

Mr Chairman honorable representatives of international and local research and development agencies distinguished scientists ladies and gentlemen it is my pleasure today as always to be among people like you I am pleased especially because your activities in addition to advancing knowledge on our resources and capacities aim to reduce food insecurity in developing countries

It is well known that a basic and common problem of developing-country governments especially in Africa is how to attain food security This even though the world around us contains tremendous wealth of plant and animal resources which have continued to provide materials for food agriculture (including fish culture) forestry and fisheries In addition groups of these organisms constitute untapped resources for humans to develop and use in various ways

In almost all cases the search for causes of insufficient or reduced food production in developing countries has identified as a factor the lack of critical/scientific knowledge on available resources to serve as a basis for their management development and sustainable exploitation

Among food resources fish mainly from capture fisheries are an important and cosmopolitan source of animal protein in the diets of humans FAO estimates indicate that in 1990 world fish production from capture fisheries (marine brackish- and freshwater) was approximately 85 million metric tons while production from culture was almost 12 million This was not sufficient for the world to talk of global food security with reference to fish

Since 1990 however various estimates show that over 60% of the world s capture fisheries resources are overexploited At the same time there is a growing demand from an ever-expanding human population for increased fish production

The need to increase fish production is even more crucial in the developing world where over 40 countries including Ghana depend on fish for over 50% of animal proteins in diets Coupled with this our high rates of population growth make it obligatory on us to identify fishes with high production potential

Mr Chairman ladies and gentlemen among fishes that have provided food and a basis for economic engagements of several families and communities in tropical Africa the fresh- and brackishwater group of fishes commonly called tilapias is well recognized The socioeconomic significance of tilapias in Africa where they are widely distributed is still largely based on their capture fisheries Although the culture potential of tilapias has been envisaged since the 1960s evidence of this is being demonstrated more in countries outside the range of tilapias' natural distribution such as Asia (e g China the Philippines and Thailand)

Many reasons may be given for our apparent lack of tilapia culture development to industrial status as demonstrated in Asia Of all possible reasons a principal one is that Africa has not taken advantage of or recognized the potential genetic resources in tilapias To improve this situation requires long-term research

In the 1980s UNESCO estimated that about 88% of the world s scientists were concentrated in technologically developed countries It is therefore not surprising that 90% of developmental research products have originated from the advanced countries and their research associates

With respect to tilapias this is now very evident in Asia which has invested in long-term research partnerships with advanced country institutions and nongovernmental international institutions such as the University of Wales Swansea and Stirling University in Scotland and ICLARM respectively A product of such associations is the super tilapia presented to the world in 1993 by ICLARM and its research partners

Mr Chairman ladies and gentlemen as I have said African developing countries have greater urgency to increase local fish production With capture fisheries resources declining options for the required increase can only be expected through stock enhancement and culture programs From the experiences of advanced countries that have already taken the options it has become increasingly clear that for maximum and long-term benefits genetic knowledge of fishes to be involved in either program is a basic requisite This is also in line with various recent international conventions and action plans especially the Convention on Biological Diversity and Agenda 21 from the UNCED

The term biological diversity has come to encompass organic variability at all levels of organization at the base of which is genetic diversity Thus to ensure appropriate conservation strategies and sustainable utilization of our fish genetic resources knowledge of genetic characteristics including labeling and documentation are important requisites Deliberations during the next three days will center on approaches for doing this for tilapias in particular and for fishes in general with special reference to low-technology laboratories as found in most developing countries

Mr Chairman invited guests scientists ladies and gentlemen my Ministry which among other things manages biodiversity issues in this country will look forward to the outcome of your deliberations and assist wherever possible My copy of the program indicates there is a lot to be accomplished in these few days Therefore I deem it fit to end now and declare the workshop open

Abstract

The ICLARM project Research on Tilapia Genetic Resources of Ghana for Their Future Conservation and Management in Fisheries and Aquaculture was implemented from January 1991 to June 1996 in partnership with the Institute of Aquatic Biology (IAB) Accra and the Zoologisches Institut und Zoologisches Museum (ZIM) Universitat Hamburg funded by BMZ/GTZ Its main output was the *Biochemical Laboratory Manual for Species Characterization of Some Tilapiine Fishes* copublished in 1996 by ICLARM and GTZ This manual was presented in draft form for critical review and improvement at the four-day international workshop on the Characterization of Ghanaian Tilapia Genetic Resources for Use in Fisheries and Aquaculture held 4-7 June 1996 at IAB Accra under the auspices of the project The workshop was attended by 51 participants from 17 countries Belgium Benin Côte d Ivoire Egypt Ghana France Germany Republic of Guinee Kenya Malawi Mali the Philippines Senegal South Africa Uganda the United Kingdom and the USA In addition to evaluating the ICLARM-GTZ manual the workshop provided the project team invited experts and participants an opportunity to discuss common interests in tilapia biodiversity and genetic resources conservation and sustainable use and to consider further research and training needs The participants presented papers on international concerns with respect to fish biodiversity and genetic resources management techniques to discriminate tilapiine species and populations and the results of the ICLARM-IAB-ZIM and other projects Three working groups gave recommendations for the conservation of aquatic genetic resources for improvement of the ICLARM-GTZ manual and for aquaculture and biodiversity conservation The workshop proceedings are copublished by ICLARM and GTZ

SESSION 1 - INTRODUCTORY PAPERS
Chairman **Prof J C Norman**

International Concerns on Fish Biodiversity and Genetic Resources Management[a]

R S V PULLIN
International Center for Living Aquatic Resources Management
MCPO Box 2631 0718 Makati City
Philippines

PULLIN R S V 1997 International concerns on fish biodiversity and genetic resources management p 1 2 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Aquatic biodiversity at the gene species and ecosystem levels provides the genetic resources that are the basis of aquaculture and fisheries The diversity of exploited aquatic organisms is very high over 5 000 of the known 24 600 species of finfish are used by humans However this diversity is poorly documented especially below the species level Characterization evaluation and use of aquatic genetic resources in breeding programs have not progressed very far for most farmed aquatic organisms Moreover the genetic impacts of most capture fisheries have scarcely been studied at all It is however becoming more widely recognized that the conservation and sustainable use of aquatic genetic resources are interdependent and that aquatic genetic resources are being lost through human interventions For example freshwater fishes are the most threatened of all vertebrate groups exploited by humans About 160 species are endangered and about one species per year becomes extinct The threats include water abstraction pollution overfishing and the impacts of exotic species Such threats to the conservation and sustainable use of aquatic organisms require analysis not just at the species level but also at the intraspecific and local population levels Different populations may be recognized as evolutionarily significant units and as management units

The most important and essential resource for effective conservation and sustainable use of aquatic genetic resources is accurate up-to-date and readily accessible information This is often lacking because it is unstructured scattered and held in a multiplicity of formats Modern information technology (e g relational databases CD-ROMs and the Internet) can help to remedy this for scientists and policymakers General information must also be accessible to the public not just to scientists and those in authority because public awareness support and participation are vital for the effective conservation and sustainable use of natural resources

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Conservation of aquatic genetic resources will be largely *in situ* in open waters though *ex situ* collections of broodstocks are possible for some species and collections of cryopreserved spermatozoa are expected to be used for some conservation and breeding programs of finfish species In all of these areas there is a need to develop accurate and cost-effective field and laboratory methods for the genetic characterization of aquatic organisms The traditional taxonomic methods are not adequate to identify aquatic genetic resources especially below the species level Biochemical methods are emerging to fill this need

Review of Techniques Applied to the Characterization of Tilapiine Species and Populations

K-A NAISH AND D O F SKIBINSKI
School of Biological Sciences
University of Wales Swansea
Singleton Park Swansea SA2 8PP
United Kingdom

NAISH K A and D O F SKIBINSKI 1997 Review of techniques applied to the characterization of tilapiine species and population p 3 5 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Research into the genetic variation of tilapiine species and populations has tended to follow trends in the development of molecular methods Prior to 1980 most work involved the study of variation in proteins mainly allozyme electrophoresis The methods employed were simple and were used to characterize species but also proved useful in certain population studies

In the early 1980s many population geneticists examined variation in the DNA itself using methods such as sequencing hybridization and the study of restriction fragment length polymorphisms using restriction enzymes Two classes of DNA have been used for studying tilapiine species and populations mitochondrial DNA and repeat regions Mitochondrial DNA is inherited maternally The higher mutation rate and absence of any recombination allows the study of recent evolutionary events Variation in the mitochondrial genome of tilapiine species has been used to support classification of *Oreochromis* subspecies and to identify the origins of cultured strains Repeat regions are found throughout the nuclear genome These are highly variable and of particular interest to those interested in recently diverged populations or in kinship studies Repeat regions comprise a core sequence which may be simple in its structure (microsatellites) or more complex (minisatellites) The regions are highly susceptible to length mutations which result in a number of length alleles at a single locus Initial attempts to characterize repeat variation involved hybridization and resulted in a complex multiband pattern known as a multilocus DNA fingerprint DNA fingerprint studies in tilapia have been concerned primarily with kinship studies but have also been used to characterize strain variation However these fingerprints have been superseded by further innovations in molecular methods

In the mid-1980s a technique used to amplify regions of the genome the polymerase chain reaction (PCR) enhanced genetic studies considerably The ability to target any region of the genome allowed the use of simple methods to characterize genetic variation PCR studies in tilapia have to date involved two such techniques the random amplification of polymorphic DNA (RAPD) and microsatellites RAPD involves amplification of anonymous regions of the genome using short primers Polymorphisms in the patterns produced are dependent on the primers binding to a given site RAPDs have been used

to study genetic variation within farmed strains of tilapia but are limited in that the markers are dominant and heterozygotes cannot be discriminated from homozygotes Microsatellites are repeat regions single loci are simple to characterize and are amplified in a PCR reaction Their highly variable nature makes them ideal for population and kinship studies Over 150 tilapia microsatellites have been isolated to date and have been used to study populations variation within strains and for genome mapping A certain class of microsatellite tetranucleotides is of particular interest to laboratories seeking a simple means to study genetic variation Several tetranucleotides have been isolated in tilapias

Allozyme electrophoresis remains the method of choice in characterizing tilapia species the method is informative and technically simple However the use of PCR allows regions of mitochondrial DNA to be amplified and the method may prove more sensitive Genetic variation within populations is probably best characterized using microsatellites Simplified DNA methods may prove useful in many laboratories in the future

Methods for characterizing variation in tilapiine species and populations this table summarizes findings from methods used in the tilapias and may not be applicable to other species

Type of study	Allozyme electrophoresis	Mitochondrial DNA	Multilocus repeats	RAPD	Microsatellites
Phylogenetics	Good	Good[1]	Not suitable	Not suitable	Not suitable
Hybridization between species	Good	Good[3]	Not suitable	Good	Fair[4]
Population genetics	Good	Good	Fair[5]	Fair[5]	Excellent
Pedigree analyses	Not suitable	Not suitable	Excellent	Not suitable	Excellent

Notes

[1] Some caution should be exercised mitochondrial phylogenetic relationships between some species of *Oreochromis* differ from allozyme phylogenies This phenomenon may be due to the persistence of an ancient shared genotype (J F Agnese pers comm)

[2] Useful for obtaining species specific markers

[3] Useful when combined with information from nuclear DNA

[4] Information from microsatellites is very useful provided that the locus specific primers amplify microsatellite loci across species

[5] Heterozygotes cannot be discriminated from homozygotes the markers are dominant

Discussion

Dr Odei Have you applied your techniques for field use anywhere yet—for example in your work in the Philippines—and found them successful?

Dr Naish Yes We are interested in methods for use in less developed nations The methods have worked in the Philippines and India

Mr Padi Have you produced any transgenic tilapia and have you evaluated them?

Dr Naish Yes We have their F1 and F2 generations and we are now checking their growth rates

Dr Satia Of the three techniques DNA methodology seems to have wide application and would be the best to promote

Dr Naish Yes it s better not to use complicated techniques A lot of the techniques are simple and can be used in developed countries PCR may be used However you need to choose a technique appropriate to what you are looking for

On the Species-specific Composition of Molecules from Muscle and Blood of Specimens of the Tilapia Group

L RENWRANTZ, T M FALK, O HALLAS AND W VILLWOCK
Zoologisches Institut und Zoologisches Museum
Universitat Hamburg
Martin-Luther-King-Platz-3
20146 Hamburg
Germany

RENWRANTZ L T M FALK O HALLAS and W VILLWOCK 1997 On the species specific composition of molecules from muscle and blood of specimens of the tilapia group p 6 11 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

In addition to classical morphometric and meristic investigations enzyme analyses are used to differentiate between species of the Tilapia group (Abban 1988 Teugels and Thys van den Audenaerde 1992 Pouyaud and Agnese 1995) Evidence is presented that genetic variations between species are also expressed in the composition of blood and muscle proteins

Erythrocyte Membrane Molecules

Comparable to the human blood grouping technique erythrocytes from different species were probed with lectins (carbohydrate binding molecules that cause agglutination reactions when specific sugars occur on the cells surface) Out of seven Tilapia species only *Sarotherodon melanotheron* erythrocytes were clumped by both *Limulus* and *Ricinus* 120 lectin In addition a positive reaction of serum bovine albumin to erythrocytes of *Tilapia zillii* is specific to this species among the tilapias investigated After neuraminidase treatment of erythrocytes two additional lectins identified *Oreochromis aureus* *O niloticus* and *S galilaeus* (Oberst et al 1988 Oberst et al 1996)

These indications of species specific variations in erythrocyte antigens were supported by cross-immunization experiments *S galilaeus*-specific antibodies (IgM-class) were obtained by injecting erythrocytes of this species into *O niloticus* (Oberst et al 1989) However it was found that repeated freezing of the tetrameric IgM caused denaturation of the macromolecules For this reason a simple procedure for IgM isolation was developed to split the tetramers into more stable monomers Their apparent molecular weight was determined to be 216 kDa their H chain of 80 kDa and their L chain of 26 kDa

In addition monoclonal antibodies were raised against erythrocyte antigens for testing against specific erythrocyte membrane proteins Membrane isolation and electrophoretical analyses have been accomplished

Hemoglobins

Vertebrate hemoglobins are usually tetrameric molecules the monomers each

composed of a heme group and a globin chain These polypeptides may vary in their molecular weight and/or amino acid composition Thus hemolysates of eleven tilapia species were electrophoretically tested and up to six different major globin chains were identified often varying between species Differing combinations of these globin chains in the composition of tetramers would result in a relatively high number of hemoglobin types occurring in one species This hypothesis was supported by isoelectric focusing of the hemoglobin from different species Up to 20 major hemoglobin bands were obtained per species and species-specific band patterns were shown to occur When singular tetrameric hemoglobins from *S melanotheron* were isolated for investigation by disc electrophoresis variations were detected in their globin chain composition revealing for example $1a_1$ $1a_2$ $1b_1$ and $1b_2$ chain or $2a_1$ and $2b_1$ chains or $2a_1$ $1b_1$ and $1b_2$ chain (Falk 1994)

Parvalbumins

Acidic molecules from muscle extracts possessing relatively low molecular weight and heat stability comprise the group of parvalbumins (Lehky et al 1974 and Blum et al 1977) When muscle extracts obtained from individuals of different species were heated (70°C) and analyzed by disc electrophoresis and isoelectric focusing species specific differences were obtained

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Discussion

Dr Elghobashy How can we detect the purity of the fish species from agglutination methods using lectins? Can you test for all species or just the ones you are interested in?

