colloques et séminaires

Genetics and Aquaculture in Africa

Éditeur scientifique

Jean-François Agnèse

Genetics and Aquaculture in Africa

Actes du colloque Génétique et aquaculture en Afrique
Abidjan, 1er - 4 avril 1997

Genetics and Aquaculture in Africa

Éditeur scientifique
Jean-François Agnèse

Éditions de l'Orstom
INSTITUT FRANÇAIS DE RECHERCHE SCIENTIFIQUE POUR LE DÉVELOPPEMENT EN COOPERATION

collection Colloques et séminaires

Paris, 1998

Mise en page
Sébastien Lavoué, Jean-François Agnèse

Traduction
Suzanne Otémé, Ziriga Otémé, Jean-François Agnèse

Fabrication
Catherine Plasse

Maquette de couverture
Michelle Saint-Léger

Maquette intérieure
Catherine Plasse

Photo de couverture
Orstom/Jean-François Agnèse : « *Sarotherodon melanotheron* de Côte d'Ivoire ».

La loi du 1er juillet 1992 (code le la propriété intellectuelle, première partie) n'autorisant, aux termes des alinéas 2 et 3 de l'article L. 122-5, d'une part, que les « copies ou reproductions strictement réservées à l'usage du copiste et non destinées à une utilisation collective » et, d'autre part, que les analyses et les courtes citations dans le but d'exemple ou d'illustration, « toute représentation ou reproduction intégrale ou partielle faite sans le consentement de l'auteur ou de ses ayants droit ou ayants cause, est illicite » (alinéa 1er de l'article L. 122-4).

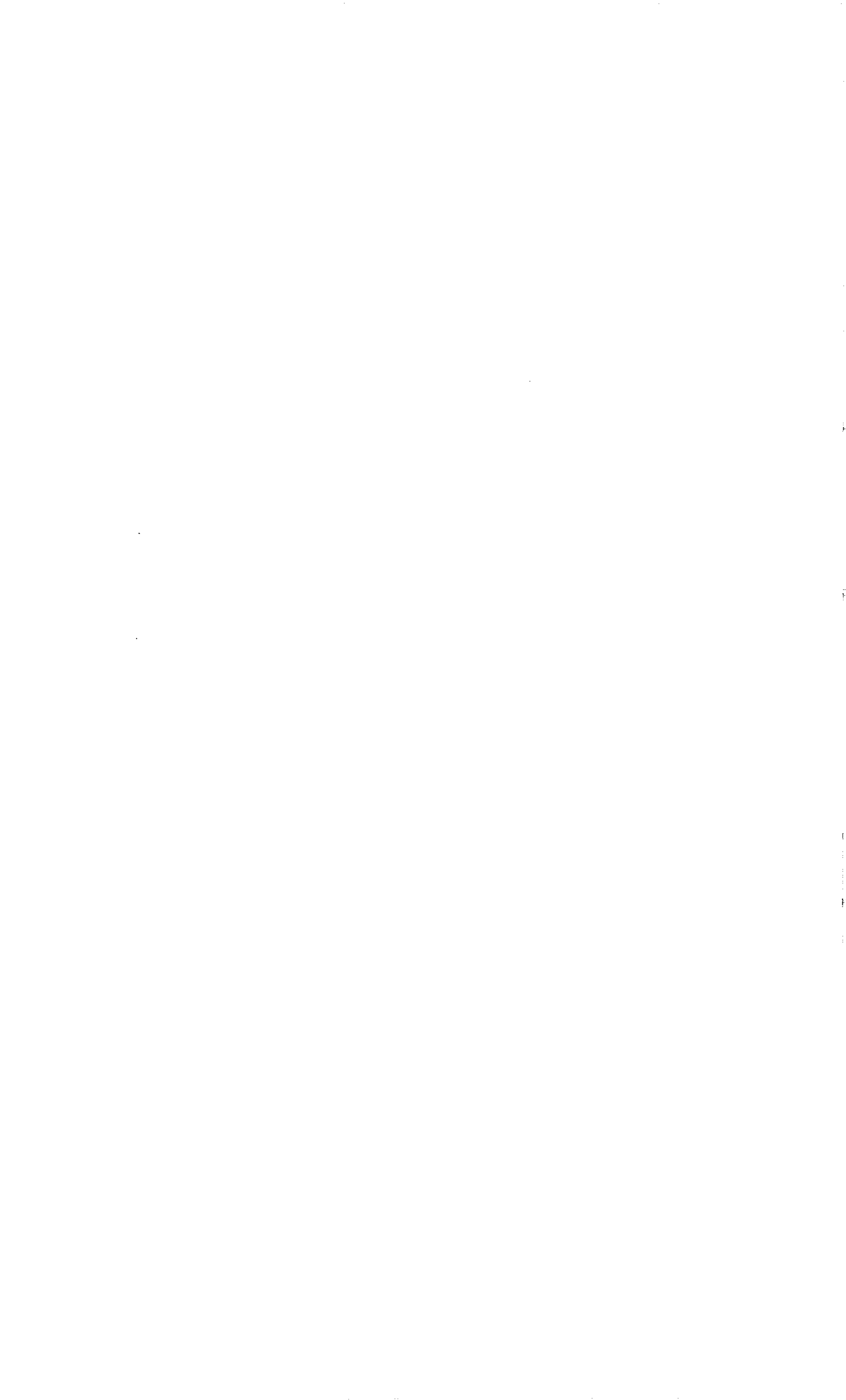
Cette représentation ou reproduction, par quelque procédé que ce soit, constituerait donc une contrefaçon passible des peines prévues au titre III de la loi précitée.

