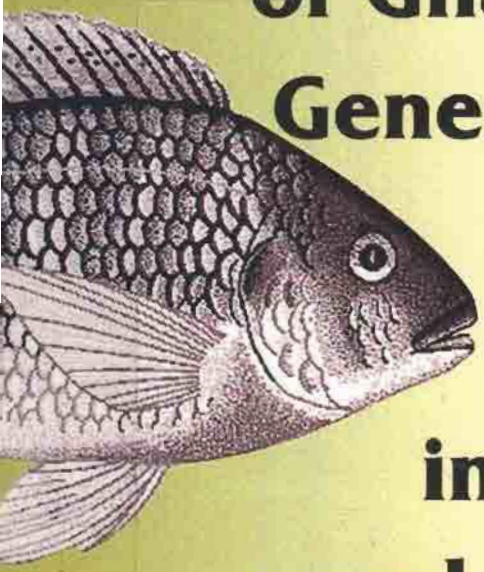
Characterization of Ghanaian Tilapia Genetic Resources for Use in Fisheries and Aquaculture



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

INSTITUTE OF AQUATIC BIOLOGY
(CSIR)
Institute of Aquatic Biology/
Council for Scientific
and Industrial Research

UNIVERSITÄT HAMBURG
Zoologisches Institut
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Contents

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, Universität Hamburg (ZIM), Hamburg, Federal Republic of Germany, have been, since 1991, partners in a project supported by the Bundesministerium für Wirtschaftliche Zusammenarbeit und Entwicklung (BMZ)/ Deutsche Gesellschaft für 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
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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 für 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
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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 für 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), Universität 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 Guineé, Kenya, Malawi, Mali, the Philippines, Sénégal, 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]

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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.

[a]ICLARM Contrib. No. 1273.

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

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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[2]	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. Agnèse, 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

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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 Agnèse 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 Côte d'Ivoire. For all the tilapia species we have worked on, we have samples also from Côte d'Ivoire. The collaboration has been on the personal level with Dr. Agnèse. 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 Department 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 Department 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. Agnèse: 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)

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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. Agnèse, conducted at the IDESSA fish station in Bouaké.

Four different strains of *O. niloticus* were assessed: the Bouaké (BKE) strain, which was obtained from the IDESSA fish station; the Niger (NIG) strain, from the Niger river; the Sénégal (SEN) strain, from the Sénégal 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 Sénégal and Bouaké 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 Sénégal strain to have the best rate, followed by the Bouaké 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 Bouaké strain and the Sénégal strain. Taking the observations from the three parameters studied, it would seem that the Sénégal strain is the best fish for culture, followed by the Bouaké strain, the Niger strain and the Ghana strain. The results are, however, not yet conclusive and the study is continuing.

Fish Genetics in Egypt

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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

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DIALLO, A. 1997. Biodiversity and genetics of farmed tilapias in Sénégal. 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.

Sénégal, 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 Sénégal 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 (Sénégal 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 Sénégal river and the Guiers lake. *T. guineensis* and *S. melanotheron* are euryhaline; these are present in the Sénégal river, Saloum and Casamance estuaries and Guiers lake. *S. melanotheron* is also found in waterbodies (Niayes) around Dakar city.

Fish culture experience in Sénégal began with projects around the Sénégal 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 Océanographique 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 Sénégal 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 Sénégal, Côte d'Ivoire and Congo are an example. G.G. Teugels and J-F. Agnèse have suggested that *S. melanotheron paludinosus* is probably *S. melanotheron heudelotii*. The genetic distance among *S. melanotheron* populations from several places in Sénégal and among *O. niloticus* populations from Sénégal and Bouaké 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^{-1}, 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 Sénégal. Was it accidental that different species of tilapia are cultured in different areas of Sénégal?

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

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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

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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

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COULIBALY-SYLLA, F. 1997. Tests of fish stocking in bancôtières 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 "bancôtières," 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 bancôtières 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 bancôtières in both villages. The fish that were fed grew by an average of 0.41 g·day. The weight of fish declined in bancôtières without supplementary feed. The results suggested that, for effective culture of *C. anguillaris* in these bancôtières, 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 bancôtières. 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 bancôtières 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

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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)

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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, Sénégal 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 Sénégal 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 Sénégal. Important affinities were also demonstrated between *C. anguillaris* populations from West Africa and *C. gariepinus* populations from Lake Victoria (Teugels 1982, 1986; Risch 1992; Agnèse 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 (Adépo-Gourène 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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Agnèse, J.-F., G.G. Teugels, P. Galbusera, R. Guyomard, F. Volckaert and E. Zouros. Four independent and simultaneous approaches to characterize sympatric catfish populations of *Clarias gariepinus* and *C. anguillaris* (Siluroidei: Clariidae) from Sénégal: morphometry, allozymes, microsatellites and RFLP of mitochondrial DNA. Molecular systematics. (In press).