Prof Renwrantz Yes you can use lectins There are six lectins with which you can identify all the species considered in this investigation

Dr Pullin Most of the techniques for describing differences between species and populations use neutral markers and tell us nothing about their physiological functions or their importance for aquaculture performance Perhaps the multiple

hemoglobins offer the best chance of finding markers that actually relate to performance

Prof Norman The National Agriculture Research Project of Ghana is supporting research on freshwater fishes including tilapia Three hundred thousand US dollars has been voted for this research

Prof Alhassan I wonder why the project concentrates on Ghanaian tilapia genetic resources Are the resources in neighboring countries like Côte d Ivoire completely different from those of Ghana? Shouldn t we encourage regional cooperation in such characterization studies?

Dr Abban The word Ghanaian in the project title is not exclusive We used Ghanaian tilapias to do the work but we have been collaborating with Cote d Ivoire For all the tilapia species we have worked on we have samples also from Cote d Ivoire The collaboration has been on the personal level with Dr Agnese What is needed is more collaboration on the national level

Dr Pullin Every country has its own activities and plans But countries and groups also come together and do things An example is the Species 2000 Project which is a global project The objective is to set up a checklist of all living things on this planet covering about 1 75 million species This is the first step towards a global inventory Through modern information technology this is very likely to be possible in the next generation We are also proposing a regional study on *S melanotheron* in its natural habitats as a case study in conservation and use of tilapia

Mr Biney The project is coming to an end soon What effect has it had on tilapia production? What information did you pass on to the farmer and has this information helped in tilapia production? What fora did you use to disseminate information?

Dr Abban In the process of doing this work with reference to the farmer at least we know where what species or strain is Most farmers have come to know that the Nile tilapia is the best species to cultivate But among its populations in Ghana there is a population around Kpandu whose growth rate is higher than the others so we advise that farmers take their broodstock from there Even though the objective of this project does not immediately deal with the farmer such as crop research which develops seed [like maize] for farmers we will ultimately from this research develop fish seed for the farmers We are now supplying some tilapia seed to farmers But the farmers and the public will have to wait for some time for us to develop better seed

Dr Pullin This present project has been aimed at methods to characterize better the fish resources in Ghana for use now and the future Unless there are good methods the policies for increasing fish production cannot be well framed

Dr Abban We have organized seminars for fish farmers who normally collect fish from anywhere to stock their ponds We have also sensitized people on germplasm movement We know what germplasm there is and how it is moving The Institute of Aquatic Biology (IAB) has also been looking into conservation so that development

plans do not interfere with valuable germplasm which could be lost in the process IAB is doing this with the Departme nt of Game and Wildlife In Ghana fish have never been considered as wildlife But through seminars we have told people that because fish live in the natural environment they are wildlife We are also educating people that as we move towards a policy on conservation we should not move species into river basins where they were originally not there For instance we have tilapia now in the Densu where it was not originally found The effects of this we do not know With respect to management and conservation we are providing the information on what to conserve

Mr Biney The Institute of Aquatic Biology cooperates with the Depaitment of Game and Wildlife towards the conservation of the manatee We have researched and found out that the manatee which was thought to have been extinct lives in certain parts of Ghana In this same vein we hope future cooperation work will be towards conservation of fish

Dr Brummett If practical applications for genetic characterization depend on National Action Plans what concrete steps might a country take to move from the information we now have to the formulation of Action Plans?

Dr Pullin Wherever fish are bred breeders need good scientific information As far as this relates to farmers the latter are not interested in Latin names The kind of results that are going to impact on farmers are different from those needed by future breeders For instance in the Philippines better fish have been bred from a research project The Government wants to take this further with fish farmers

Mr de Graft-Johnson What is the project doing in the area of international collaboration to ensure that the genetic purity of our species is not contaminated by tilapias from our [upstream] boundaries—e g from Burkina Faso Togo and Benin?

Dr Abban The present project does not include managing resources upstream We are however aware that countries upstream are also interested in conserving the purity of their stocks

Dr Pullin A major reason why freshwater species are threatened is that the water they live in is used for other things Forty percent (40%) of the world s population is faced with water shortage and money voted for water supply improvement does not really include provisions for the living things in the water From this project the various countries that share water with Ghana could also take methods for the documentation of their tilapia genetic resources that is why we have produced a manual

Dr Odei With respect to Action Plans I take the stance that the Convention on Biological Diversity opens the doors for biologists to be involved in matters related to biodiversity conservation and management They must therefore be involved with the policy aspects of biodiversity issues It is not enough for them to throw their hands up in despair They must be involved If they have plans for conservation of fish they must put them forward to Government They should submit Action Plan proposals

What about ex situ conservation of threatened fishes? If it is difficult to do in situ conservation we should work out proposals for ex situ conservation of tilapias under threat of extinction

Mr Biney Dr Pullin indicated that the difference between aquatic biodiversity and genetic resources is that the latter must have a use But is not being part of biodiversity processes alone not useful?

Dr Pullin For something to be described as a resource it should have use The problem with the huge array of diversity in aquatic systems is that only a small proportion has been investigated so there is a huge information gap The dividing line between what is aquatic biodiversity and what are aquatic genetic resources is not clear

Dr Falk There is one promising aspect that I would like to add for discussion the functional properties of tilapia hemoglobins and their value for aquaculture It seems likely that the heterogeneity of tilapia hemoglobins as demonstrated by Prof Renwrantz may be directly linked with a functional heterogeneity The most acidic and most alkaline hemoglobin components should display such functional differences For example it is well demonstrated that salmonid hemoglobins are functionally distinct in character Hemoglobin components with acidic pI s exhibit strong Bohr- and Root-effects and are sensitive to temperature and pH That means increasing temperatures and decreasing pH values reduce their O_2-affinity In contrast alkaline hemoglobin components of the same species are insensitive to temperature and pH changes Their O_2-affinity is also higher Moreover they are not modulated by organophosphates like ATP and UTP That means they could serve as an emergency system under hypoxic and hypocapnic condition a critical situation that often occurs in pond culture For aquaculturists it could be interesting to select species that display a high number of these so-called high O_2-affinity temperature insensitive hemoglobins

Prof Renwrantz Immune system studies with antibodies will also lead us to tilapias that are more sensitive or less sensitive to disease

Dr Agnese With regard to methods the important thing is to choose methods depending on what you need to know Dr Naish said if you want to know about the phylogeny then you choose electrophoresis to recognize species immunological techniques for populations microsatellites for individuals fingerprinting

Prof Renwrantz Morphometric methods will be the most important for people to use in the field Biochemical investigations benefit from the collection of as many data as possible to confirm the conclusions To be able to use biochemical methods in the field the methods will have to be as simple and as fast as possible for instance not requiring centrifugation etc just adding two drops of lectin to blood is an example

Dr Pullin Very often those who receive fish from others do not check their identification They assume that what the supplier says about the fish is

right This is not always so With the advent of the Convention on Biological Diversity there is a need for the capability to check the identification This need not involve very elaborate equipment and techniques

Dr Odei We hope the fish scientists are not going to look into the nitty gritty of the fish so much that we will have to wait for so long without having fish on the table to eat During this workshop we have been shown some of the systems in China and Ghana In Ghana with our little feed resources how does the farmer identify the best fish to grow from the methods elaborated?

Ms Duthie Perhaps in our circumstances and environment after the initial testing of strains of *O niloticus* and evaluation and choosing the best strains we can use a quick lectin test and then advise the farmers that such a test identifies the best strain to use?

Prof Norman In agriculture there are very quick tests for say soil quality We believe very quick testing tools should also be developed for fish identification Perhaps the big-time commercial fish growers can use these tools but we are not there yet Perhaps some of these techniques may be used by fish hatchery operators

SESSION 2 - COUNTRY PRESENTATIONS
Chairman **Dr. B. Satia**

Preliminary Results on the Zootechnical Characterization of Four Strains of *Oreochromis niloticus* (Linnaeus 1758)

G GOURENE
Universite de Cocody Laboratoire d Hydrobiologie
22 BP 582 Abidjan 22
Côte d Ivoire

O ASSEMIEN
Institut Des Savannes
BP 633 Bouaké 01
Côte d Ivoire

AND

J -F AGNESE
Laboratoire de Genetique des Populations
Centre de Recherches Océanologiques/ORSTOM
BP V18 Abidjan
Côte d Ivoire

GOURENE G O ASSEMIEN and J F AGNESE 1997 Preliminary results on the zootechnical characterization of four strains of *Oreochromis niloticus* (Linnaeus 1758) p 12 13 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Oreochromis niloticus is the most important species used in fish culture in Africa particularly in Côte d Ivoire It is highly esteemed for its breeding potentials (ease of propagation rapid growth etc) and its organoleptic qualities This study is part of a Genetics Research Program under Dr J -F Agnese conducted at the IDESSA fish station in Bouake

Four different strains of *O niloticus* were assessed the Bouake (BKE) strain which was obtained from the IDESSA fish station the Niger (NIG) strain from the Niger river the Senegal (SEN) strain from the Senegal river and the Ghana (GHA) strain from Lake Volta Eleven 50 m^2 ponds were used with three replicates per strain except for the Ghana strain which had only two The ponds were stocked with 110 fish each (2 2 fish m^2) Fish were fed with a granulated feed (T2GE) which contained 30% protein including 10% from animal sources Three parameters were examined for three months the average body weight the daily growth rate and the indices of consumption The average body weights of the four strains were investigated and results showed that the Senegal and Bouake strains were significantly heavier than the Ghana and the Niger strains after three months The daily growth rates were also evaluated A positive increase in the daily growth rate was observed for all strains from the start of the experiment up to 58 days after which the daily growth rate started decreasing At 88 days the daily growth rates of the four strains

showed the Senegal strain to have the best rate followed by the Bouake strain the Niger strain and the Ghana strain The indices of consumption were compared to assess the strain that consumes the least food to produce a kilo of fish The Ghana strain had the highest rate of consumption immediately followed by the Niger strain the Bouake strain and the Senegal strain Taking the observations from the three parameters studied it would seem that the Senegal strain is the best fish for culture followed by the Bouake strain the Niger strain and the Ghana strain The results are however not yet conclusive and the study is continuing

Fish Genetics in Egypt

H ELGHOBASHY
Central Laboratory for Aquaculture Research
Abbassa-Abu Hammad Sharkia
Egypt

ELGHOBASHY H 1997 Fish genetics in Egypt p 14 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Egypt has great potential for freshwater and brackishwater aquaculture Historically fish production in Egypt has been derived from natural marine and freshwater systems Aquaculture production provides about 15% of the total fish production in Egypt and fish farming may provide 10% of the needed animal protein The polyculture system is the most practical aquaculture system in Egypt It includes different species such as tilapias mullet common carp Chinese carps and catfish The most important of these are tilapias and mullet However the numbers of mullet wild fry have decreased because of increasing water pollution Tilapias are the preferred freshwater species nowadays in the aquaculture system due to their excellent growth rate disease resistance environmental adaptability and high market acceptability They now represent the most demanded freshwater fish in the Egyptian markets

Fish related research has always been oriented towards increasing fish production reduction of production cost and other problems in the aquaculture industry Fish genetics research in Egypt has a relatively short history during which efforts have concentrated on the following firstly electrophoretic studies of tilapia aimed at identifying biochemical markers for the discrimination species to support morphological identification and secondly attempts to study the effect of salinity on gene expression in tilapia species In both studies SDC-polyacrylamide gel electrophoresis and different isozyme systems (eg Peroxidase and LDH) proved to be useful in discriminating species and confirmed effect of salinity on gene expression

Currently fish genetic research in Egypt includes growth evaluation of native stocks and strains of *Oreochromis niloticus* Four major strains of *O niloticus* involved in the studies are the Aswan strain from Kafr fish hatchery in southern Egypt El Zawya strain from the Kafr El Sheikh in the north of the Delta Mariut strain from Alexandria in north Egypt and the Abbassa strain from Sharkia mid-Delta area

As part of evaluation efforts are made to trace the history of populations where study materials were obtained Strain samples have been held and spawned separately Known hybrids were also produced Electrophoresis and DNA fingerprinting studies on the strains and their hybrids are in progress Preliminary results indicate considerable genetic and performance difference among strains of the species

Current research interest also is focused on transgenic *O niloticus* using electroporation The rate of DNA integration of the rainbow trout growth-hormone gene (rtGH1cDNA) in surviving electroporated embryos was about 20% or higher The mean weight of transgenic fish was three to seven times higher than nontransgenic fish

Biodiversity and Genetics of Farmed Tilapias in Sénégal

A DIALLO
Centre de Recherche Oceanographique de Dakar - Thiaroye
BP 2241 Dakar
Sénégal

DIALLO A 1997 Biodiversity and genetics of farmed tilapias in Senegal p 15 16 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Senegal like many west African countries has six native *Tilapia* species *Oreochromis aureus O niloticus Tilapia guineensis T zillii Sarotherodon galilaeus* and *S melanotheron heudelotii* These are naturally distributed in the Senegal river and the Guiers lake in the north the Saloum estuary in the middle and the Casamance estuary in the south Three have been used for fish culture and production *O niloticus* used by projects in the north (Senegal river) since 1979 and *T guineensis* and *S melanotheron* in the south (Casamance estuary) since 1988 where they have been trapped in ponds for several months before harvest *O niloticus* is naturally present in the Senegal river and the Guiers lake *T guineensis* and *S melanotheron* are euryhaline these are present in the Senegal river Saloum and Casamance estuaries and Guiers lake *S melanotheron* is also found in waterbodies (Niayes) around Dakar city