© Orstom éditions, 1998

ISSN : 0767-2896
ISBN : 2-7099-1390-9

Remerciements

La préparation et la réalisation de ces premières rencontres intitulées « Génétique et aquaculture en Afrique » ont été assurées par le Centre de recherches océanologiques d'Abidjan. Un grand nombre de personnes ont participé activement (ou passivement en supportant mon irritabilité inaccoutumée) et il m'est impossible de toutes les nommer. J'aimerais cependant rendre hommage au travail effectué par deux d'entre eux, Antoinette ADINGRA et Jean-Baptiste AVIT qui se sont montrés des collaborateurs aussi efficaces que discrets.
La Direction de l'aquaculture et des pêches ainsi que le gérant de la société Carpivoire nous ont permis de visiter leurs installations. Je les remercie pour cette journée fort agréable de « travaux pratiques » sur la lagune Ebrié.
Mes remerciements vont aussi aux orateurs qui ont ensuite accepté avec enthousiasme de me donner sans tarder un texte, avec figures et tableaux, de leur communication ce qui m'a permis de réaliser cet ouvrage.
La traduction des textes a été confiée à Suzanne OTEME (secondée de Ziriga OTEME) qui a effectué avec rapidité (malgré une messagerie électronique défaillante) un travail de qualité.
Ensuite, il a fallu mettre tous les textes en forme pour arriver à ce résultat final. Pour réaliser ce travail, Sébastien LAVOUE m'a été d'une aide très précieuse.
A tous deux, cette expérience de l'édition nous aura beaucoup appris. Nous le devons en particulier à Catherine PLASSE et Elisabeth LORNE qui nous ont familiarisés avec les polices Zapf dingbats, les gouttières, les belles pages et autres veuves et orphelines et qui immanquablement ont repéré nos mises en page prohibées et césures incorrectes.
Enfin, dans une entreprise comme celle-ci ou le soutien financier est essentiel, il m'est agréable de remercier l'Union européenne (programme Genetics), l'Orstom, le CRO d'Abidjan et le programme BAD Ouest pour leur aide.



Liste des auteurs

Eddie Koffi **Abban,** Généticien, Water Research Institute, P.O. BOX M 32, Accra, Ghana.

Béatrice **Adepo-Gourène,** Généticienne, Centre de Recherches Océanologiques, BP V18, Abidjan, Côte d'Ivoire. email : adepo@cro.orstom.ci

Jean-François **Agnèse,** Généticien, Orstom, Laboratoire Génome et populations, CC 63, Université Montpellier II, place E. Bataillon, 34 000 Montpellier, France. email : agnese@crit.univ-montp2.fr

Aggrey J. D **Ambali**, Généticien, University of Malawi, Chancellor College, Box 280, Zomba, Malawi.

Jean-Baptiste **Amon Kothias,** Hydrobiologiste, Centre de Recherches Océanologiques, BP V18, Abidjan, Côte d'Ivoire.

Olga **Assemien,** Hydrobiologiste, Idessa, Station piscicole, 01 BP 633 Bouaké 01, Côte d'Ivoire.

Herman **van der Bank**, Généticien, Rand Afrikaans University, Auckland Park Johannesburg, P.O. Box 524, Auckland Park, Afrique du Sud. email : hvdb@rau3.rau.ac.za

Devin **Bartley,** Hydrobiologiste, F. A. O., Inland Water Resources and Aquaculture Service, Viale delle Terme di Caracalla, Rome 00100, Italie.

Kostas **Batargias**, Généticien, Institute of Marine Biology of Crete, 71 003 Iraklion, Grèce.

Christine **Casal,** Hydrobiologiste, Iclarm, Bloomingdale Bldg, 205 Salcedo st. Legaspi Village, 1200 Makati City, Philipines. email : c.casal@cgnet.com

Sebastino **Da Costa,** Hydrobiologiste, Idessa, Station piscicole, 01 BP 633 Bouaké 01, Côte d'Ivoire. email : dacosta@bouake.orstom.ci

Roger **Doyle,** Généticien, Marine Gene Probe Laboratory, Dalhousie University, Halifax, NS, Canada B3H 4J1.

Thomas **Falk**, Généticien, Zoologisches Institut und Zoologisches Museum, Universität Hamburg, Martin Luther-King Platz 3, 20146 Hamburg, Allemagne. email : tmfalk@pin-net.de

Sylvain **Gilles,** Hydrobiologiste, Orstom, Centre de Recherches Océanologiques, BP V18, Abidjan, Côte d'Ivoire. email : gilles@cro.orstom.ci

Germain **Gourène,** Ichtyologiste, Faculté des Sciences et Techniques, laboratoire d'Hydrobiologie, 22 BP 582, Abidjan 22, Côte d'Ivoire.

Marc M. **Hanssens**, Ichtyologiste, Musée Royal de l'Afrique Centrale, laboratoire d'Ichtyologie, B-3080, Tervuren, Belgique.

Thomas **Kocher,** Généticien, Department of Zoology, Rudman Hall, 46 College road, Durham, NH 03824-2617, Etats Unis d'Amérique. email : kocher@unh.edu

Giogos **Kotoulas**, Généticien, Institute of Marine Biology of Crete, 71 003 Iraklion, Grèce. email : kotoulas@nefeli.imbb.forth.gr

Sébastien **Lavoué,** Généticien, Laboratoire d'Ichtyologie générale et appliquée, Muséum National d'Histoire Naturelle de Paris, 43, rue Cuvier, 75 005, Paris, France.