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Discussion

Dr. Pullin: The results that you have described for the Bouaké 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

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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.

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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 inbreeding.

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]

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We summarize here the results of four studies at the Genetics Laboratory of the Centre de Recherches Océanologiques (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 Sénégal 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

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

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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 Ayamé, 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. Agnèse: 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 Ayamé 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 Ayamé, the hybrids have become the most important commercially fished population.

Dr. Abban: Since there is an introduced species of tilapia in Lake Ayamé, 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. Agnèse: 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. Agnèse: Definitely.

Genetic Characterization of Malawian Tilapias

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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.

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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]

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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

[a]ICLARM Contribution No. 1361.

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

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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

[a]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. Agnèse: 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: F.H. van der Bank, G. Gourène, 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. Agnèse
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. Gourène, 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.

APPENDICES

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2. The Utilization of Allozymes to Study Population of Tilapias

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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 Océanologiques (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 Sénégal 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 Ayamé, Côte 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

[a]Phylogenetic relationship among 21 species of three tilapiine genera *Tilapia*, *Sarotherodon* and *Oreochromis* using allozyme data. L. Pouyaud and J.-F. Agnèse. 1995. J. Fish Biol. 47: 26-38.

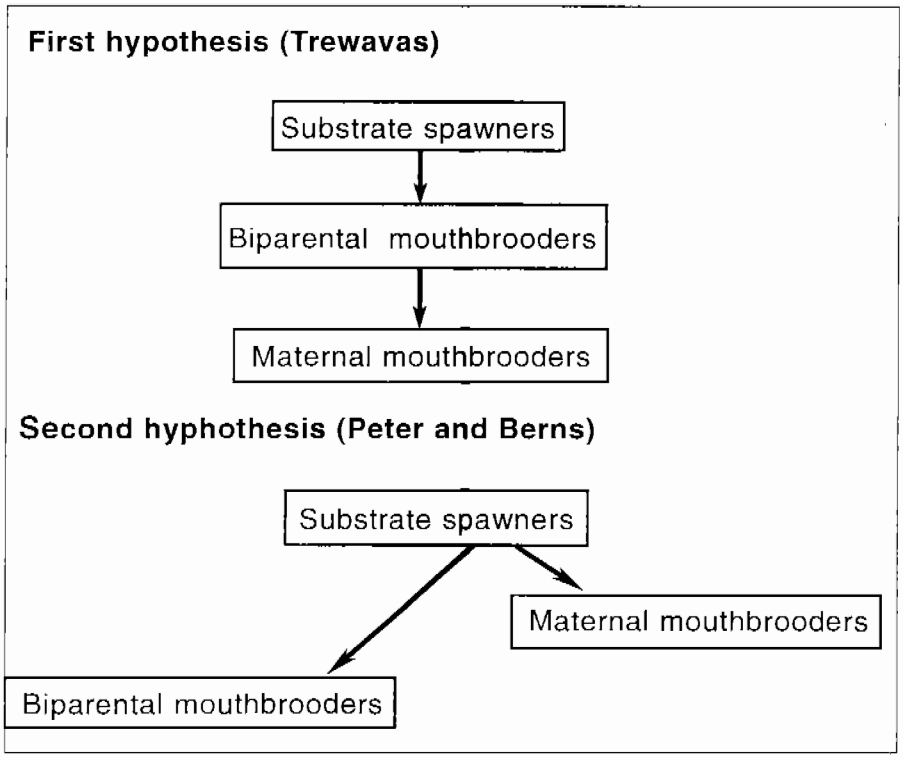

Fig. 1. Alternative hypotheses for the reproductive behavior of tilapias. Arrows indicate the origin of groups.