Fish culture experience in Senegal began with projects around the Senegal river supported by the Catholic Relief Service and USAID in 1979 During this period *O niloticus* from Côte d Ivoire was introduced Fish were raised in ponds and cages but aquaculture failed because of technical environmental and socioeconomic constraints Since 1994 organized fishermen in cooperation with government (Eaux et Forêts) and research advisers established further fish culture projects in ponds and cages Initial results gave a mean production of 166 kg 400 m^2 pond year and 45 000 fingerlings year In the Casamance research on fish culture was started in 1988 by the Centre de Recherche Oceanographique de Dakar - Thiaroye (CRODT) supported by the International Development Research Centre (IDRC) of Canada Constraints potentials and socioeconomic aspects were identified Two local species *T guineensis* and *S melanotheron* were used for trials and production and yields increased from 569 to 1960 kg ha^{-1} $year^{-1}$ Integrated fish farming was developed it increased family incomes up to 400% Recent trials on fish pen culture with wild *T guineensis* and *S melanotheron* gave a mean yield of 1400 kg ha^{-1} $year^{-1}$

Aquaculture in Senegal is expanding using *O niloticus* in the north where there is enough freshwater from the river (a dam was built to increase water availability for agriculture irrigation systems) and the Guiers lake The development of agriculture will provide agricultural by-products that can be used for fish feeds at low costs In the mid-Casamance dams were built in valleys (to increase rice production by up to 50% was the main goal) and fresh- and brackishwater will be now available for eight to nine months per year *O niloticus* (introduced) *T guineensis* and *S melanotheron* will be

used in a well-integrated farming system According to available agricultural by-products in each zone research on feeds and growth performance is in progress at fish culture stations

Many tilapias are common to different African countries with varying environments To improve basic knowledge on their genetics and diversity regional research programs must be developed Comparative performance trials on the growth and production of three strains of *S melanotheron* populations from Senegal Côte d Ivoire and Congo are an example G G Teugels and J-F Agnese have suggested that *S melanotheron paludinosus* is probably *S melanotheron heudelotii* The genetic distance among *S melanotheron* populations from several places in Senegal and among *O niloticus* populations from Senegal and Bouake is not great

International collaborative research must be improved because tilapias are now widely distributed in the world and are the most widely cultured fish A program like the GIFT project (ICLARM and collaborators) must be developed for African scientists working on tilapia culture

By maintaining the level of landings (fisheries) and with the population increase of 4% y ' fish culture using *O niloticus S melanotheron* and *T guineensis* in well managed integrated farming systems is the best way to cover the gap of protein demand and to increase income and welfare for smallholders mainly in the countryside

Discussion

Dr Teugels I did not synonymize *S melanotheron paludinosus* with *S m heudelotii* On the basis of the sample that was at my disposal I was not able to recognize it Perhaps it was just not present in the sample or perhaps it has disappeared since it was described More research is necessary

Dr Abban You indicated aquaculture was in various parts of Senegal Was it accidental that different species of tilapia are cultured in different areas of Senegal?

Dr Diallo It was planned some are more appropriate in the south where the salinity is high in the estuary There is freshwater near Dakar

East African and International Concerns over the Declining Fish and General Biodiversity in Lake Victoria

F W B BUGENYI
Fisheries Research Institute
National Agricultural Research Organization
PO Box 343 Jinja
Uganda

BUGENYI F W B 1997 East African and international concerns over the declining fish and general biodiversity in Lake Victoria p 17 18 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

There has been a growing need (in East African countries) for action in the key areas of land degradation water supply environmental pollution marine and freshwater resource management habitat conservation and biodiversity and deforestation - a whole ecosystem (including land atmosphere and water) that calls for management Kenya Uganda and Tanzania are among the poorest countries in the world They experience shortages of nutritious foods for ordinary people These people are able to survive only through small-scale/subsistence agriculture that is naturally destructive of the land with the consequent deterioration of the aquatic environments Their economies are heavily dependent on agriculture with a number of cash crops (including exports of fish) and a high level of subsistence fishing and agriculture Their populations are rapidly increasing while the resources to support them are declining Lake Victoria the lake basin common to the three countries is used as a source of food energy and drinking and irrigation water and as shelter transport and repository for human agricultural and industrial waste

Although there are many features of Lake Victoria that are of intense interest to international and local scientists it is fish that receive the most attention for their nutritious food value (particularly to the locals) and because of their changing ecology (to the scientists) Most of the fish species now in the lake also originally lived in the adjacent west-flowing rivers but cichlids in particular had a remarkable burst of speciation in response to the change from river to lake conditions They are capable of more rapid genetic change and speciation than other groups of African fish

One of the main events of importance to the lake system in the past thirty years was the introduction of new species into the lake This changed the species composition of the lake s aquatic system which together with other factors (environmental degradation overfishing and use of destructive fishing gears) led to the decline of fish biodiversity Scientists from East Africa and from the international community at various institutions in and outside the region are engaged in research aimed at the sustainable utilization of the lake basin s natural resources

Discussion

Dr Owino How are you going to ensure the conservation of catchment areas especially on the Kenyan side given the high population increase in the area? How and why was Nile perch introduced? Was there prior sound scientific research undertaken?

Dr Bugenyi The Nile perch which is a good table fish was introduced to replace the haplochromine species which were abundant in the lake but not valued as table fish The Nile perch was introduced accidentally by a British national who saw its value over the haplochromine species No research was undertaken before the introduction Kenya has one of the largest catchments and a number of rivers from there bring pollution from cities and farms into the lake All the East African countries are in good collaboration They are also collaborating with Agricultural and Extension officers who educate farmers to reduce the impact of silt and other waste into the rivers and lakes

Dr Pullin Is your Institute doing or planning to do research on the genetic impacts of overfishing in Lake Victoria?

Dr Bugenyi The government is more interested in projects that address the immediate problems of fishermen including fish production

Dr Pullin If someone made a proposal on a collaboration on genetic impacts of Lake Victoria fisheries would it be supported?

Dr Bugenyi Every project has to address the national needs of increased fish production But we scientists can write the proposal in such a way that it can include genetic studies

Tilapia Biodiversity, Genetic Resources Conservation and Sustainable Use: a South African Perspective

F H VAN DER BANK
Research Unit for Aquatic and Terrestrial Ecosystems
Department of Zoology Rand Africaans University
PO Box 524 Johannesburg 2006
South Africa

VAN DER BANK F H 1997 Tilapia biodiversity genetic resources conservation and sustainable use a South African perspective p 19 20 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Cichlids of southern Africa comprise eight genera and 41 species (Skelton 1993) Adult tilapias range from 10 to 45 cm standard length and the largest tilapia caught was 6 123 kg Rod and line subsistence fishermen each crop 1-2 kg of fish per day and about 300 tons of tilapia are produced per year from Lake McIlwaine (Zimbabwe) alone Up to 16 000 tons of tilapia are caught annually in Lake Kariba and 117 000 angling licenses were issued in 1977-1978 in South Africa Because tilapias are very popular as table fish tilapia demand currently exceeds supply in southern Africa Furthermore due to the pressure on marine fish resources it is expected that the demand for freshwater fish will increase Despite these facts very few productive fish farms have so far been developed

In South Africa tilapia production increased from 11 to 40 metric tons from 1988 to 1991 (Hecht and Britz 1993) The reasons for the low production statistics include management shortcomings as well as the severe drought that prevailed over the last 10 years The lack of control over breeding and stocking of tilapias has resulted in overpopulation hybridization and stunting of stock (Skelton 1993) Nevertheless the potential of indigenous tilapias for aquaculture remains high Although significant advances have been made during the last few years there is an urgent need to increase protein production because of the fast increase in human population This would be possible with the optimal use of water resources and an appropriate Aquaculture Development Plan Such plans have had a major impact on progress of fish farming in other countries — e g Egypt Israel and Nigeria Policymakers legislators and conservation officers should get involved in developing management plans and strategies to ensure the protection and sustainable use of natural tilapia populations which are being overexploited in neighboring countries For example in Namibia there is uncontrolled access to gillnets with no prescribed mesh size limits

Not only do we need to protect biodiversity but the quality of our genetic resources should also be conserved The genetic integrity of wild tilapia species is threatened by hybridization with translocated made lakes unintentional translocations via intercatchment connections and the introduction for biological control of nuisance plants and animals (De Moor and Bruton 1988) The erosion of genepools (through hybridization) should be avoided

Isozyme studies have been predominantly used to assess the genetic variation and differentiation of tilapia species in southern Africa — e g Van der Bank and Ferreira (1987a 1987b) Lizemore et al (1989) Van der Bank et al (1989) Oosthuizen et al (1993 in press) and Van der Bank (1994) Allozyme data can be used to identify hybrids and provide an important species identification tool because it is extremely difficult to identify young tilapias which do not show typical secondary sexual characteristics by using only morphological characteristics Biochemical keys can be constructed using fixed allele mobility differences between species to identify six of southern African tilapia species (Van der Bank et al 1989 Oosthuizen et al 1993)

Although allozyme data are still being employed advanced methods such as microsatellite and randomly amplified polymorphic DNA (RAPD) markers should also be used to assess genetic variation for utilization in selection programs Unpublished results from an RAPD study by Clifford Nxomani (Ph D student Rhodes University South Africa) revealed no significant differentiation among six populations of *Tilapia sparrmanii* and among five different color morphs of *T guinasana* The latter result was attributed to incomplete assortative mating (C Nxomani pers comm)

Strong institutional collaboration in research training information distribution and funding is needed to strengthen national fish genetic resource ma-nagement and to develop programs for the sustainable use of freshwater fish resources in the whole southern African region It would also be valuable to establish an African tilapia resource center where information and research materials could be obtained Researchers are welcome to contact the author if they wish to obtain endemic tilapia species from South Africa and Mr Clinton Hay (fax 09264 661-361) for freshwater fish species from Namibia

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Tests of Fish Stocking in Bancôtières in Mali

F COULIBALY-SYLLA
Rural Economy Institute
Program Resources Halieutiques (CRRA/Mopti)
BP 47 Mopti
Mali

COULIBALY SYLLA F 1997 Tests of fish stocking in bancotieres in Mali p 21 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Preliminary studies were carried out on fish stocking in temporary ponds called bancôtieres the results of diggings for construction purposes These ponds are generally made on clayish soil These studies were done to address the need for an increased protein source for the villages on the banks of the Niger River after a decline in aquatic production following a period of drought In 1993-1994 the bancotieres of one village were stocked with *Clarias anguillaris* given daily thereafter a ration with an 11 33% protein feed Fish stocked in another village were not given any supplementary feed Ducks inhabited the bancôtieres in both villages The fish that were fed grew by an average of 0 41 g day The weight of fish declined in bancôtieres without supplementary feed The results suggested that for effective culture of *C anguillaris* in these bancotieres at least six months growout period (implying early stocking of fingerlings) an appropriate breeding cycle in captivity and assurance of regular feeding are needed

This study led to another in 1994-1995 using the same ponds and stocking at 2 and 3 fish m^2 with two treatments (with compost and compost + bran as feed) Tilapia breeders were introduced to these ponds such that the hatched juveniles became a natural source of supplementary protein for *C anguillaris* Cattle manure was added at 5 kg week and a movable compost system was adopted as fertilizer source compost was put in baskets made by the farmers to float on the bancôtieres The daily growth rates for *C anguillaris* range from 0 18 to 0 70 g day The results suggested that a density of 2 fish m^2 was more efficient However several problems were encountered the availability of bran which is essentially used for domestic animals the difficulty in maintaining the compost systems on the water surfaces and irregularity of compost renewal

In the pursuit of developing a viable aquaculture system in this rural setting experiments were continued in 1995-96 this time using a better system of fertilization and excluding bran However the study was terminated due to severe drought A follow up of the study will be performed for the 1996 farming season We conclude that the culture of fish in bancôtieres is possible These ponds are of potential importance in the Central Delta of the Niger River

The Genus *Tilapia* and its Potential for Aquaculture in the Republic of Guinea

K CAMARA
Departement de Biologie
Université de Kankan BP 209
Republic of Guinea

CAMARA K 1997 The genus *Tilapia* and its potential for aquaculture in the Republic of Guinea p 22 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Four tilapia species recorded from the upper Niger have potential for aquaculture *Oreochromis niloticus niloticus* (Linnaeus 1758) *Sarotherodon galilaeus galilaeus* (Linnaeus 1758) *Tilapia dageti* (Thys van den Audenaerde 1971) and *T zillii* (Gervais 1848) *O niloticus niloticus* are frequently found at Niandan and at Milo *S galilaeus galilaeus* is more abundant at the stations of Sankaram Dion and Milo than at Niandan *T dageti* is abundant in the stations of Sankarani in Mandiana where the largest and heaviest fish (134-250 mm 243-785 g) have been found *T zillii* is relatively abundant at the Dion station at Baramana and less common at the stations at Niandan and Milo The species diversity of tilapias is important and in the inshore basin the species most frequently found are *S caudomarginatus S melanotheron melanotheron T brevimanus T guineensis* and *T louka* Those that are less abundant are *S galilaeus galilaeus T dageti* and *T zillii*

The possibilities for aquaculture vary according to the ecological sites of the four natural regions of the country the upper middle and lower reaches of the Guinea river and the forest region In order to promote aquaculture there are needs to make the public aware of its benefits to encourage fish farmers by providing them a lot of support to encourage research on genetic resources of fish used in aquaculture (e g catfish tilapias) and to have a rational management of agricultural byproducts that could be utilized in aquaculture

SESSION 3 - PRESENTATIONS ON CHARACTERIZATION AND CONSERVATION OF FISH SPECIES AND POPULATIONS

Chairman **Prof L. Renwrantz**

Morphometric Characterization of Populations and Strains of *Oreochromis niloticus, Sarotherodon melanotheron* (Cichlidae), *Clarias anguillaris, Clarias gariepinus* (Clariidae) and *Chrysichthys nigrodigitatus* (Claroteidae)

G G TEUGELS

Musee Royale de L'Afrique Centrale Africa-Museum
Section Vertébrés Laboratoire Ichthyologie Leuvensesteenweg 13
B-3080 Tervuren
Belgium