Antonios **Magoulas**, Généticien, Institute of Marine Biology of Crete, 71 003 Iraklion, Grèce.

Frans **Ollevier**, Biologiste, Université catholique de Louvain, laboratoire d'écologie et d'aquaculture, Institut de zoologie. Naamsestraat 59, B-3000, Louvain, Belgique.

Ziriga Josué **Otémé,** Hydrobiologiste, Centre de Recherches Océanologiques, BP V18, Abidjan, Côte d'Ivoire. email : oteme@cro.orstom.ci.

Maria Lourdes **Palomares,** Hydrobiologiste, Iclarm, Bloomingdale Bldg, 205 Salcedo st. Legaspi Village, 1200 Makati City, Philipines. email : m.palomares@cgnet.com

Laurent **Pouyaud**, Généticien, Orstom, Intalasi penelitian Perikanan Air Tawar, Jl. Ragunan, Pasar Minggu, P. O. Box 7220/Jkspm, Jakarta 12540, Indonesia.

Roger **Pullin**, Hydrobiologiste, Iclarm, Bloomingdale Bldg, 205 Salcedo st. Legaspi Village, 1200 Makati City, Philipines. email : r.pullin@cgnet.com

Lothar **Renwrantz**, Généticien, Zoologisches Institut und Zoologisches Museum, Universität Hamburg, Martin Luther-King Platz 3, 20146 Hamburg, Allemagne.

Benedict **Satia,** Hydrobiologiste, F. A. O., Inland Water Resources and Aquaculture Service, Viale delle Terme di Caracalla, Rome 00100, Italie.

Guy G. **Teugels,** Ichtyologiste, Musée Royal de l'Afrique Centrale, laboratoire d'Ichtyologie, B-3080, Tervuren, Belgique. email : teugels@africamuseum.be

Wolfgang **Villwock**, Généticien, Zoologisches Institut und Zoologisches Museum, Universität Hamburg, Martin Luther-King Platz 3, 20146 Hamburg, Allemagne.

Filip **Volckaert,** Généticien, Université catholique de Louvain, laboratoire d'écologie et d'aquaculture, Institut de zoologie. Naamsestraat 59, B-3000, Louvain, Belgique. email : filip.volckaert@bio.kuleuven.ac.be

Emmanuel J. **Vreven,** Ichtyologiste, Musée Royal de l'Afrique Centrale, laboratoire d'Ichtyologie, B-3080, Tervuren, Belgique. email : vreven@africamuseum.be

Elefterios **Zouros**, Généticien, Institute of Marine Biology of Crete, 71 003 Iraklion, Grèce. email : zouros@nefeli.imbb.forth.gr

Sommaire

Introduction 15

General topics

Genetic resources for aquaculture:
ownership and access 21
R. Pullin

Transgenic fish. The future of fish with novel genes 33
F. Volckaert *et al.*

Genetic mapping of Tilapiine fishes 59
T. D. Kocher

Genetic markers in marine biology
and aquaculture research : when to use what 67
A. Magoulas *et al.*

The utilization of ancient DNA
to assess fish biodiversity: example of Mormyridae 79
S. Lavoué *et al.*

Natural hybridization in tilapias 95
J.F. Agnèse *et al.*

A database approach to illustrate
genetic trends in fishes 105
M. L. D. Palomares *et al.*

The paradox of international introductions
of aquatic organisns in Africa 115
B. P. Satia *et al.*

Genetic impact of some fish species introductions
in African freshwaters 125
J.F. Agnèse

Cichlids

An overview of the biological diversity
and culture of tilapias (Teleostei, Cichlidae) 137
G. Gourène *et al.*

Hemoglobin variations in some tilapiine species (Teleostei, Cichlidae) of the genera *Oreochromis* and *Sarotherodon* 147
T. M. Falk *et al.*

Genetic differentiation among natural populations of the Nile tilapia *Oreochromis niloticus* (Teleostei, Cichlidae) 153
J. F. Agnèse *et al.*

Preliminary results on morphometric differentiation between natural populations of the Nile tilapia *Oreochromis niloticus* (Perciformes, Cichlidae) 165
G. G. Teugels *et al.*

Morphometric and allozyme variation in natural populations and cultured strains of the Nile tilapia *Oreochromis niloticus* (Teleostei, Cichlidae) 175
E. J. Vreven *et al.*

Zoo-technical characterization of four strains of *Oreochromis niloticus* 183
O. Assemien *et al.*

Morphological and genetic differentiation of West African populations of *Sarotherodon melanotheron* Ruppel, 1852 (Teleostei, Cichlidae) 189
B. Adépo-Gourène *et al.*

Comparison of brackish water growth performances of *Sarotherodon melanotheron* (Cichlidae) from three West African populations 199
S. Gilles *et al.*

Genetic diversity analysis of *Oreochromis shiranus* species in reservoirs in malawi 211
A. J. D. Ambali *et al.*

Catfishes

Biodiversity and aquaculture of African catfishes (Teleostei, Siluroidei): an overview 229
G. G. Teugels *et al.*

Intra- and interspecific morphometric variation in *Clarias gariepinus* and *C. anguillaris* (Siluroidei, Clariidae) 241
G. G. Teugels

Comparison of growth performances of the Niger and Bouaké strains of *Clarias anguillaris* 249
S. Da Costa