(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, Sénégal and Volta rivers and many lakes like those of the Rift Valley: Albert, Baringo,

[b]J.-F. Agnèse, B. Adépo-Gourène, 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 Côte 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 Côte 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, Sénégal, 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, Sénégal 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, Sénégal 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

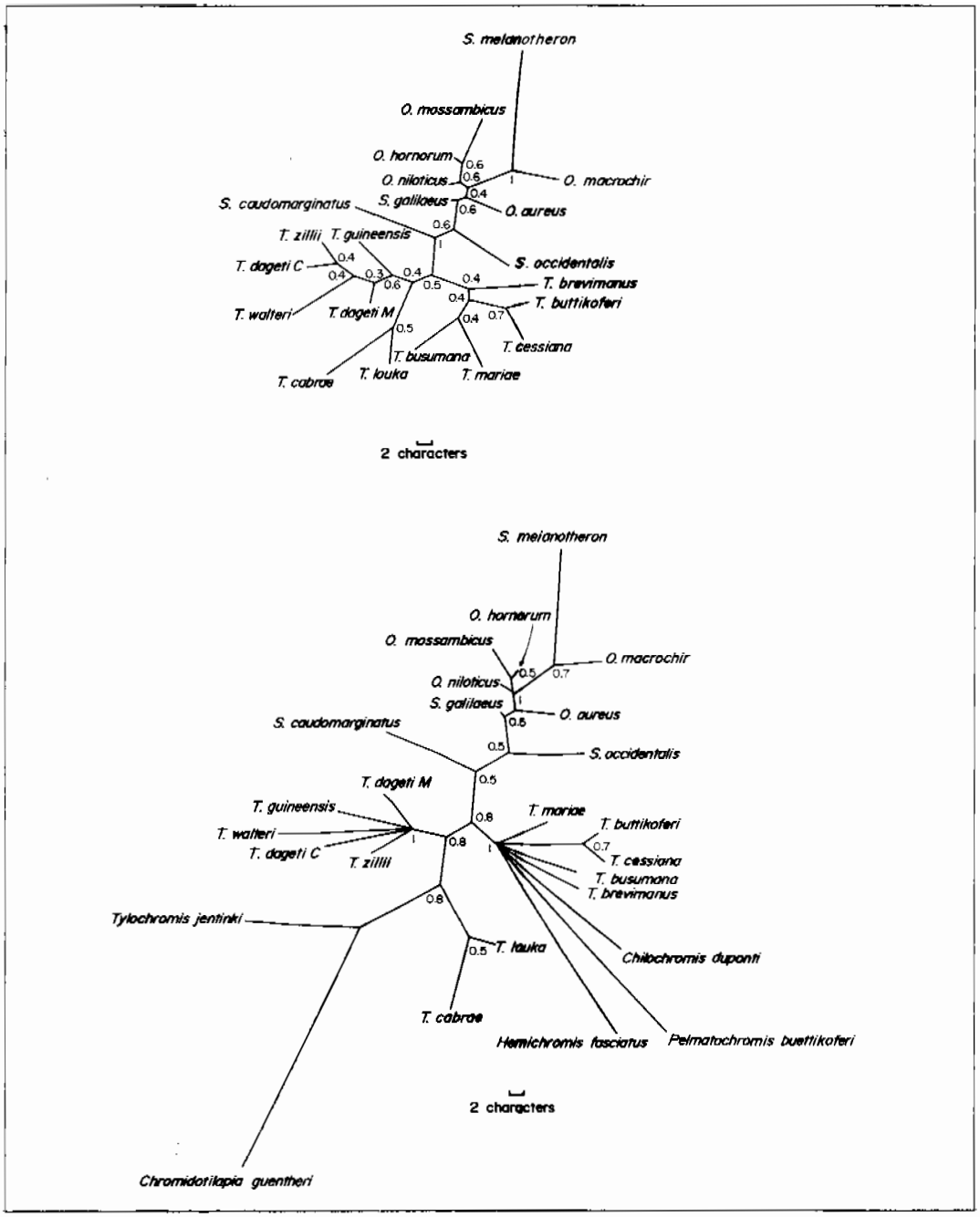

Fig. 2. Genetic relationships (adapted from Pouyaud and Agnèse 1995): (a) among 20 tilapia species and (b) between these species and five species of other genera. Lengths of the different branches of the networks have been calculated by a parsimony algorithm (MIX) and are based on the number of allelic differences (characters). *T. guineensis* C and *T. guineensis* M represent two different populations of this species from Côte d'Ivoire and Mali, respectively.