TEUGELS G G 1997 Morphometric characterization of populations and strains of *Oreochromis niloticus Sarotherodon melanotheron* (Cichlidae) *Clarias anguillaris Clarias gariepinus* (Clariidae) and *Chrysichthys nigrodigitatus* (Claroteidae) p 23 24 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Since 1993 studies have been undertaken on the morphometric characterization of populations and strains of fish species used in aquaculture in Africa as part of two ongoing multidisciplinary research projects financed by the European Union (Project Genetics-STD3) and the Belgian National Fund for Scientific Research (Project Catfish)

Tilapia and catfish specimens from various localities in Burkina Faso Chad Congo Côte d Ivoire Gambia Guinea Kenya Mali Senegal and Swaziland were examined Each specimen was individually labeled The same materials were independently studied by other teams using genetic and parasitologic approaches For morphometric analysis several measurements and meristic counts were taken from each specimen The selection of these variables was based on their diagnostic value if the taxonomy of the group was well known If not more characters were studied Data were subjected to univariate and multivariate analysis

Results obtained so far make the researchers doubt the validity of the subspecific division of the Nile tilapia *Oreochromis niloticus* as introduced by Trewavas (1983) and Seyoum and Kornfield (1992) particularly for some East African subspecies For the same species the supposed natural geographic origin of the aquaculture strains examined could also be challenged Populations of *O niloticus niloticus* from Egypt showed closer affinity with other East African subspecies than with populations of the same subspecies originating from West Africa For *Sarotherodon melanotheron* a brackishwater tilapia present from Senegal to Congo one of the five subspecies recognized by Trewavas (1983)

could not be distinguished and its validity is also challenged A range extension was found for another subspecies

Comparison between populations of the catfish species *Clarias anguillaris* and *C gariepinus* led to the recognition of a hybrid specimen in the population from an artificial canal in Senegal Important affinities were also demonstrated between *C anguillaris* populations from West Africa and *C gariepinus* populations from Lake Victoria (Teugels 1982 1986 Risch 1992 Agnese et al in press) *Chrysichthys nigrodigitatus* populations from the extremes of the distribution range could easily be distinguished populations in between and in particular those from lagoons showed an important polymorphism and were greatly overlapping (Adepo-Gourene et al in press)

In conclusion morphometric characterization although sometimes considered classical and therefore not a very relevant technique is still a powerful method to characterize populations and strains All the results mentioned above have been confirmed by more recent genetic approaches

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Discussion

Dr Pullin The results that you have described for the Bouake strain of *O niloticus* cast doubt on the discussions about the origins and attributes of this strain published in ICLARM Conference Proceedings Vol 16 (Pullin 1988) This strain has been widely distributed Would a museum like yours be interested in extending further your studies on the populations under domestication rather than just on wild populations?

Dr Teugels Yes of course if the resources are available

Mr Padi Have you seen any wild red tilapia in Uganda I am asking this because somebody mentioned the presence of wild red tilapia in Uganda at a conference in the USA

Dr Teugels I did not find any red tilapia in Uganda

Genetic Characterization of the Indian Major Carp, *Catla catla*, Using Tetranucleotide Microsatellite Repeats

K A NAISH, D O F SKIBINSKI AND G C MAIR
School of Biological Sciences
University of Wales Swansea
Singleton Park Swansea SA2 8PP
United Kingdom

NAISH K A D O F SKIBINSKI and G C MAIR 1997 Genetic characterization of the Indian major carp *Catla catla* using tetranucleotide microsatellite repeats p 25 26 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

The Indian major carp (*Catla catla*) has been farmed in the southern Indian state of Karnataka for over forty years Since its introduction for aquaculture from source populations in north Indian rivers there have been few if any re introductions Spawning of the species is induced and prolonged Typically early maturing broodstock are used to produce fingerlings for food production while later maturing fish have been used to produce broodstock for future generations As a result it is possible that slow growing late-maturing fish have been inadvertently selected as broodstock for several hatchery strains of *C catla* (Eknath and Doyle 1990)

One of the first steps in improving the genetic management of exploited stocks is to estimate the level of variation in domesticated and wild stocks Such a test would provide information on the changes in genetic characteristics due to broodstock management practices and as a result selective breeding techniques may then be developed Microsatellite repeats are highly polymorphic and offer a means of discriminating both individuals and closely related populations (O Reilly and Wright 1995) Although dinucleotide repeats are more frequently used in population studies the polymerase chain reaction amplification of tetranucleotide loci is less likely to produce spurious products and these loci are more readily scored using simple electrophoresis methods Several such tetranucleotides have been isolated in *C catla* DNA These loci have been used to estimate genetic variability in fingerlings from four hatchery populations in Karnataka

Results so far suggest that the biggest hatchery believed to have the largest effective population size of mating individuals produces fingerlings which are the least variable of the strains examined Such a result may reflect broodstock management practices the use of a small founder population when the hatchery was first established or the sampling of a small number of families during the present study Further findings indicate that certain strains are more variable than the putative source population and these strains contain alleles not recorded in the wild population Either reintroductions occurred from other sources or rare alleles became dominant in the strains following domestication

The technique has provided useful information which will allow hatchery managers to produce a breeding program for genetic improvement Tetranucleotide loci in this species may be used in future studies involving selective breeding microsatellites are useful in establishing

kinship In a program which selects individuals for desirable characteristics, it is possible to cross related individuals and reduce genetic variation in the resultant offspring Using microsatellites to identify potential unrelated broodstock will prevent such an outcome This practice known as walkback selection (Doyle and Herbinger 1995) is expected to play an important role in future selective breeding programs

References

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Eknath A E and R W Doyle 1990 Effective population size and rate of inbreeding in aquaculture of Indian major carps Aquaculture 85 293 305

O Reilly P and J M Wright 1995 The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture J Fish Biol 47(A) 29 55

Discussion

Mr Padi What mating system is used in the Indian hatcheries? Is it random assortative or pedigreed? I ask because the system will affect how the effective population size (N_e) is estimated and consequently in-breeding

Dr Naish Fish are mated as they mature but this is not random

Dr Ambali N_e estimation using models of migration is inappropriate in domesticated populations because the exchange of genetic material does not follow the assumptions in the models of migration Exchange of material depends on the socio-economic relationships among the farmers

The Utilization of Allozymes to Study Populations of Tilapias[a]

J -F AGNESE[b]
B ADEPO-GOURENE

AND

L POUYAUD[b]
Laboratoire de Génétique
Centre de Recherches Océanologiques/ORSTOM
BP V18 Abidjan
Côte d Ivoire

AGNESE J F B ADEPO GOURENE and L POUYAUD 1997 The utilization of allozymes to study populations of tilapias p 27 28 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

We summarize here the results of four studies at the Genetics Laboratory of the Centre de Recherches Oceanologiques (CRO) Côte d Ivoire using allozymes to study tilapia populations The first study showed that the genus *Tilapia (sensu stricto)* has the largest number of ancestral characters and confirmed the hypothesis that the mouthbrooders (*Sarotherodon* and *Oreochromis*) arose from the substrate spawners (*Tilapia*) In addition the results confirmed the hypothesis that all mouthbrooders arose from a single speciation event and that species of the genus *Sarotherodon* appear to have been the first to split from the substrate spawner group because in the resulting phylogenetic tree they are situated between the species of the genera *Tilapia* and *Oreochromis*

The second study dealt with the genetic characterization of *O niloticus* populations These were clustered into three major groups first composed of the Nile drainage (the Nile and Lake Edward) and the Kenyan Rift Valley populations (the Suguta river and Lakes Baringo and Turkana) second the Ethiopian Rift Valley populations (the Sodore hot springs and Lakes Awasa Koka and Ziway) and third West African populations Lake Chad and the Chari Niger Volta and Senegal rivers We suggest that *O niloticus* originated from the Nile from where individuals have been able to colonize independently East and West Africa

The third study revealed that the non-native Lake Victoria population of *O niloticus* possess alleles common to both

Because of the relevance and importance of this workshop contribution additional text and illustrations are presented as Appendix 2 (pp 46-56)

[b]Another address CNRS UPR 9060 Universite Montpellier II F34095 Montpellier CEDEX 5 France

Nile and Kenyan populations and probably resulted from a mixing of these two From this study it seems highly improbable that there has been hybridization between *O niloticus* and *O esculentus*

The fourth study revealed the presence of natural hybrids of *T guineensis* and *T zillii* in Lake Ayame Côte d Ivoire probably caused by their confinement and close association in this artificial lake

Discussion

Dr Brummett Are there paleontological explanations for how Nile populations might have crossed the desert to reach West Africa?

Dr Agnese I am not the best person to answer this But we know there had been a lot of communication between Chad and the Nile There are a lot of species common to Chad and the Nile Some time ago there was no desert between Chad and the Nile *Tilapia zillii* is common to both

Dr Teugels You have given one hypothesis There is another hypothesis that of Paleochad In West Africa tilapia speciation is very hypothetical

Dr Pullin Your example of hybridization between *Tilapia guineensis* and *T zillii* in Lake Ayame could be important because such hybridization between sympatric species as a result of human intervention (e g making a dam) is not usually considered as a potential environmental impact Perhaps we should look at the recent reviews on introgressive hybridizations in open water populations and see whether any of these also correlate with human interventions We could then enter this information in Fishbase

Dr Agnèse Yes in this case (unlike all others cited we know of with respect to hybridization) there was no transfer of the species only the making of the dam *Sarotherodon melanotheron* was introduced but this could not have caused the hybridization because we found hybrids in other artificial lakes where *S melanotheron* had not been introduced

Dr Teugels In Lake Ayame the hybrids have become the most important commercially fished population

Dr Abban Since there is an introduced species of tilapia in Lake Ayame is it possible that hybridization of the introduced species (*S melanotheron*) with any of the two (*T zillii and T guineensis)* could have caused the occurrence of hybrids of *T zillii and T guineensis?*

Dr Agnese We did not find *S melanotheron* genes in these studies of tilapia hybrids

Dr Pullin This shows that when people build dams that could bring a change of species

Dr Agnese Definitely

Genetic Characterization of Malawian Tilapias

A J AMBALI
Biology Department Chancellor College University of Malawi
PO Box 280 Zomba
Malawi

AND

R W DOYLE
Gene Probe Laboratory Dalhousie University
Halifax Nova Scotia
Canada B3H 4J1

AMBALI A J and R W DOYLE 1997 Genetic characterization of Malawian tilapias p 29 30 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

In Malawi the genus *Tilapia* is represented by *T rendalli* (Boulenger) while the genus *Oreochromis* is divided into two subgenera *O (Oreochromis)* and *O (Nyasalapia)* The *O (Oreochromis)* flock comprises *O shiranus* (subdivided into *O sh shiranus* [Boulenger] and *O sh chilwae* [Trewavas]) *O mossambicus* (Peters) and *O placidus* (Trewavas) The *O (Nyasalapia)* species flock is composed of *O karongae* (Trewavas) *O lidole* (Trewavas) *O saka* (Trewavas) and *O squamipinnis* (Gunther) *O shiranus* sp an indigenous mouthbrooding tilapia is the most widely cultured species in the country it has been widely distributed in fish farms and reservoirs However the subspecies of *O shiranus* have been difficult to distinguish morphologically

Microsatellite DNA analysis was carried out to provide data for postulating the genetic relationships of *O shiranus* sp populations found in the natural waterbodies in the country and to determine genetic distances between *O shiranus* sp and other tilapia species *O mossambicus O Ny karongae* and *O niloticus O niloticus* is not found in Malawi Five sets of primers were tested on other species of tilapia of the genera *Oreochromis Sarotherodon* and *Tilapia* With a few exceptions the loci analyzed were polymorphic in most of the species The total number of alleles varied among loci ranging from 18 to 30 alleles per locus in five species and was higher than reported in studies on allozymes in the same species (Ambali 1996)

Calculation of Cavalli-Sforza and Edwards (1967) chord distance showed that Lake Chiuta population was closer to the Lake Chilwa population (D = 0 034) than to Lake Malombe population (D = 0 07) A dendrogram constructed using the UPGMA method produced two major groups the *mossambicus* group of tilapias (*O shiranus* sp and *O mossambicus*) clustered together the second cluster was that of non- *mossambicus* tilapias Lake Chilwa and Chiuta populations clustered together as *O sh chilwae* the Lake Malombe population formed a second cluster as *O sh shiranus* The results of clustering analysis were similar

to species and subspecies groupings done by principal components analysis and multidimensional scaling Ordination analysis especially principal component analysis suggested that although *O karongae* and *O niloticus* formed a clade in the UPGMA dendrogram the two species were genetically very different

The classification of populations into *O sh chilwae* and *O sh shiranus* is supported by the known history of geological events associated with the lakes Lakes Chilwa and Chiuta constituted a single open lake which became partitioned by a sand bar during the early Holocene humid phase There was no connection between the two lakes and Lakes Malawi-Malombe drainage system where *O sh shiranus* was found The waterfalls on the Shire river form a natural barrier preventing interspecific hybridization between *O mossambicus* and *O sh shiranus* The former species occurs in the lower course of the river the latter is distributed in the upper course

References

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Carvalli Sforza L L and A W F Edwards 1967 Phylogenetic analysis models and estimation procedures Evolution 32 550 570

Discussion

Mr Padi Have you observed any hybrids between *O shiranus shiranus* and *O shiranus chilwae?*

Dr Ambali No such hybrids have been observed What we had at the main research station was *O sh shiranus* Now that *O sh chilwae* has been brought from the lake the two have come together and may hybridize Before they were never together

Dr Teugels What is the period of origin of Lake Malawi?