Sensitivity to inbreeding and sperm cryopreservation in the catfish *Heterobranhus longifilis* Valenciennes, 1840 257
Z. J. Otémé

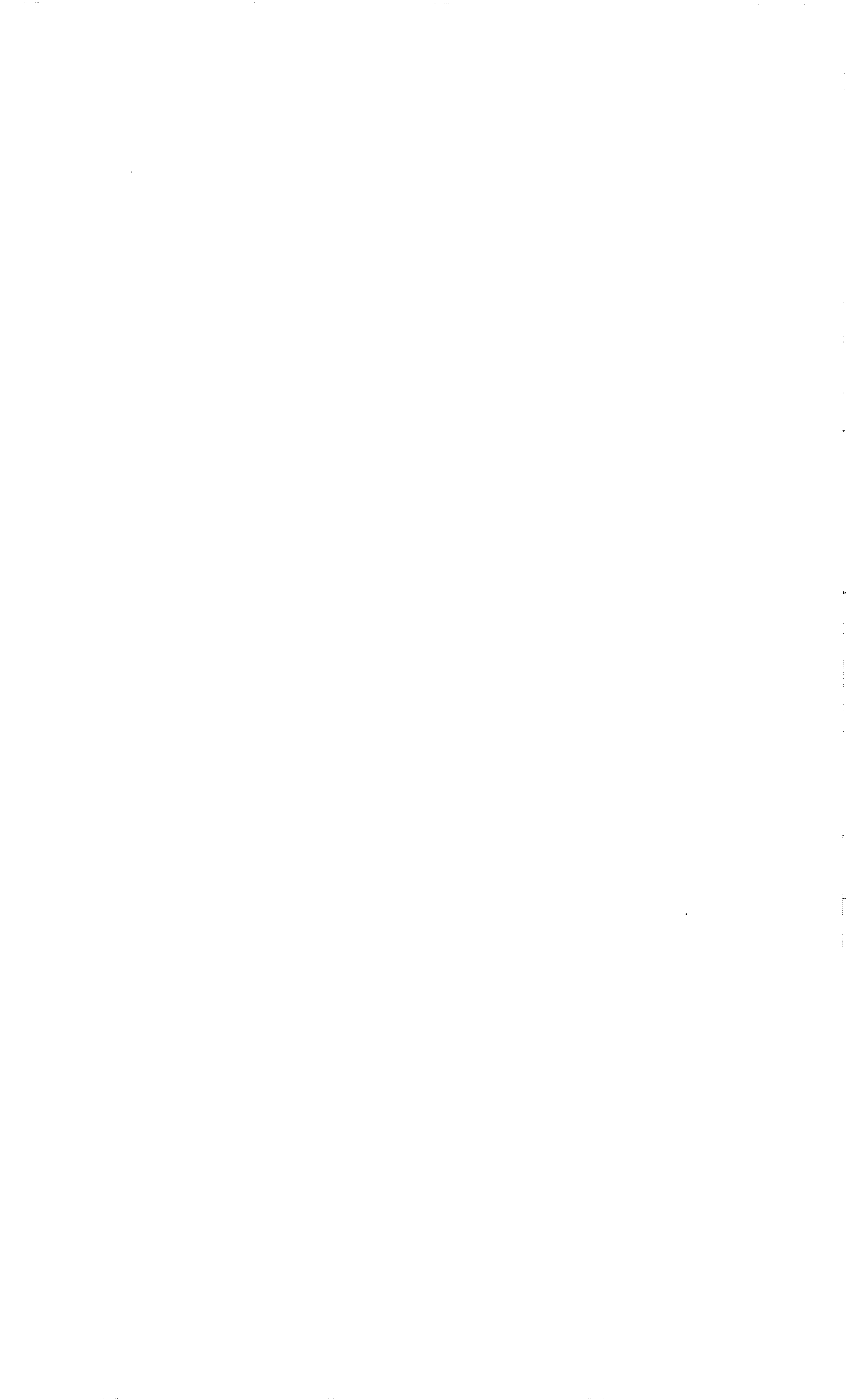
Allozyme comparisons of fish used in aquaculture in South Africa 269
H. van der Bank

Morphologic and genetic differentiation of natural populations of *Chrysichthys nigrodigitatus* (Siluroidei, Claroteidae) 277
B. Adépo-Gourène *et al.*

Microsatellite variation in the african catfish *Crhysichthys nigrodigitatus* (Siluroidei, Claroteidae) 285
G. Kotoulas *et al.*

Résumés........ 289

Abstract 309



Introduction

Research to develop aquaculture in Africa was at its peak in the 1960's. Several dozens of cultured species were tested and still are being tested to determine their adaptability or their aptness for rural and urban Africa.

The identification of potentially interesting species, the mastery of their biological cycles, and the optimization of culture conditions are the research axes which have progressed the most in the last few years. As a consequence, we have seen all sorts of aquacultural activities appear, from growers with one pond to the large of industrial projects. We see very simple aquacultural activities like the grow out of harvested wild fingerlings and others more complex requiring specialized knowledge and qualified personnel. Trials have been attempted in all environments, in rivers, ponds, dam reservoirs and lagoons.

Despite all these efforts, the development of aquaculture in Africa has lagged behind, especially when compared to that of other tropical particularly in Asia. The example of tilapia culture is very demonstrative on this subject. Even though Africa is the cradle of all the species, it is responsible for only a few percentages of the global production of tilapia. Far from discouraging us, the examples of these other countries give us objectives that we must reach.