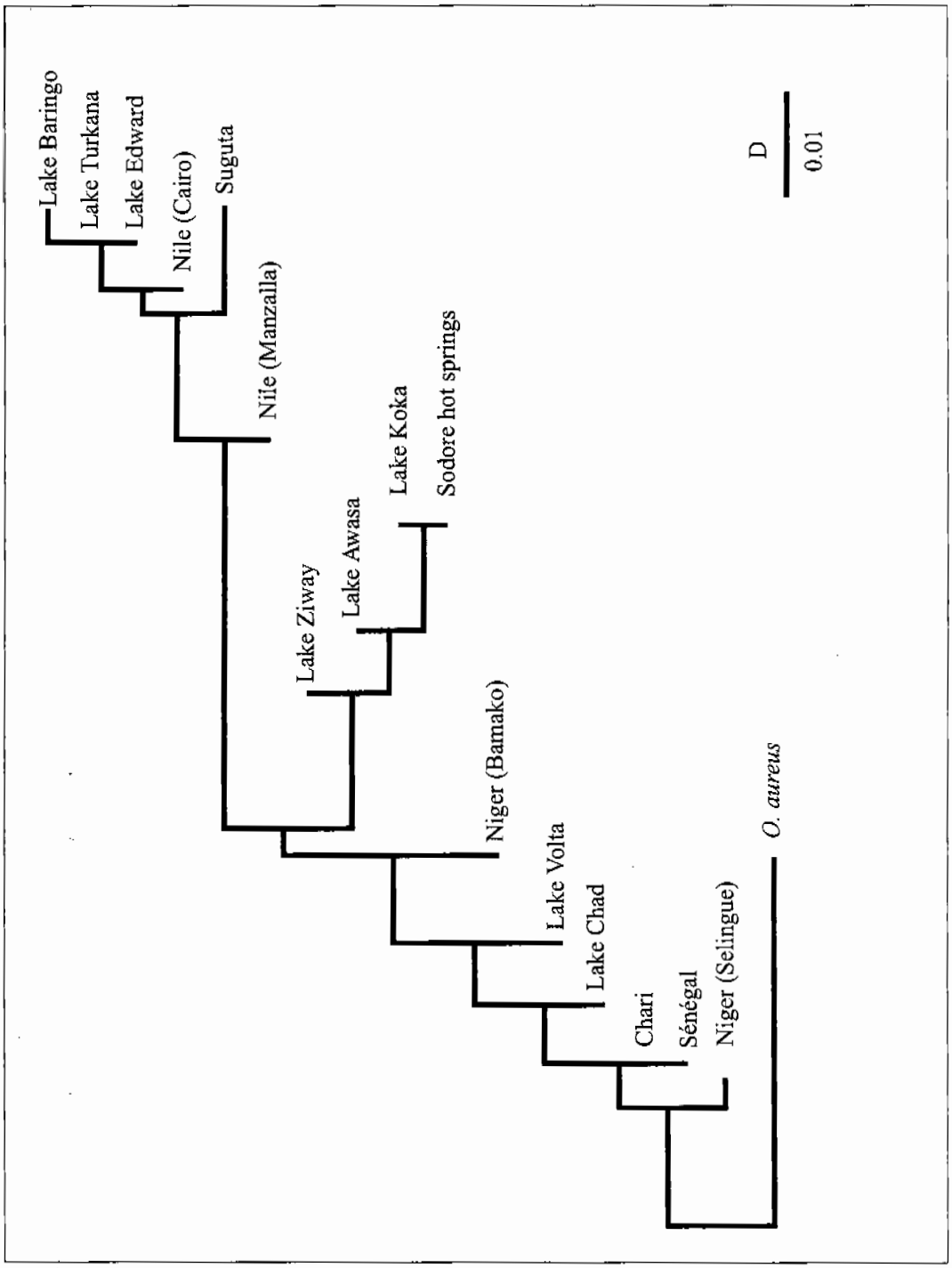

Fig. 3. Genetic relationships among different populations of *Oreochromis niloticus* with *O. aureus* for comparison) (adapted from Agnèse et al., in press) using the neighbor-joining method on a Nei (1978) genetic distances matrix.

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. Agnèse, B. Adépo-Gourène, J. Owino and R. Amán.