Dr Ambali I have no idea about its age There was some idea before that Lake Malawi was fed into the Lake Chilwa This has been disproved there was never a connection between Lake Malawi and Lakes Chilwa and Chiuta

Using and Protecting Malawi's Indigenous Fishes[a]

RANDALL E BRUMMETT
ICLARM/GTZ Aquaculture Project
PO Box 229 Zomba
Malawi

BRUMMETT R E 1997 Using and protecting Malawi s indigenous fishes p 31 32 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Policymakers need options which allow them to make economic decisions about conservation and use of biodiversity Utilizing indigenous germplasm for food production is a simple way out of the dilemma between economic growth and conservation In other words if a resource is demonstrably valuable there will be good economic reasons to manage it sustainably A first step in increasing the value of aquatic biodiversity is an assessment of the usefulness of indigenous fish species in aquaculture and water resources management ICLARM is developing an approach to selecting aquaculture candidates matching the culture unit s characteristics with those of indigenous fish species

Characterizing the Pond

Rather than using the classical approach of labeling food items according to their taxonomy or ecological niche pond food resources are broadly categorized based loosely on the properties of the food which affect their selectivity by consumers For the farmed fish species and pond systems in Malawi food resources are categorized as either plankton (phyto- and zoo-) macrophytes (including filamentous algae) or benthic invertebrates (including detritus) The logic of using such categories is twofold (1) these grouped components of the food web are fairly easy to quantify so data can be collected from a large number and wide variety of ponds and (2) fish may not actually be choosing individual foods as much as they choose a feeding habit or lifestyle which then predetermines which sorts of food items they will gather

Characterizing the Fish

Initial screening is done through literature review of species and examination of specimens captured from the wild Capture data external features (e g dentition body size and form gill raker structure) are qualitatively evaluated to identify species which might survive grow and reproduce in small ponds Stomach contents are quantified to determine what the fish eats in its natural environment This process has so far identified about 30 species which seem to have culture potential

Matching the Fish with the Culture Environment

To give an indication of how well a particular fish species might fit into the pond environment the frequencies of materials from the food groups described

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above in the stomach and/or gut and in the pond system to which they would be stocked are compared Pond food items are converted into dry matter per square meter Relative frequency of dry matter is then calculated to show where food resources are concentrated Fish stomach contents are tabulated according to the relative frequency of the food groups Comparing *Barbus paludinosus* *Lethrinops furcifer* *Oreochromis shiranus* and *Tilapia rendalli* stomach contents to conditions in small ponds fed with chopped grass

	Plankton	Macro phytes	Benthos/ detritus
Grass fed pond	0 02	0 56	0 42
B paludinosus stomach	0 93	0 07	0 00
L furcifer stomach	0 02	0 06	0 92
O shiranus stomach	0 67	0 28	0 05
T rendalli stomach	0 01	0 88	0 11

Taking the average of the absolute value of the difference between food available and food eaten for each food group gives a general indication of the fishes food fit (F_f) with the proposed culture environment A perfect fit using this method would be represented by an average F_f of 0 0 A perfect mismatch would give an F_f of 0 66 In this case *T rendalli* (F_f = 0 21) would be the best grass-fed pond culture candidate of the three followed by *L furcifer* (F_f = 0 33) *O shiranus* (F_f = 0 43) and *B paludinosus* (F_f = 0 61) The method is tentative and needs much refinement hopefully without making it too complicated

Discussion

Ms Entsua-Mensah You showed us a number of slides of Malawian fishes which you said could not be identified Does it mean they did not have local names too?

Dr Brummett I showed the slides to some fishermen but they did not seem to know the names In terms of choosing indigenous species for aquaculture all the fishes for aquaculture in Malawi are indigenous but the problem is improving on the stocks

Dr Teugels How do you integrate into your theory the consideration that fish change diets from young to adult fish?

Dr Brummett One of the things we do is to characterize the materials in fish stomachs with age

Genetic Characterization of Tilapiine Stocks in the Lake Victoria Region

M WILSON
Makarere University Uganda

F BUGENYI
Fisheries Research Institute (FIRI)
National Agricultural Research Organization (NARO)
Jinya Uganda

L KAUFMAN
Boston University Marine Program
Department of Biology
Boston University
5 Cummington Street
Boston MA 02215
USA

AND

P FUERST
Department of Molecular Genetics
The Ohio State University
484 W 12th Avenue
Columbus OH 43210-1292
USA

WILSON M F BUGENYI L KAUFMAN and P FUERST 1997 Genetic characterization of tilapiine stocks in the Lake Victoria region p 33 34 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Genetic variabilities within and among allopatric populations of three species of the genus *Oreochromis* [*O esculentus* (endemic to Lakes Victoria and Kyoga) and *O leucostictus* and *O niloticus* (two exotic species introduced to Lake Victoria in the late 1950s to supplement the failing fisheries)] were examined to compare their population structure assess the magnitude of interspecific hybridization and provide data for a conservation and aquaculture development strategy for *O esculentus* Samples (>10 individuals/species/locality) were taken from Lake Victoria and eight satellite lakes in the Victoria basin Lakes Kayanja Kayugi Manywa and Nabugabo in the Nabugabo system Lake Kanyaboli in the Yala-Nzoia Systems and Lakes Kachira Kiyanebalola and Mburo in the Koki-Lakes system Among the species examined *O niloticus* (0 818) had the highest population gene diversity followed by *O leucostictus* (0 786) with *O esculentus* (0 765) having the lowest *O esculentus* (0 801) exhibited

the highest degree of population subdivision followed by *O niloticus* (0 740) then *O leucostictus* (0 686) All six of the *O esculentus* populations examined exhibited evidence of *O niloticus* alleles The most highly introgressed population was that of Lake Mburo (35 27) the least Lake Kanyaboli (6 72) The three Nabugabo satellite lakes [Lakes Kayanja (12 94) Kayugi (14 39) and Manywa (13 73)] and Lake Kachira (14 11) showed similar levels of introgression from *O niloticus* into *O esculentus* Gene introgression from *O esculentus* into *O niloticus* was generally lower than in the other direction Lake Victoria *O niloticus* (0 91) showed little evidence of *O esculentus* alleles though some introgression was apparent in the remaining three populations [Lake Kachira (8 20) Lake Mburo (6 67) and Lake Nabugabo (21 10)] *O niloticus* in Lake Nabugabo where *O esculentus* has been extirpated displayed surprisingly high levels of introgression and retention of *O esculentus* alleles

The introduced *O niloticus* had high genetic diversity as compared with its congeners (*O esculentus* and *O leucostictus*) There was evidence of introgression of *O niloticus* into *O esculentus* As a result of hybridization since the introduction of *O niloticus* to the Lake Victoria region it is possible that no pure stocks of *O esculentus* exist today

SESSION 4 - DISCUSSION OF THE ICLARM-IAB-ZIM PROJECT RESULTS AND RECOMMENDATIONS

Chairman **Dr R S V Pullin**

Dr Brummett How do you use the manual to interpret data when you get mixed results such as pure strains and hybrids?

Dr Abban What we are indicating in the manual is that if you follow such and such procedures this is the type of results you get and this is how to interpret them

Prof Renwrantz The type of results you get with hybrids has not been described in the manual because it can be very complicated The manual says these are the results you get with pure strains so that when you are confronted with hybrids you can infer this from the results for pure strains But we could indicate in the manual a list of published papers one could refer to

Dr Pullin We can add a paragraph to that effect

Dr Falk We are focusing on species - characterization but for example hemoglobin and globin chain analysis provide information about hybridization These results and figures are included in the manual

Prof Renwrantz To identify hybrids you have to do a comparative study It is difficult to identify one individual as a hybrid But comparison can identify and differentiate individuals of pure strains from hybrids Taking this manual as a cookbook then there should be a little more information on steps to use for such identification

Dr Falk The markers are species-specific You can improve the resolution power of each technique to get the subspecies We did it with East African *Oreochromis n sugutae/O n eduardianus* and *O n niloticus* by the use of hemoglobin and globin chain analysis We were quite successful

Dr Abban We had the problem of distinguishing species before the problem of subspecies It was not our intention to go into subspecies in the manual

Prof Renwrantz We cannot give you a clear answer on which of the procedures to use to test subspecies But you can use isoelectric focusing at pH 3-10 and also antibodies for agglutination But so far we do not know which of these techniques are most sensitive for subspecies

Dr Agnèse Include references of all people who have done work similar to those in the manual—e g E Trewavas for morphometrics It will also be good to indicate references of similar work done in Africa To whom is the manual addressed? People who do not know anything about the subject of the manual and need to be informed?

Dr Pullin There is a section in the Draft Manual which is missing It includes key words references and overviews

of expert work Some of the present suggestions will be included With respect to end-users we are trying to teach biochemical methods to people who want to use them but are apprehensive

Dr Abban For West Africa for instance I do not know many laboratories that have tools and equipment to do electrophoresis This manual shows that the technique is not magic and that millions of dollars are not needed to do it That is why we have not provided infrastructure but only shown how people can improvise to use the techniques University students who may have read about a technique but are scared to do it can benefit from the manual

Prof Renwrantz Even with people who have biochemical knowledge you have to decide where to start from You can get indications about which of the procedures in the manual will be promising depending on what you are looking for

Dr Brummett It is worth pointing out that the manual is not a key It is a component of a key You have to select the technique based on what you want to achieve

Dr Ambali So add data analysis and also have a phylogenetic tree included

Dr Abban This is not a key it is a manual For example it is the basis to discriminate between two species When one is faced with discriminating among many species then it is a key The manual is nowhere near building phylogenetic relationships We never decided to build a key or phylogenetic trees This is a manual you can use to identify or differentiate between this and that With agglutination you can identify between species without reference But for other methods you need references

Dr Renwrantz My suggestion is that you consider the different approaches in the manual and then relate it to the condition of your laboratory If the manual is not appropriate let us know so we can improve it

Dr Pullin I hope some of the methods in the manual will be tried by groups in this region We have not had time and money to put it into test and training exercises - this is a gap to be filled My impression is that when people get tilapia from somewhere they do not bother to check identification whether through looking at taxonomic monographs or morphometrics We do not have any idea of how widely the existing tilapia differentiation tools are being used Has any one used the key that Dr Rosemary Lowe-McConnell put together after the Bangkok Tilapia Conference[a]? Maybe we need to do some research on how the manuals are being used?

Dr Van der Bank We need to know the resources of fish material and where these can be easily obtained

Dr Abban It will be good to have a source say a laboratory where everybody can go and collect some fish sample We could do this if for instance we ask anybody coming from an indigenous

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Pullin R S V Editor 1988 Tilapia genetic resources for aquaculture ICLARM Conf Proc 16 108 p (French edition available from 1989)

tilapia area to bring 10 samples of a species

Dr Pullin How would Dr Van der Bank propose the establishment of the tissue bank?

Dr Van der Bank A decentralized network of people

Dr Ambali In Malawi keeping samples in alcohol will not be difficult But keeping them in a refrigerator will be difficult

Dr Agnese It is better to have collaboration because besides the nonavailability of money to fund a centralized system most scientists will prefer their specimens to be close to them in their laboratories

Dr Brummett Research stations should make sure that the lines they maintain are pure lines

Ms Duthie Compilation of an inventory of who has what material will also be useful

Dr Pullin It can be difficult to transfer live fish But perhaps transferring just a piece of muscle will not be difficult With the former one will have to take note of the sovereign rights of nations and all the legal requirements of shipping biological material

Dr Abban I see it as a simple problem If for instance Dr Ambali has a muscle sample of *O shiranus chilwae* in alcohol and I contact him in a letter he can easily send it to me But transferring live fish is difficult

Dr Pullin We will have to explore to what extent this (biochemical material) can be left out of legal control

Dr Brummett In addition to maintaining biochemical specimens let us maintain the living organisms as well

Dr Satia As scientists we should be careful to take note of legal aspects of material transfers and the requirements of the Convention on Biological Diversity The people who own the material have rights We should rather concentrate on transfer of knowledge and that is how this manual comes about

Dr Pullin Because the exchange of sample containing DNA is becoming increasingly complicated we should build on exchanging results ideas standard pictures of gels etc

Prof Renwrantz Colleagues working on the same molecules say LDH can compare results from different regions and different laboratories if the sample is analyzed with respect to molecular weights and not with respect to how far dyes travel The latter is influenced by laboratory conditions

Dr Pullin With respect to our objective to develop a field kit we would like to have your opinions on whether or not we should keep it as a future objective?

Dr Satia Our discussion has given us some leads on simple techniques to include such as techniques from Dr Naish and Prof Renwrantz We should however include those from other scientists not present at this forum

Prof Renwrantz We should not expect too much from the simple methods developed so far because the methods cannot identify hybrids

Besides most museums etc prefer conservative methods It is only recently that they have included biochemical approaches

Dr Pullin Does anybody know of any field kit that could be used at collection sites?

Dr Brummett Most people go to the field and they do not know the type of fish they see and have to carry it to the laboratory

Dr Ambali A field kit is important Most African countries do not know the fish they are dealing with But it is important to keep the kit very simple A bit of morphological information should be included with the biochemical methods

Dr Teugels A morphological approach is good but has limitations otherwise there would be no need for any other We have equipment and tools for electrophoresis and chromosome studies in our laboratory But biochemical techniques alone cannot give all the answers Morphology and morphometrics still have a future for describing new taxa etc

Dr Renwrantz Do you Dr Teugels subscribe to a field kit?