Certainly, we understood very early on that aquaculture in Africa would not develop without natural resources management. By natural ressource management we mean firstly an understanding of these resources. We cannot imagine that aquaculture could be attempted without adequate knowledge of its principal material, fish. Countries like France and Belgium have a fairly long history of ichthyological research in tropical habitats. Orstom in France and the Musée Royal de l'Afrique Centrale in Belgium have been working together and with their African partners for a long time towards a better knowledge of African fauna, principally Western and Central Africa. This research has allow us to become aware of the specific riches present on this continent, and at the same time, its fragility. Today, almost all of the continental aquactic environments are threatened by climactic modifications and by man's actions.

More than ever before we run the risk of seeing a great number of natural environments vital to the maintenance of fish populations disappear. For we scientists, we must quickly add to our knowledge of these environments, of these populations, before they disappear and in the hope of saving the essential by our actions.

Perhaps ten years ago, some of us in Ghana, in South Africa, in Mali and in Côte d'Ivoire, began genetic research on fish populations. Very rapidly the first results showed the interest of these works and these first trials didn't take long to multiply. In 1980 we counted fewer than 5 publications on natural African fish population genetics; in 1990 this number was greater than 20 and today it is more than 80.

This increase reflects the scientific community's preoccupation with and the growing interest of financiers in the bettering of our knowledge of the fauna of African fresh waters. With this in mind, it seemed useful to reunite the greatest number of principal actors in today's research to discuss the latest works and to create cooperative dynamics between these researchers, a cooperation made necessary by the multiplicity of techniques used and the frequently consequential geographic dimensions of the research programs.

In fact, as we will see in the communications to come, some of them have required travel in almost a dozen countries, while others have had one biological resource surrounded by two, three or even four different teams.

Concerning population genetics research in West Africa, the Genetics program, financed by the European Community, will no doubt mark the 1990's. Its objectives were the development of the most recent genetics tools on fish species of interest to aquaculture in Africa, the study of genetic variability in natural populations, and the study of zoo-technical performances of genetically differentiated populations. This program began four years ago and finishes today. It will have mobilized 7 research teams in 4 countries: Côte d'Ivoire, Belgium, Greece and France. It will have allowed the development of genetics tools like microsatellites for example, used with most of the African species of aquacultural interest: Tilapia, Clariidae, Claroteidae; it will have allowed the realization of population studies on a continental scale, 16 African countries having been the

object of sampling. Finally, it will also have allowed collaboration between twenty different specialists, the confrontation of their results and their ideas. Almost half of the communications that will be presented in these encounters will present original results coming from this program.

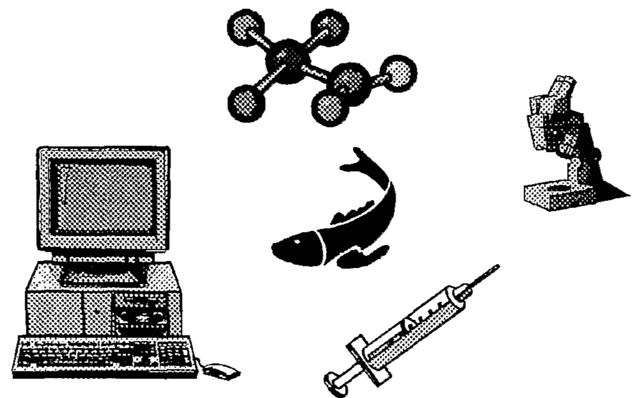
On this subject, it must also be noted the fairly broad definition given to the term population genetics because several communications actually concern works on morphological differentiation or comparisons of zoo-technical aptitudes between populations. If we are justly interested in a fish's genes, the morphology of the individual is the first characteristic that we perceive. Recognizing a species or a population is the first thing a researcher or grower must be able to do. Morphological studies have proven their worth, we would not be able to work without them, even if today genetics allow noticeable progress in this domain, the study of morphology remain indispensable to a number of population genetics studies. We will see a very demonstrative sample with the work on populations of *Clarias sp.* Without the morphological study which accompanies them, no population genetics study would make any sense.

On the comparison of the zoo-technical aptitudes of populations, it is a sort of culmination of most works of population genetics in aquaculture.

So we have numerous communications which will be presented over three days here in Grand Bassam. Researchers from North America, Europe, Asia and of course Africa are present. The three most active African laboratories in this field are represented. The Institut of aquatic biology of Accra, the University of Johannesburg, and of course the Centre de Recherches Océanologiques of Abidjan. I thank all the participant for being here and I hope you enjoyable and fruitful encounters at Genetics and Aquaculture in Africa.

General topics

part 1



Genetic resources for aquaculture: ownership and access

Roger S.V. Pullin
Biologist

Introduction

The regulations, protocols and practices that govern access to and ownership of the world's genetic resources (microorganisms, plants and animals) are complex and are still evolving rapidly. In order to appreciate the current situation with respect to aquatic genetic resources and to forecast likely future trends, we must first consider the recent history, the current picture and future scenarios for genetic resources in general. Up to the 1980s, most genetic resources were considered as being in the public domain: a common heritage of humans, with open access and without intellectual property rights (IPR). This view has since changed progressively as various parties have sought ownership of and regulated access to genetic resources: *e.g.* the national sovereignty acquired over all biodiversity within national boundaries by Parties to the Convention on Biological Diversity (CBD) (CBD, 1994; TINKER 1995), the recognition of farmers' and breeders' rights over distinct varieties and breeds, and attempts by indigenous peoples to secure rights to the genetic resources upon which their livelihoods have traditionally depended and to their local knowledge about these resources (*e.g.* GADGIL *et al.*, 1993; Crucible Group, 1994). As a further illustration of this trend towards defined ownership, it was suggested at the fifth session of the Global Biodiversity Forum, held

1-3 November 1996 in conjunction with the third Conference of the Parties of the CBD in Buenos Aires, that the term "wild resources/species" should be replaced with the term "non-domesticated resources" so as to avoid giving the impression of no ownership of such resources.