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 Ayamé 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 Ayamé 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 Ayamé. 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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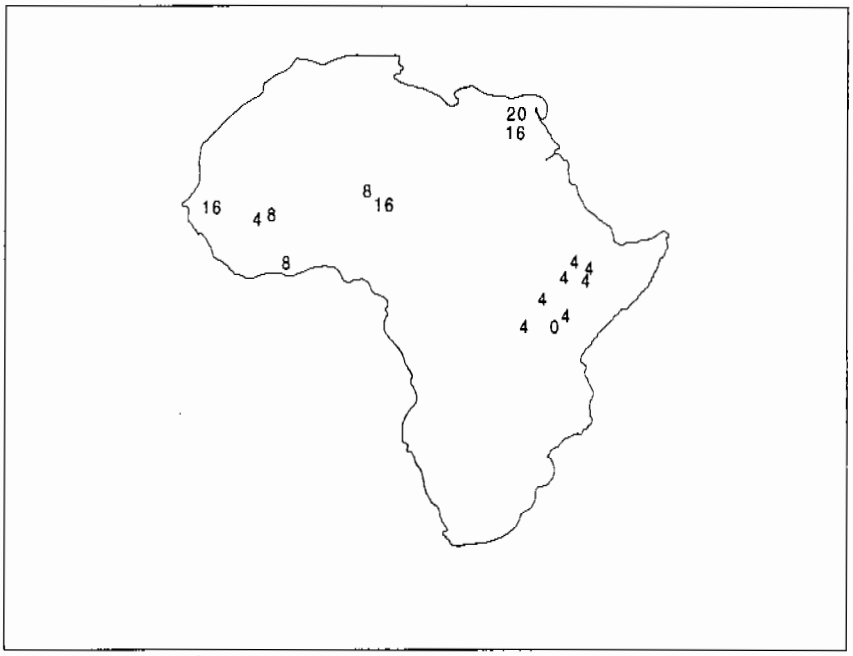

Fig. 4. Values of P (number of polymorphic loci observed divided by the number of all loci studied) in percent, observed in all the populations investigated.

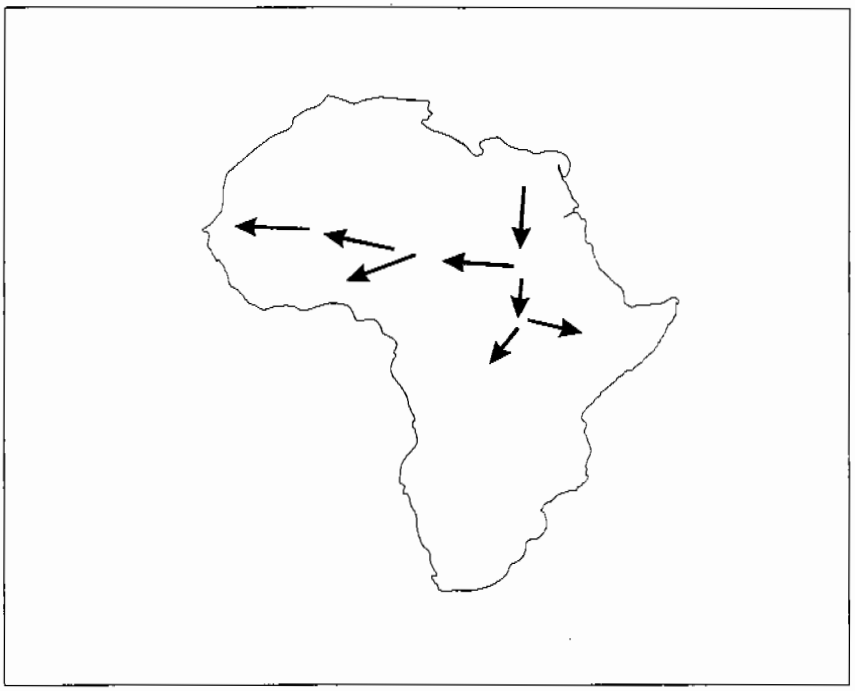

Fig. 5. Possible colonization events that led to the current distribution of *Oreochromis niloticus*. From the Nile, individuals have independently colonized East and West Africa.