Dr Teugels The idea of a field kit is good

Dr Brummett It seems to me biochemical techniques override morphometrics because the former can be useful in the analysis [separation] of hybrids and pure strains

Dr Pullin Since tilapia will be moving around with the possibilities of formation of more and more hybrids we have to promote more identification of tilapia than has been done It is to such efforts that this project has tried to contribute

SESSION 5 - REPORTS OF WORKING GROUPS

Conservation of Aquatic Genetic Resources

Chairman Dr B Satia
Members FH van der Bank G Gourene J Owino K Camara and A A Asso

Freshwater fishes are the most threatened of widely exploited vertebrates Most if not all aquatic genetic resources in the region resemble the wild types Approaches to the conservation of these resources should be based on these premises

Recommendations

- Enhance public awareness and seek or secure the support of the different stakeholders
- Develop and use easy cost-effective and reliable morphological and biochemical keys since knowledge of what is to be conserved is imperative
- Encourage countries to implement appropriate guidelines for biosafety and proper use of genetic resources
- Encourage countries to respect existing Codes of Practice on the introduction and transfers of aquatic species Where such introductions and/or transfers are necessary they should be based on explicit protocols
- Assist countries in ensuring the proper utilization and development of local aquatic genetic resources
- Encourage governments to create sanctuaries reserves and restricted areas for conserving local genetic resources
- Adopt a multidisciplinary and holistic approach involving all stakeholders in conserving aquatic genetic resources

The Manual Prepared from the ICLARM-IAB-ZIM Project: Methodologies

Chairman Dr J -F Agnese
Members A Ambali A Abban K-A Naish L Renwrantz T Falk C M V Casal and A Asamoah

Recommendations

- Include in the introduction (1) target audience (2) a statement that the manual evaluates current biochemical methods and (3) the importance of biochemical techniques to be used together with morphometric methods in the identification of species
- Add a chapter on the evaluation of the techniques as well as a matrix of species and techniques so that comparisons can be easily made Add page numbers for easy reference
- Incorporate flow diagrams at the beginning of each chapter to allow quick reference At the bottom of the page mention basic requirements in conduct of methodology (e g freezing of samples)
- Include a discussion chapter to expound on the use of heterozygosity to evaluate population status
- Include future directions of morphological and biochemical techniques
- Provide approximate costs for performing biochemical analyses and a list of laboratories capable of carrying out these tests
- Emphasize the presence of similar studies which used other methods and point out that the work has to be continued
- Cite all important literature English and French to include morphometric references and other biochemical methods and analysis particularly those carried out in West Africa

Aquaculture and Biodiversity Conservation

Chairman Dr R S V Pullin
Rapporteur Dr R Brummett
Members A Diallo O Assemien
A Lovell F Attipoe F Coulibaly-Sylla
F Amevenku B A Gourene H Elghobashy
G Teugels and F Bugenyi

This group discussed three general topics concerning the relationship between aquaculture development and the conservation and wise use of aquatic biodiversity

1 ***Introduction of Exotic Species and Development of Indigenous Species***

There can be serious negative consequences for local biodiversity when exotic species imported into a country or region for purposes of aquaculture escape into natural waterbodies In addition many introductions fail to achieve sustained increases in aquaculture production When introductions do succeed however there can be substantial positive benefits in terms of income generation and food production Making informed decisions about the relative costs and benefits of importing exotic species will require careful consideration of the role which exotics might play in both local economies and ecosystems

Aquaculture policy planners and entrepreneurs have often viewed exotic species as magic bullets which will overcome other often more immediate constraints and engender rapid aquaculture development This has been done without regard for the often large available number of potentially valuable aquaculture species indigenous to an area Consequently few of these species have been adequately studied either in the wild or under culture conditions The use of indigenous species offers real potential to improve local aquaculture output without the risks associated with importation of exotics

Recommendation

In future rather than introducing alien species for aquacultural purposes a maximal use should be made of indigenous species in order to conserve local genetic resources and biodiversity The selection of possible aquaculture candidates should be based on thorough studies of their general biology in the wild and in captivity

2 ***The Place of Aquaculture in the Environment***

Aquaculture both affects and is affected by the external aquatic environment with which it interfaces While clean water is essential the wastes from aquaculture facilities often degrade the bodies of water into which they fall This may have serious consequences to biodiversity which depends on those waterbodies for survival Thus the very biodiversity on which aquaculture depends for the

provision of new genetic material may be negatively affected by the very presence of the aquaculture itself There may thus be clear justification for not locating aquaculture facilities in pristine areas or sites designated as biological reserves

Recommendation

The siting of aquaculture operations should be planned with thorough consideration of their probably impacts on biodiversity and the environment This may preclude aquaculture development in some areas of high conservation importance Conversely aquaculture should be protected against pollution and other negative impacts

3 The Role and Danger of Genetically Modified Organisms

Genetically modified organisms in particular transgenics are unknown quantities both in their potential to improve aquaculture output and their impact on local biodiversity At present the more conservative methods for modifying the genotypes of aquaculture species (e g selective breeding) still offer substantial room for improving productivity The organisms modified by these methods arguably pose fewer environmental risks than do those which are the result of gene transfer or other more radical methods

It is at present virtually impossible to determine the environmental impacts of the release of genetically modified organisms into the environment Substantial research into this question is needed before informed decisions can be made

Recommendation

As domestication of aquatic species and their genetic improvement for aquaculture performance proceed it is important that their products whether produced by selection or genetic manipulations do not have adverse impacts on biodiversity and the natural environment

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The following were inadvertently omitted in Appendix 1 - Principal participants from collaborative institutions and from other countries

Mme O Assemien
Institut Des Savannes
BP 633
Bouake 01
Cote d Ivoire
Tel (225) 63 31 39
Fax (225) 63 20 45

Dr C A Biney
Acting Director
Institute of Aquatic Biology
PO Box 38 Achimota
Accra Ghana
Tel (233-21) 775135
Fax (233-21) 777170

Dr A J Ambali
Biology Department
Chancellor College
University of Malawi
PO Box 280
Zomba Malawi
Tel (265) 522 046 523 056
Fax (265) 522 046
E-mail
aambali@unima wn apc org

Our apologies

The Editors

APPENDICES

1. Principal Participants from Collaborative Institutions and from Other Countries

Dr E K Abban
Institute of Aquatic Biology
PO Box 38 Achimota
Accra Ghana
Tel (233-21) 775135 775511
Fax (233 21) 777170
E-mail
william_anim_dankwa@ghastinet gn apc org

Dr B Adepo Gourene
Centre de Recherches Oceanologiques
BP V18
Abidjan Côte d Ivoire
Tel (225) 355016
Fax (225) 351155

Dr J -F Agnese
Laboratoire de Genetique des Populations
Centre de Recherches Oceanologiques/
ORSTOM
BP V18
Abidjan Cote d Ivoire
Tel (225) 355014 355880
Fax (225) 351155
E mail agnese@abidjan orstom ci

Prof W S Alhassan
Council for Scientific and Industrial
Research
PO Box M32
Accra Ghana
Tel (233-21) 777651 to 54
Fax (233-21) 777655

Dr R E Brummett
ICLARM/GTZ Aquaculture Project
Integrated Farming Systems
PO Box 229
Zomba Malawi
Fax (265) 522733
E-mail r brummett@cgnet com

Dr F W B Bugenyi
Fisheries Research Institute
National Agricultural Research
Organization
PO Box 343
Jinja Uganda
Fax (256) 043 21727 22050
E-mail fbbugenyi@mukla gn apc org

Mr K Camara
Departement de Biologie
Universite de Kankan
BP 209
Republic of Guinea

Ms C M V Casal
International Center for Living Aquatic
Resources Management
MCPO Box 2631
0718 Makati City
Philippines
Tel (632) 812-8641 818-0466
Fax (632) 816-3183
E-mail iclarm@cgnet com
c casal@cgnet com

Mme F Coulibaly-Sylla
Rural Economy Institute
Programme Resources Halieutiques
(CRRA/Mopti)
BP 47
Mopti Mali
Fax (223) 223775

Dr A Diallo
Centre de Recherches Oceanologique de
Dakar - Thiaroye
BP 2241
Dakar Senegal
Fax (221) 342792
E-mail adiallo@isra isra sn

Dr H Elghobashy
Central Laboratory for Aquaculture Research
Abbassa Abu Hammad Sharkia
Egypt
Tel (055) 400497
Fax (055) 400498

Dr T M Falk
Zoologisches Institut und Zoologisches Museum
Universitat Hamburg
Martin Luther King Platz 3
20146 Hamburg Germany
Tel (040) 4123 3873 or 4123 3880
Fax (040) 4123 3937 or 4123 2449
E mail tmfalk@pin-net de

Dr G Gourene
Universite de Cocody
Laboratoire d Hydrobiologie
22 BP 582
Abidjan 22 Cote d Ivoire
Tel (225) 257336/449000
Fax (225) 440412

Mr A Lovell
Department of Biological Sciences
University of Warwick
Conventry CV4 7AL
United Kingdom
Fax (44 203) 524619
E mail fawx@dna bio warwick ac uk

Dr K A Naish
School of Biological Sciences
University of Wales Swansea
Singleton Park Swansea SA2 8PP
Wales United Kingdom
Fax 0792 295447
E mail k naish@swansea Ac UK

Prof J C Norman
Agricultural Research
Council for Scientific and Industrial Research
PO Box M32
Accra Ghana
Tel (233 21) 775135
Fax (233 21) 777170

Dr C Amoako Nuama
Ministry of Environment Science and Technology
PO Box M232
Accra Ghana
Fax (233 21) 666828

Dr M A Odei
Council for Scientific and Industrial Research
PO Box M32
Accra Ghana
Tel (233 21) 775135
Fax (233 21) 777170

Dr J Ouake
Direction de Peches
CARDER MONO
BP 383
Lokossa
Cotonou Benin
Tel (229) 331551
Fax (229) 313837

Dr J Owino
National Museum of Kenya
Center for Biodiversity
PO Box 40658
Nairobi Kenya
Tel (254) 274 2131 4
742161 4 ext 251/249/252
Fax (254) 274 1424

Dr R S V Pullin
International Center for Living Aquatic Resources Management
MCPO Box 2631
0718 Makati City
Philippines
Tel (632) 812 8641 818 0466
Fax (632) 816 3183
E mail iclarm@cgnet com
r pullin@cgnet com

Prof L Renwrantz
Zoologisches Institut und Zoologisches Museum
Universitat Hamburg
Martin Luther-King -Platz-3
20146 Hamburg Germany
Tel (040) 4123-3873 or 4123 3880
Fax (040) 4123-3937 or 4123-2449

Dr B Satia
Programme for Integrated Development of Artisanal Fisheries in West Africa
BP 1369
Cotonou Benin
Tel (229) 330925 330624
Fax (229) 330519 313149
Tlx 5291 FOODAGRI

Dr G G Teugels
Musee Royale de L Afrique Centrale
Africa-Museum
Section Vertebres
Lab Ichtyologie
Leuvensesteenweg 13
B-3080 Tervuren
Belgium
Tel (32) 02 769 5640
Fax (32) 02 767 0242
E-mail teugels@africamuseum be

Dr FH van der Bank
Research Unit for Aquatic and Terrestrial Ecosystems
Department of Zoology
Rand Africaans University
PO Box 524
Johannesburg 2006
South Africa
Tel (+27) 11 489 2450
Fax (+29) 11 489 2286
E mail FHVDB@rau3 rau ac za

2 Additional Participants from Ghana

Mr A A Addo
Fisheries Department (MOFA)
Box 630 Accra

Ms G Bansa
Encyclopaedia Africana
Accra

Mr S H Biney
Council for Scientific and Industrial Research
CSIR Secretariat
PO Box M32 Accra

Mr M K Dade
Department of Oceanography and Fisheries
University of Ghana Legon

Dr C Gordon
Volta Basin Research Project
PO Box 209
Legon Accra

Mr W Q-B West
Food and Agriculture Organization
PO Box 1628 Accra

Mr FYK Attipoe
Mr J N Padi
Mr J K Teye
Institute of Aquatic Biology
PO Box 139 Akosombo

Mr S A Agbenyo
Mr R Agbobli Jr
Mr J S Amakye
Mr FK Amevenku
Ms P Ameyaw
Mr J A Ampofo
Mr A Asamoah
Mr R Asmah
Mr K Atsakpo
Mr H R Dankwa
Ms M Duthie
Ms Entsua-Mensah
Mr K A A de Graft-Johnson
Mr W J Ntow
Institute of Aquatic Biology
PO Box 38 Achimota

2. The Utilization of Allozymes to Study Population of Tilapias

J -F AGNESE
Laboratoire de Génétique
Centre de Recherches Océanologiques
ORSTOM BP V18 Abidjan
Cote d'Ivoire

B ADEPO-GOURENE
Laboratoire de Génétique
Centre de Recherches Océanologiques
ORSTOM BP V18 Abidjan
Côte d Ivoire

AND

L POUYAUD
CNRS UPR 9060 Université Montpellier II
F34095 Montpellier CEDEX 5
France

AGNESE J F B ADEPO GOURENE and L POUYAUD The utilization of allozymes to study population of tilapias p 46 56 *In* R S V Pullin C M V Casal E K Abban and T M Falk (eds) Characterization of Ghanaian tilapia genetic resources for use in fisheries and aquaculture ICLARM Conf Proc 52 58 p

Abstract

We summarize here the results of four studies at the Genetics Laboratory of the Centre de Recherches Oceanologiques (CRO) Cote d Ivoire using allozymes to study tilapia populations The first study showed that the genus *Tilapia (sensu stricto)* has the largest number of ancestral characters and con firmed the hypothesis that the mouthbrooders (*Sarotherodon* and *Oreochromis*) arose from the substrate spawners (*Tilapia*) In addition the results confirmed the hypothesis that all mouthbrooders arose from a single speciation event and that species of the genus *Sarotherodon* appear to have been the first to split from the substrate spawner group because in the resulting phylogenetic tree they are situated between the species of the genera *Tilapia* and *Oreochromis*

The second study dealt with the genetic characterization of *O niloticus* populations These were clustered into three major groups first composed of the Nile drainage (the Nile and Lake Edward) and the Kenyan Rift Valley populations (the Suguta river and Lakes Baringo and Turkana) second the Ethio pian Rift Valley populations (the Sodore hot springs and Lakes Awasa Koka and Ziway) and third West African populations Lake Chad and the Chari Niger Volta and Senegal rivers We suggest that *O niloticus* originated from the Nile from where individuals have been able to colonize independently East and West Africa

The third study revealed that the non native Lake Victoria population of *O niloticus* possess alleles common to both Nile and Kenyan populations and probably resulted from a mixing of these two From this study it seems highly improbable that there has been hybridization between *O niloticus* and *O esculentus*

The fourth study revealed the presence of natural hybrids of *T guineensis* and *T zillii* in Lake Ayame Cote d Ivoire probably caused by their confinement and close association in this artificial lake

Studies

1 Phylogenetic relationships among three tilapiine genera[a]

The classification of Tilapias is controversial Thys van den Audenaerde (1970 1971 1978 1980) grouped all Tilapias under the genus *Tilapia* within which he created a number of subgenera Trewavas (1966 1973 1980 1981 1982a b) divided the genus *Tilapia (sensu lato)* into three genera *Tilapia* *Sarotherodon* and *Oreochromis* based on their meristic morphometric and ethological characteristics and especially on their reproductive behavior genus *Tilapia* are substrate spawners genus *Sarotherodon* paternal or biparental mouthbrooders and genus *Oreochromis* maternal mouth-brooders Trewavas (1983) produced a detailed monograph on the mouthbrooding genera

The evolution of the reproductive behavior of tilapias is also open to debate Trewavas (1982a) suggested that a mouthbrooding ancestor diverged from the ancestral group of substrate spawners to give rise to the biparental mouthbrooders *(Sarotherodon)* and then the maternal mouthbrooders *(Oreochromis)* (Fig 1) An alternative hypothesis proposed by Peters and Berns (1978 1982) is that the present mouthbrooders have different ancestors and were the result of multiple evolutionary events from substrate spawners According to their hypothesis maternal mouthbrooders were the first to split from the substrate spawners