These developments and debates have been and continue to be dominated by consideration of plant genetic resources, especially those for the major human food crops and for species with proven or potential worth for pharmaceutical use. The resulting literature on plant genetic resources is large and complex and is growing rapidly. A review of this is beyond the scope of this paper, but selected references are appended to illustrate the scope of work still needed for aquatic genetic resources. Public opinion might swing away from widespread privatization of genetic resources if the exercising of ownership and access rights is ultimately found to be not worth its cost. In other words if, as is likely to be the case for many species, the "pot of gold" for genetic resource owners is small and the cost of collecting payments is large. For example, it was estimated at a recent international consultation on fish genetic resources (PULLIN and CASAL, 1996) that of the US$700 million known profits from global trade in plant seeds in 1993, assuming (optimistically) that about 10% of these derived from materials subject to the provisions of the CBD, there would be only about US$70 million in profits to be shared among source countries and probably only about US$7 million to be shared as royalties.

For most aquatic genetic resources, significant royalties and cost-effective administration and collection of these are even harder to envisage. It is, however, understandable that biological resource-rich and cash-poor countries must seek to maximize their utilization of and returns from their genetic resources. Private sector interests, especially in the more developed countries, are pushing for more privatization and patenting of biological material and processes, mainly on the premise that these measures are necessary for the further investments in discoveries and developments that will produce from such material and processes the maximum benefits for humankind. There are, however, counterarguments. These are largely ethical but some are economic: for example, that patents do not normally stimulate invention or investment in more research to provide further advances and benefits (BUSCH, 1995). The consequent polarization of views held up the signing of the CBD by

some parties, on the grounds that this would damage the interests of their biotechnology industries. It has also given rise in Europe, most recently in 1996, to controversial proposals for European Parliament and Council Directives on the legal protection of biotechnological inventions (*e.g.* Commission of the European Communities, 1996). The 1996 proposal is currently being opposed, on ethical and moral grounds, as an undesirable step towards the privatization of nature and lifeforms and as a constraint to research (DALTON *et al.*, 1997). An earlier directive on the patenting of biological material (published on 12 October 1988) was rejected by the European Parliament on similar grounds, after strong protests by nongovernmental organizations, developing-region representatives and farmers' organizations (GRAIN, 1995). For at least the next decade, it is unlikely that these controversies will be fully resolved. Moreover, although focused on plants and microorganisms, the emerging protocols and mechanisms governing ownership of and access to genetic resources will probably be deemed to apply also to livestock and to aquatic animals.

The former FAO Commission on Plant Genetic Resources has now become the FAO Commission on Genetic Resources for Food and Agriculture (CGRFA) (FAO, 1995a). Its 1997 meeting will include consideration of livestock genetic resources. Aquatic genetic resources will be on its agenda for future meetings. FAO has already established a program for the Management of Global Animal Genetic Resources, meaning farm livestock, and a CD-ROM has already been produced (FAO, 1995b; FAO/Unep, 1995; IDAD-FAO, 1996). The FAO CGRFA is renegotiating its International Undertaking on Plant Genetic Resources with the holders in trust of collections of genetic resources, especially with those crop centers of the Consultative Group on International Agricultural Research (CGIAR) that have ongoing agreements with FAO by which "designated" germplasm of plants is held *ex situ*. The purpose of this renegotiation is to bring the Undertaking and these agreements more into harmony with the CBD. The CBD's Article 15 covers access to genetic resources. Its key elements, paragraphs 4 and 5, require that access shall be on "mutually agreed terms" and subject to "prior informed consent". These terms are intended to apply to the Contracting Parties, which, under the CBD, are sovereign States. However, this approach can be extended to other parties (institutions, groups or individuals)

(GLOWKA, 1995). The interpretation of Article 15 has been discussed at length by GLOWKA *et al.* (1994).

Genetic resources in aquaculture research and development

Genetic resources for aquaculture are, compared with genetic resources for agriculture, poorly documented, and their ownership and arrangements for access are poorly defined. In 1992, Iclarm convened an international meeting on International Concerns in the Use of Aquatic Germplasm (Iclarm, 1992). This meeting arose largely because of the success of Iclarm and its Philippine and Norwegian partners in pioneering a selective breeding approach to genetic improvement in tropical aquaculture: the Genetic Improvement of Farmed Tilapias (Gift) Project, using the Nile tilapia (*Oreochromis niloticus*) as a case study (GJEDREM and PULLIN, 1986; EKNATH *et al.*, 1993). In addition to using farmed Asian strains acquired in Asia and derived from historical introductions originating from Africa and within Asia, the Gift project team, supported by UNDP, collected new founder stocks from Egypt, Ghana, Kenya and Sénégal. These collections were made five years before the entry into force of the CBD. This 1992 meeting's recommendations did not address thoroughly the ownership of and access to such aquatic genetic resources. Indeed, a separately published report (ROSENDAL, 1992) highlighted its lack of clarity with respect to IPR and access to fish genetic resources. Its recommendations to Iclarm included the following:
"... that (a) Iclarm continue basic research on the genetic improvement of farm fishes to secure and improve upon the gains that have already been made and (b) the current breeding strategy [i.e. selection] is the most appropriate for maintaining genetic diversity and ensuring ease of access to the material [*i.e.* it is difficult to patent]. It should therefore be continued ... [and that] ... as demand for seed increases, it will be necessary for Iclarm to fully

transfer seed production and distribution responsibilities to national bodies."