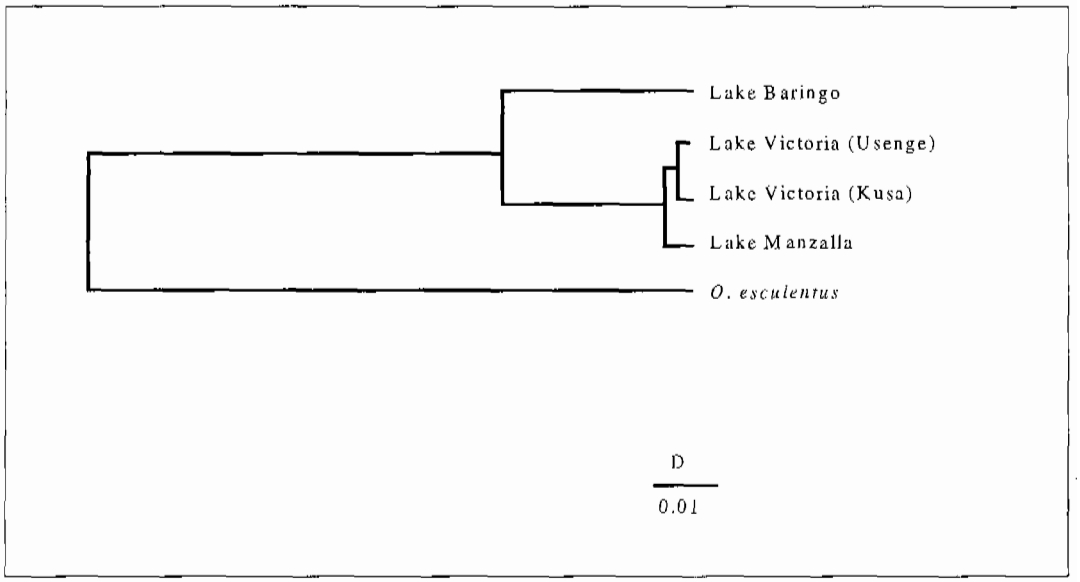

Fig. 6. Dendrogram (Nei 1972) genetic distances and UPGMA, showing genetic relationships among Lake Victoria, Kenyan and Nile populations of *Oreochromis niloticus*, and between this group of populations and *O. esculentus* from Lake Kanyaboli.

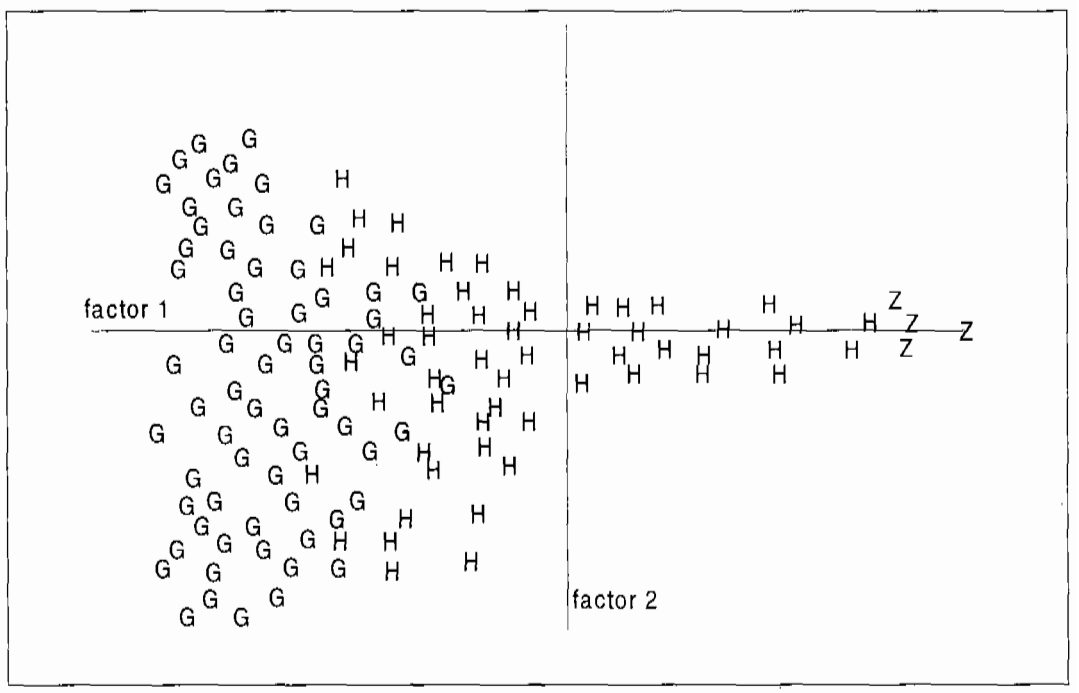

Fig. 7. Factorial analysis of *Tilapia guineensis* (G), *T. zillii* (Z) and undetermined specimens (H). Allozymes data were submitted to a correspondance analysis to assess overall relationships between samples. Multi-locus data input consisted of an unweighed sample x allele matrix, with each sample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

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