To investigate speciation in *Tilapia (sensu lato)* 24 enzyme loci were studied in five species of the genus *Oreochromis* four species of the genus *Sarotherodon* 11 species of the genus *Tilapia (sensu stricto)* and four other African cichlid species genera Results showed a close correspondence between the clustering of species and the genus to which they belong Species of the genus *Tilapia (sensu stricto)* are found at one end of the tree Three *Sarotherodon* species are found at the middle along with a clustering of species of the genus *Oreochromis* *S melanotheron* is found at the other end of the tree (Fig 2a) The addition of other cichlid species (*Chromidotilapia guntheri* *Chylochromis duponti* *Hemichromis fasciatus* *Pelmatochromis buettikoferi* and *Tylochromis jentinki*) did not change the previous phylogenetic structure (Fig 2b)

This phylogenetic tree shows that the species of the genus *Tilapia (sensu stricto)* could be considered to have the largest number of ancestral characters of all the species of *Tilapia (sensu lato)* proposed by Thys van den Audenaerde Indeed the five species added to the tree are all grouped within *Tilapia (sensu stricto)* The fact that *Tilapia (sensu stricto)* can be considered to have the largest number of ancestral characters confirms the hypothesis that mouthbrooders (*Sarotherodon* and *Oreochromis*) arose from substrate spawners In addition these results confirm the hypothesis that mouthbrooders have a *common* ancestor The phylogenetic trees obtained clearly showed the clustering of species by genus If multiple evolutionary events had given rise to the mouthbrooders this clustering of species by genus would not be observed The observation is consistent with the hypothesis that a single speciation event gave rise to all mouthbrooders Moreover as suggested by Trewavas

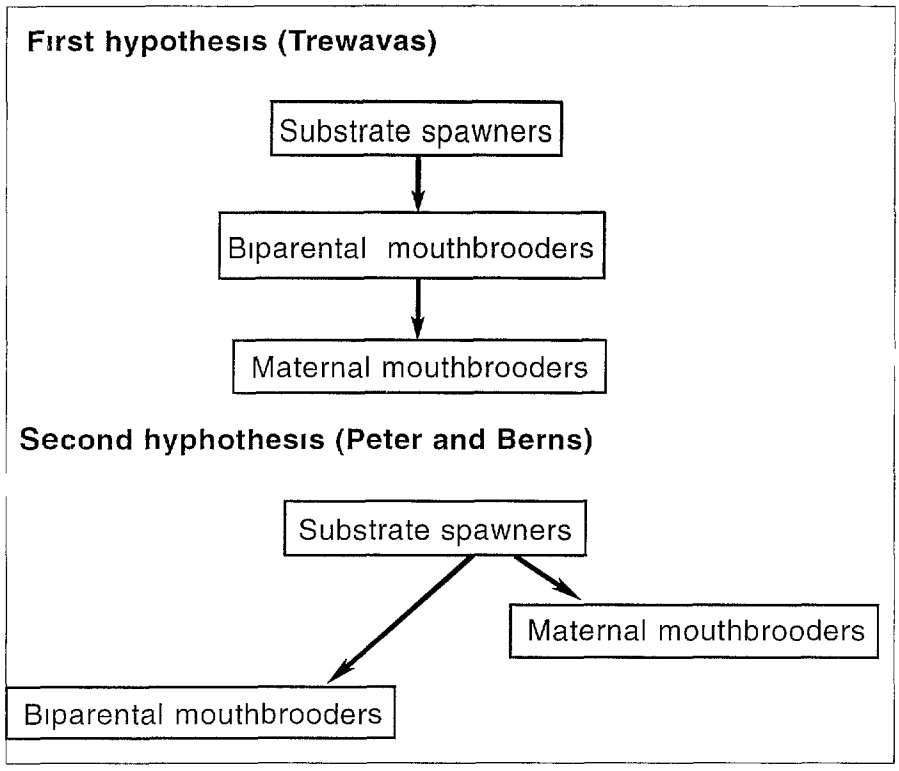

Fig 1 Alternative hypotheses for the reproductive behavior of tilapias Arrows indicate the origin of groups

(1980) species of the genus *Sarotherodon* appear to have been the first to split from the substrate spawners group since they are situated in between the species of the genus *Tilapia* and *Oreochromis* of the phylogenetic tree

Although these results clarify the phylogenetic relationships among these species of the three genera *Tilapia (sensu stricto) Sarotherodon* and *Oreochromis* questions remain concerning the relationships between *S melanotheron* and all other species This species is genetically closer to species of the genus *Oreochromis* than those of the genus *Sarotherodon* Further studies on a larger number of species in *Tilapia (sensu lato)* will be needed to elucidate the evolutionary event(s) that gave rise to this species

2 Genetic differentiation among natural populations of Oreochromis niloticus[b]

Among all tilapia species the Nile tilapia *(Oreochromis niloticus)* is the most commercially important species The natural range of *O niloticus* includes the Awash Benue Chari Gambia Niger Nile Senegal and Volta rivers and many lakes like those of the Rift Valley Albert Baringo

[b] J F Agnese B Adepo Gourene E K Abban and Y Fermon Heredity genetic differentiation among natural populations of the Nile tilapia *Oreochromis niloticus* (Teleostei Cichlidae) (In press)

Edward George Kivu Tanganyika and Turkana Trewavas (1983) using morphometrical analysis described seven subspecies *O niloticus niloticus* from West Africa and Nile *O n baringoensis* from Lake Baringo *O n cancellatus* from the Awash river system in Ethiopia *O n eduardianus* from Lakes George Edward and Tanganyika *O n filoa* from the hot springs of the Awash river *O n sugutae* from Suguta river in Kenya and *O n vulcani* from Lake Turkana Seyoum and Kornfield (1992a) described a new subspecies *O n tana* from Lake Tana in Ethiopia based upon its genetical characteristics

In Africa *O niloticus* populations from several sources have been introduced into rivers e g in Cote d Ivoire where a farmed strain (resulting from the interbreeding of individuals from the Nile and Volta basins) is now present in all major rivers Cameroon Guinea and Benin also have some introduced populations which as in Cote d Ivoire resulted from the escape of captive broodstock In many of these situations it seems that this species did not cause the disappearance of any other species particularly any related tilapia species However this was not the case in Lake Victoria where *Oreochromis niloticus* was introduced in the late 1950s (Kaufman 1992) This species and introduced Nile perch *(Lates niloticus)* have been implicated in the disappearance of a closely related species *O esculentus* which originally supported the lake s most important fishery (Ogutu-Ohwayo 1990)

Although *O niloticus* has a wide distribution and is of great economic importance the genetic characterization of natural populations has not been thoroughly accomplished This could be of great importance for research on strains for aquaculture for the protection of endangered populations (for example those in Lake Baringo or rivers like the Suguta river) and for making biogeographical inferences There has been some genetic characterization of strains used in aquaculture (McAndrew and Majumdar 1983 Basiao and Taniguchi 1983 Maracanas et al 1995) Recently some studies have been made on natural populations Seyoum and Kornfield (1992a 1992b) made a study on East African populations and Rognon (1993) and Rognon et al (in press) studied West African populations

Our study was the first in which the natural populations of *O niloticus* representing all the subspecies described in the major basins (the Awash Niger Nile Senegal Suguta and Volta rivers and Lakes Baringo Chad Edward Tana and Turkana) were investigated This was to facilitate subspecies or population characterization and biogeographic inferences We analyzed the genetic differentiation among seventeen natural populations using allozymes Sixteen of the 25 loci studied were polymorphic Dendrograms showed that the populations are clustered into three major groups the first composed of the Nile drainage (the Nile and Lake Edward) and the Kenyan Rift Valley populations (the Suguta river and Lakes Baringo and Turkana) the second composed of the Ethiopian Rift Valley populations (the Sodore hot springs and Lakes Awasa Koka and Ziway) and the third West African populations (Lake Chad and the Chari Niger Senegal and Volta rivers) (Fig 3)

The main difference between our results and Trewavas s (1983) nomenclature is the genetic differentiation observed in *O n niloticus* All the West African populations (Chad Niger Senegal and Volta basins) appear closely related whereas populations from the Nile are closely related to the East African populations (the Suguta river and Lakes Baringo Edward and Turkana) Her

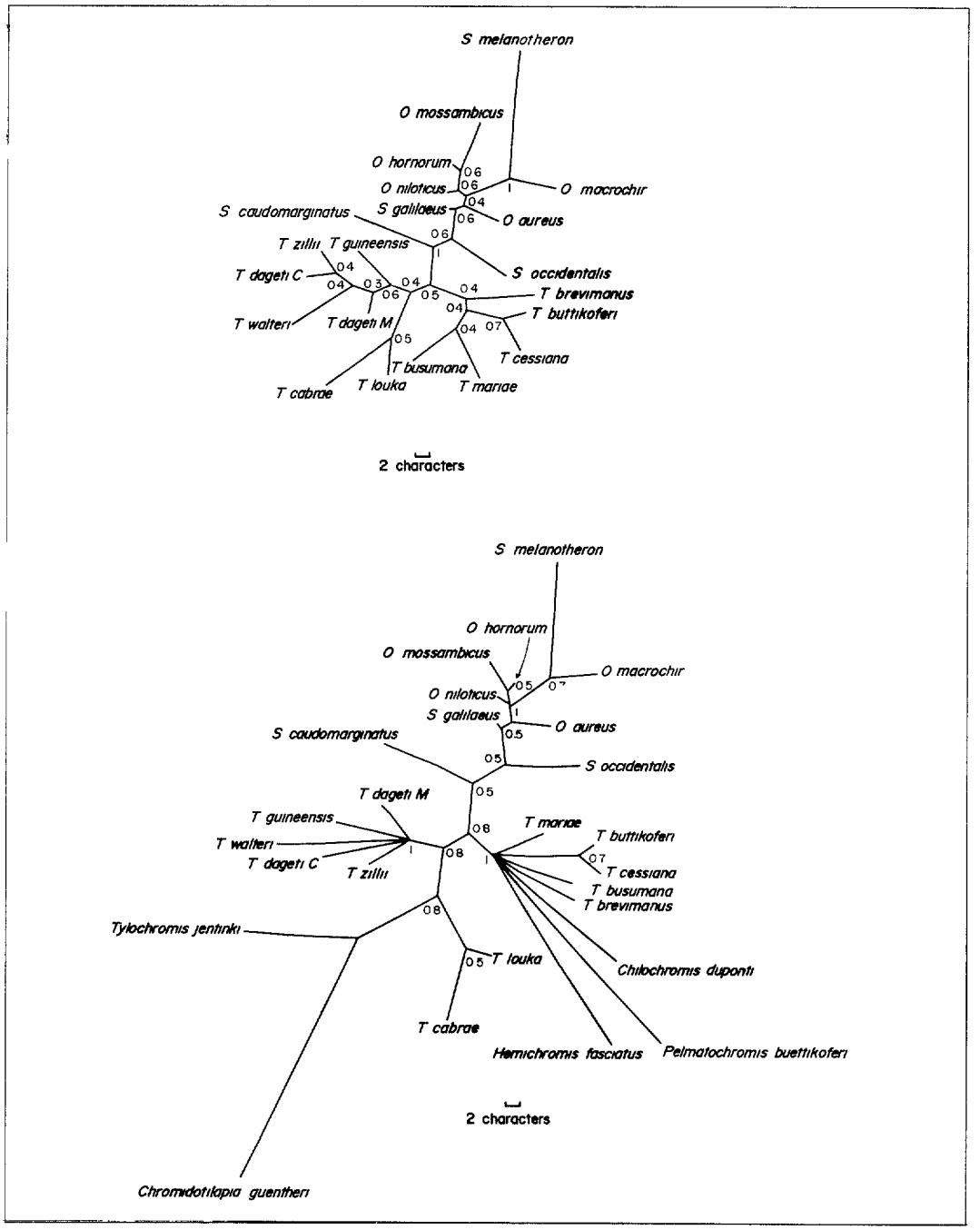

Fig 2 Genetic relationships (adapted from Pouyaud and Agnese 1995) (a) among 20 tilapia species and (b) between these species and five species of other genera Lengths of the different branches of the networks have been calculated by a parsimony algorithm (MIX) and are based on the number of allelic differences (characters) *T guineensis* C and *T guineensis* M represent two different populations of this species from Côte d Ivoire and Mali respectively

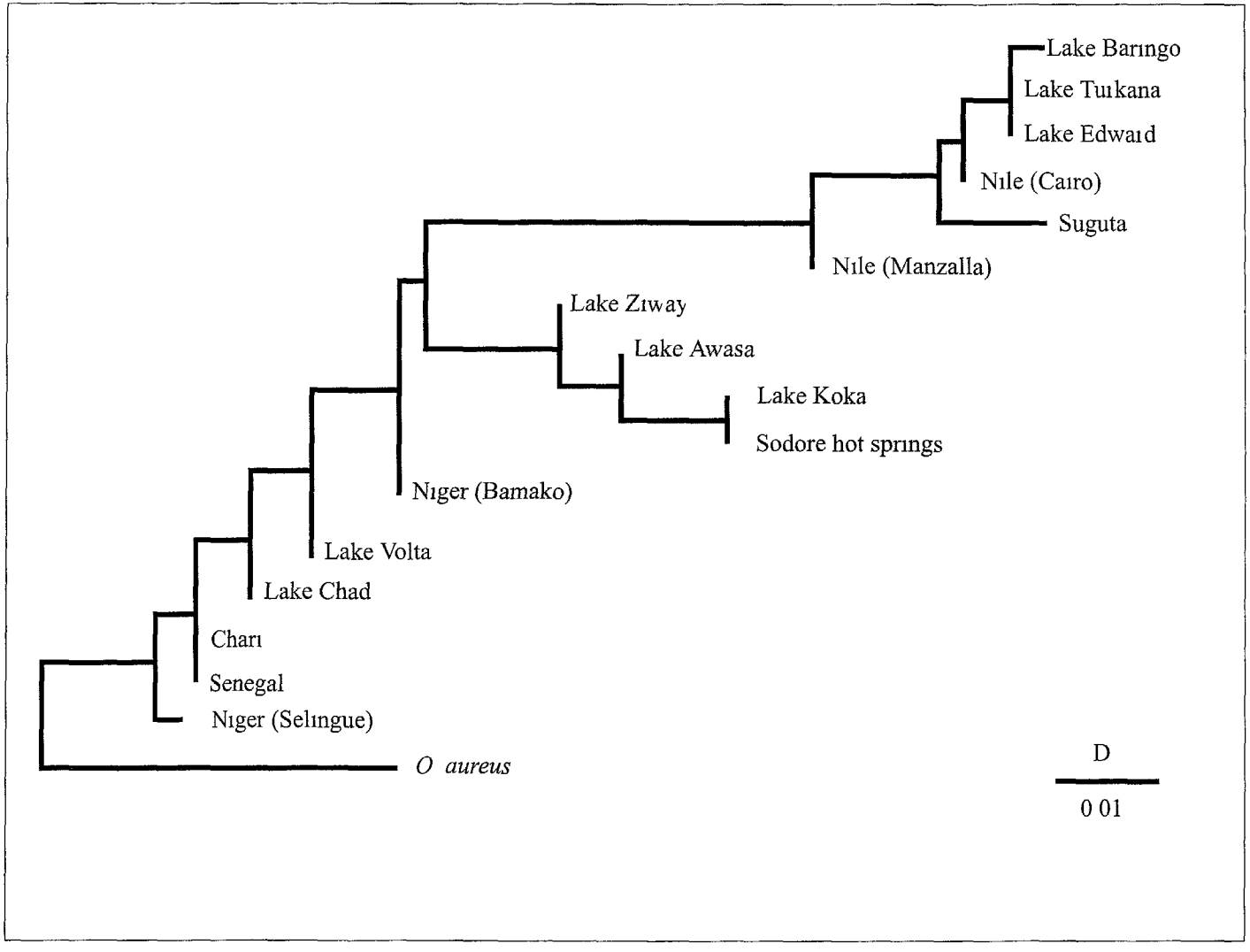

Fig 3 Genetic relationships among different populations of *Oreochromis niloticus* with *O aureus* for comparison) (adapted from Agnese et al in press) using the neighbor joining method on a Nei (1978) genetic distances matrix

subspecific nomenclature based on morphological differences is not consistent with the data from our genetic analyses