An FAO Expert Consultation on Utilization and Conservation of Aquatic Genetic Resources (FAO, 1993) then made a large number of recommendations, though not on access and ownership *per se*, and within a lengthy text and emphasized, among many other issues, the need for policy and regulations to address: "... the rights and needs of communities of users and donors to ensure that the benefits they obtain from aquatic animal genetic resources are not undermined by their distribution to others, and by the use of this germplasm by others now or in the future, and that there be a means of compensation for their contribution [...] (and) [...] ways in which recipients and users of collected germplasm may pass on the benefits derived from the use of the germplasm, and information from genetic studies, to the donor/host country scientists, local communities, farmers, fishers, and indigenous people ..."

The report of a subsequent external "Stripe Study" of genetic resources in the CGIAR (TAC/FAO 1994) recommended that: "IARC's [International Agricultural Resource Centers] involved with genetic resources of trees, animals and aquatic species should not accumulate collections of these organisms beyond the small number necessary to conduct specific research at the centres which cannot be conducted in the countries".

Iclarm was subsequently included in an external review of all CGIAR genebank operations in 1995 (SGRP, 1996 and *in press*). The review was dominated by consideration of large *ex situ* crop genebanks. Its specific recommendations to Iclarm, though useful for setting future directions in research and training (for example, realism in what can be genebanked and by what method-cryopreservation was recommended), did not address the issues of ownership of and access to germplasm used by Iclarm and its partners for research purposes. It was not clear whether limited collections of germplasm used for fixed term research projects (for Iclarm and its partners, live tilapia broodstock and cryopreserved tilapia sperm and some marine invertebrates), could be construed as genebanks *per se*. In 1996, the Iclarm Board of Trustees took a policy decision that these are germplasm collections for research purposes, kept for the duration of the work for which they are

needed, and are not genebanks per se that will be maintained indefinitely. A document stating Iclarm's policy on these collections and on intellectual property rights pertaining to aquatic genetic resources is being prepared for publication. The basis of this policy is to ensure compliance with the provisions of the CBD and to preclude claims of private ownership over germplasm held or developed by Iclarm and its partners and over related information.

Documentation of aquatic genetic resources: a prerequisite for defining ownership and access arrangements

Aquatic genetic resources are generally poorly documented except where biochemical genetic characterization methods have been used for species and groups of special significance in aquaculture and fisheries; *e.g.*, for tilapias, FRANCK *et al.*, 1992 and MACARANAS *et al.*, 1996 and for salmonids, BARTLEY *et al.*, 1992. CARVALHO and PITCHER (1995) have provided a substantial compilation of the methods available, but this is a fast developing field in which new and modified approaches are frequently described; *e.g.*, see FALK *et al.* 1996. Despite these powerful characterization tools, the accelerating pace of germplasm enhancement for aquaculture and increased interest in exchanging germplasm are creating a situation where accurate, and up-to-date accessible information about the location and status of aquatic genetic resources is not generally available. As a contribution to solving this problem, PULLIN (in press) suggested the establishment of more fish breeder's networks or associations through which public and private sector members could share germplasm and related information. These would, of course, need time, money and appropriate political and economic climates to be established and maintained. Researchers and others reporting on aquatic genetic resources and aquaculture research and development could also help by specifying more exactly the genetic

status of their fish. Researchers often give limited information on their experimental fish: usually just the species or subspecies and its "origin". It would be better in aquaculture research publications and in reports about farm performance and about trade in farmed aquatic produce, to specify the "provenance" of the material used. Provenance is a well-established concept in forestry, geology and in the world of art and antiques. In forestry, the term "provenance" refers to clonal or seed material and defines the geographical location (and hence the environment): "... in which the parent trees grew and within which their genetic constitution has been developed through artificial and/or natural selection..." (BURLEY and WOOD, 1976). The origin of material may be different. The provenance concept could be applied to tilapias to help to specify the history of material used in research and production. For example, the origin of all *Oreochromis urolepis hornorum* must be the Wami river, Tanzania or possible Zanzibar, but as this species has been moved around the world, the provenance of a given stock of fish could be institutions and farms in Brazil, Côte d'Ivoire, Israel, Malaysia, the USA and other countries (PULLIN, 1988).

Future possibilities

The conservation and use of aquatic genetic resources are interdependent as has been repeatedly stressed in recent publications (*e.g.*, MCANDREW *et al.*, 1993; MACLEAN and JONES, 1995; HARVEY, 1995; PULLIN, 1996 and in press; PULLIN and CASAL, 1996). However, ownership of and arrangements for access to aquatic genetic resources remain very poorly defined. As a result of its 1995 consultation (PULLIN and CASAL, 1996), Iclarm and FAO have proposed an international policy conference in 1998 to explore approaches and to develop tools and methods for policymakers in this field. Also in 1998, the CBD will put freshwater biodiversity high on its agenda for the first time since its entry into force. This could initiate actions among the Parties, FAO,

NGOs, the CGIAR, the private sector and others from which clearer arrangements and protocols for aquatic genetic resources might emerge. This is likely to be a lengthy process because genetic resources for aquaculture will probably continue to command less attention than those for agriculture, forestry or drug use. An indication of this is that the CBD, in working towards its first legally binding protocol, on biosafety, is restricting the scope of the protocol to genetically modified organisms (GMOs): meaning, those produced by genetic manipulation. This restriction ignores the fact that unmodified or wild alien aquatic species and aquatic breeds developed by conventional breeding methods can also be biohazards.