The distribution of tilapia species and populations in Africa depends upon historical events (climatic change geological change such as volcanic events etc) With our results it became possible to reconstruct some of the past events that led to the current distribution of *O niloticus* populations Colonization is often accompanied by bottleneck effects due to the generally small size of populations that gain access to one basin from another These bottlenecks intensify genetic drift and lead to the loss of genetic diversity In these conditions populations located at or close to the origin of the species are those which have undergone the fewest bottlenecks If we consider polymorphism (P) the populations that we studied can be divided in two groups the Nile and West African populations (subspecies *O n niloticus*) with P values never less than 0 04 and the East African populations (all other subspecies) with P values never greater than 0 04 (Fig 4) Further results from a mitochondrial DNA study showed that the Nile population can be considered as the more polymorphic one compared to the West African populations These observations led us to hypothesize that the area of origin of *O niloticus* could be the Nile From this area individuals have been able to colonize independently East and West Africa (Fig 5) To elaborate further on this colonization model more data on the genetic differentiation of populations are needed

3 Genetic characterization and possible origin of the *Oreochromis niloticus* population of Lake Victoria[c]

Oreochromis niloticus was introduced into Lake Victoria during the late 1950s (Kaufman 1992) This species and introduced Nile perch *Lates niloticus* are implicated in the disappearance of *O esculentus* which originally supported the lake s most important tilapia fishery (Ogutu-Ohwayo 1990)

Genetic characterization of *O niloticus* from Lake Victoria was performed to look for evidence of its hybridization with *O esculentus* and to explore the origins of the Lake Victoria *O niloticus* *O niloticus* specimens were collected from Usenge and Kusa in the Kenyan part of Lake Victoria from Lake Baringo and from the Nile at Manzalla *O esculentus* specimens were collected from Lake Kanyaboli a small lake very near Lake Victoria Nineteen loci were analyzed and a phylogenetic tree was produced

The two populations of Lake Victoria appeared phylogenetically close to the Nile population (Lake Manzalla) (Fig 6) Only two polymorphic loci were observed from the Lake Victoria populations The second allele of Aat 3 was present in the Nile population but absent in the Kenyan (Lake Baringo) population The second allele of Pgi 1 was absent in the Nile population but present in the Kenyan (Lake Baringo) population The Lake Victoria population possess alleles common to both Nile and Lake Baringo populations and probably results from a mixing of these two populations No specific allele of *O esculentus* was found in *O niloticus* from Lake Victoria Hence

[c] Paper in preparation by J F Agnese B Adepo Gourene J Owino and R Aman

there has probably been no hybridization between these two species

4 Interspecific hybridization of autochthonous tilapia in Côte d Ivoire[d]

Recently there was a case of hybridization between sympatric autochthonous species of *Tilapia T guineensis* and *T zillii* in an artificial lake Lake Ayame in Côte d Ivoire (Pouyaud 1994) It is easy to distinguish these species on their pharyngeal teeth (Teugels and Thys van den Audenaerde 1992) or on the coloration of the caudal fin (Pouyaud 1994) Specimens collected in Lake Ayame displayed typical species-specific and intermediate color patterns

Twenty-five loci were analyzed in more than 100 specimens from the lake Seven were polymorphic and two were discriminating between these two species See Fig 7 for results of a factorial analysis of the different genotypes observed in Lake Ayame G stands for specimens of *T guineensis* Z for *T zillii* and H for undetermined individuals The results show that the H individuals were genotypic intermediates of *T guineensis* and *T zillii* species Most of them were heterozygotes at the discriminating loci This hybridization was probably a result of their confinement and close association in the artificial lake Of five artificial lakes in Côte d Ivoire where both species are present we found such in four

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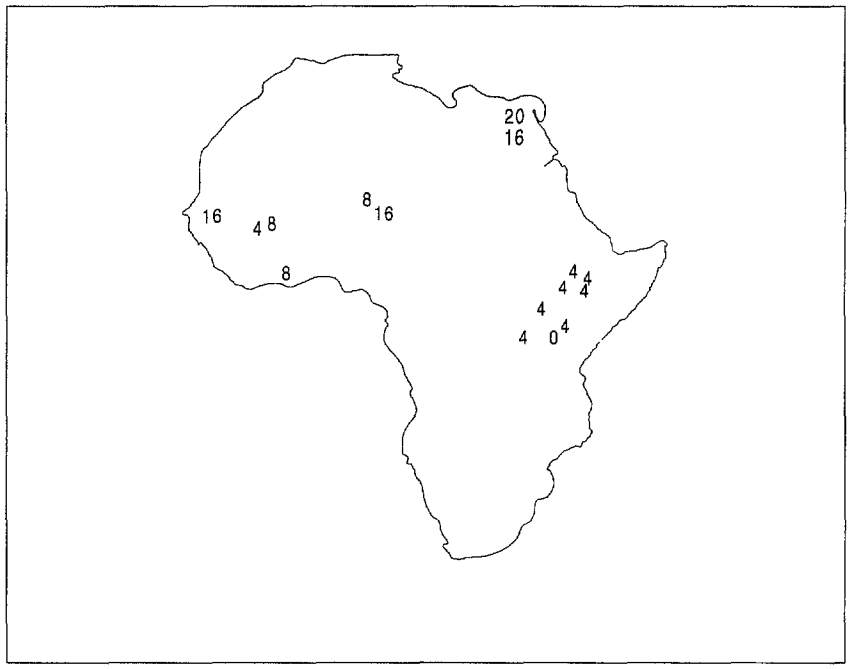

Fig 4 Values of P (number of polymorphic loci observed divided by the number of all loci studied) in percent observed in all the populations investigated

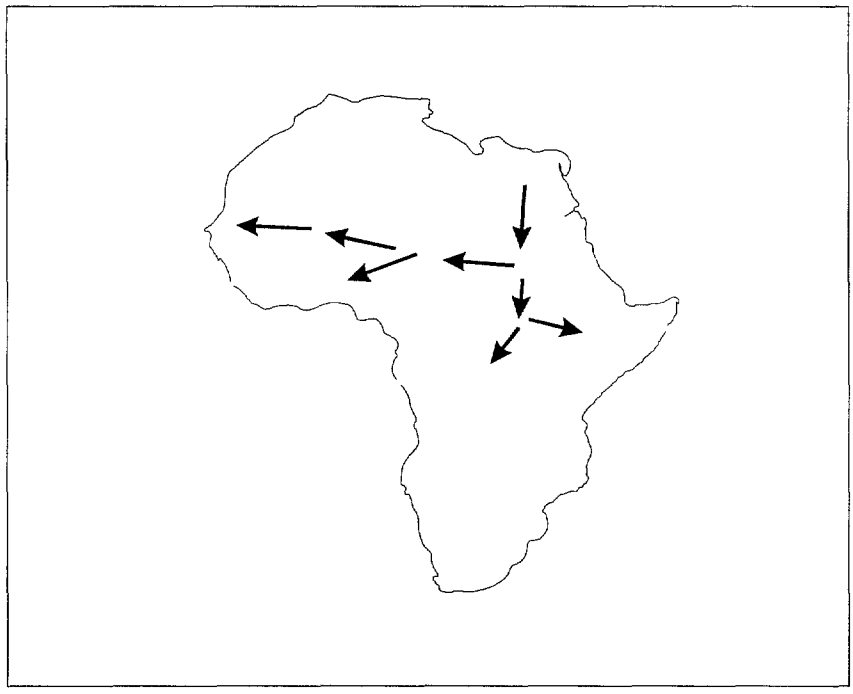

Fig 5 Possible colonization events that led to the current distribution of *Oreochromis niloticus* From the Nile individuals have independently colonized East and West Africa

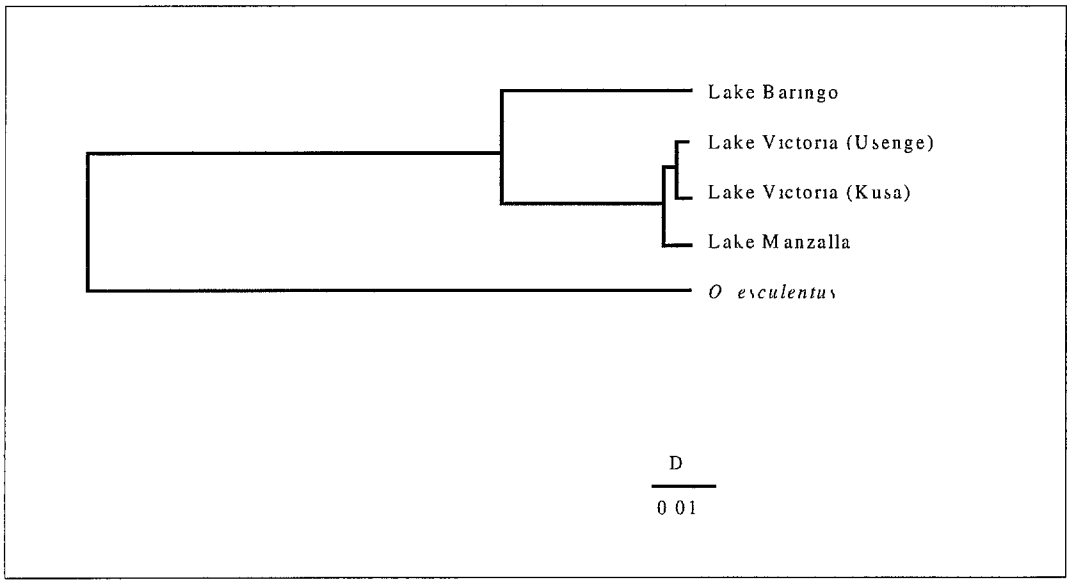

Fig 6 Dendrogram (Nei 1972) genetic distances and UPGMA showing genetic relationships among Lake Victoria Kenyan and Nile populations of *Oreochromis niloticus* and between this group of populations and *O esculentus* from Lake Kanyaboli

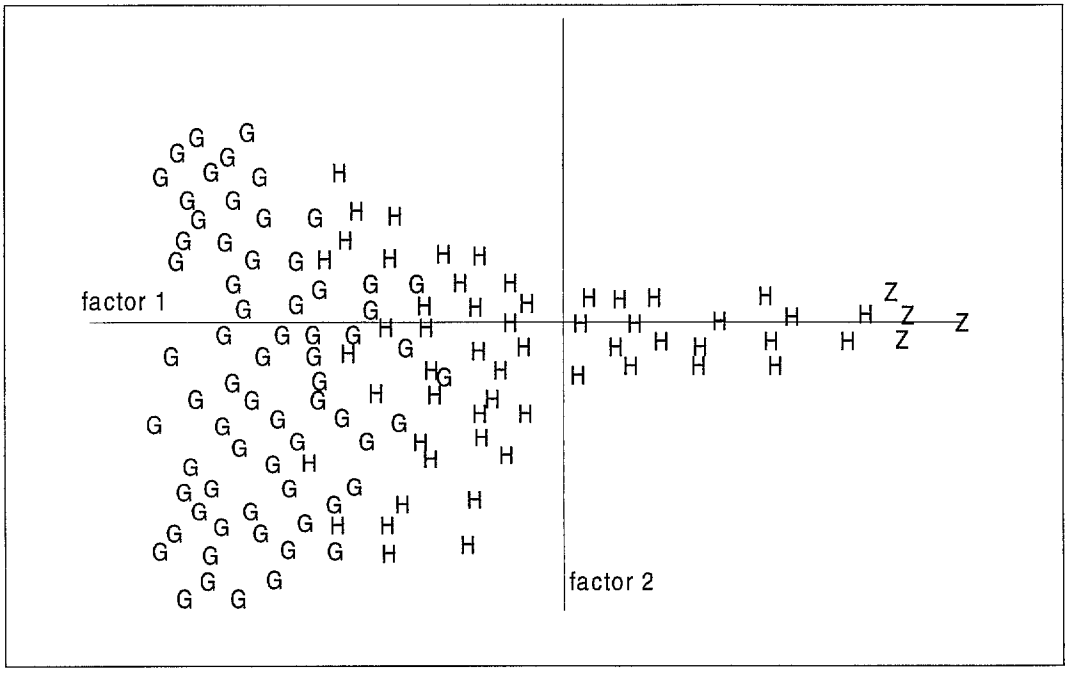

Fig 7 Factorial analysis of *Tilapia guineensis* (G) *T zillii* (Z) and undetermined specimens (H) Allozymes data were submitted to a correspondance analysis to assess overall relationships between samples Multi locus data input consisted of an unweighed sample x allele matrix with each sample defined by its allelic counts at the loci

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