While a higher profile for aquatic biodiversity and genetic resources is awaited and while insufficient sharing of data and experiences persists, policymaking and the framing of workable and equitable arrangements for their conservation and use are likely to remain *ad hoc*. Institutions, individuals, networks and the private sector will increasingly seek to document, evaluate and exchange aquatic germplasm. Some will proceed carefully and responsibly, with due regard to biosafety and quarantine [for example, as in the protocols and Manila Resolution of the International Network for Genetics in Aquaculture (Inga) (Inga, 1997)]. Others will act opportunistically and without adequate safeguards, posing threats to some aquatic biota and their habitats and to some pre-existing aquaculture and fisheries. Such moves will further hinder the gathering and sharing of accurate, up-to-date information on genetic resources for aquaculture (PULLIN, in press).

What can be done to improve this situation? The solution lies mainly in the hands of the national governments that are Parties to the CBD and to other related conventions and trade agreements. Only they can ultimately implement the national biodiversity strategies and regulations required. This difficult task requires recognition of the interests of many diverse stakeholders. Sound policies for aquatic genetic resources are therefore urgently needed.

References

BARTLEY (D.), BENTLEY (B.), BRODZIAK (J.), GOMULKIEWICZ (R.), MANGEL (M.), GALL (G.A.E.), 1992 — Geographic variation in population genetic structure of chinook salmon from California and Oregon. *Fishery Bulletin.*, U.S, 90: 77-100.

BURLEY (J.), WOOD (P.J.), Compilers, 1976 — *A manual on species and provenance research with particular reference to the tropics.* Tropical Forestry Papers N° 10. Department of Forestry, Commonwealth Forestry Institute, University of Oxford, Oxford, UK.

BUSCH (L.), 1995 — Eight reasons why patents should not be extended to plants and animals. *Biotechnology and Development Monitor.*, 24: 24.

CARVALHO (G.R.), PITCHER (T.J.), eds., 1995 — *Molecular genetics in fisheries.* London, Chapman and Hall, 141 p.

CBD, 1994 — *Convention on Biological Diversity.* Text and Annexes. Secretariat of the Convention on Biological Diversity, Montreal, Canada. 34 p.

Commission of the European Communities, 1996 — *Proposal for a European Parliament and Council Directive on the Legal Protection of Biotechnological Inventions.* COM(95)661 Final/2. Commission of the European Communities, Brussels.

Crucible Group, 1994 — *People, plants and patents: the impact of intellectual property on trade, plant biodiversity and rural society.* IDRC, Ottawa. 116 p.

DALTON (H.), GOODWIN (B.), HO (M.-W.), MCGLADE (J.), PRANCE (G.), SAUNDERS (P.), SHERATT (D.), MAYNARD-SMITH (J.), WHITTENBURY(R.), 1997 — Patent threat to research. *Nature*, 385: 672.

EKNATH (A.E.), TAYAMEN (M.M.), PALADA-DE VERA (M.S.), DANTING (J.C.), REYES (R.A.), DIONISION (E.E.), CAPILI (J.B.), BOLIVAR (H.L.), ABELLA (T.A.), CIRCA (A.V.), BENTSEN (H.B.), GJERDE (B.), GJEDREM (T.), PULLIN (R.S.V.), 1993 — Genetic improvement of farmed tilapias: the growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments. *Aquaculture*, 111: 171-188.

FALK (T.M.), OBERST (S.), VILLWOCK (W.), PULLIN (R.S.V.), RENWRANTZ (L.), 1996 — *A biochemical laboratory manual for species characterization of some tilapiine fishes.* Iclarm Educ. Ser. 17. 93 p.

FAO, 1993 — *Report of the Expert Consultation on Utilization and Conservation of Aquatic Genetic Resources.* FAO Fisheries Report 491. FIRI/R491. FAO, Rome. 58 p.

FAO, 1995a — *Broadening the mandate of the FAO Commission on Plant Genetic Resources to include other categories of organisms used for food and agriculture.* FAO Committee on Fisheries. Twenty-First Session. COFI/95/10. FAO, Rome. 8 p.

FAO, 1995b — *The management of global animal genetic resources. FAO Committee of Agriculture.* Thirteenth Session. COAG/95/5. FAO, Rome. 11 p.

FAO/Unep, 1995 — *World Watch List for domestic animal diversity.* 2nd Edition. FAO, Rome. 769 p.

FRANCK (J.P.C.), WRIGHT (J.M), MCANDREW (B.J.), 1992 —
Genetic variability in a family of satellite DNAs from tilapia (Pisces: Cichlidae).
Genome, 35: 711-725.

GADGIL (M.), BERKES (F.), FOLKE (C.), 1993 —
Indigenous knowledge for biodiversity conservation.
Ambio., 22: 151-156.

GJEDREM, (T.), PULLIN (R.S.V.), 1986 —
A new breeding program for the development of tilapia culture in developing countries. Report to the Rockefeller Foundation.
Iclarm, Manila, Philippines. 15 p.

GLOWKA (L.), 1995 —
Determining access to genetic resources and ensuring benefit sharing: legal and institutional considerations for states providing genetic resources. Paper presented at the Global Biodiversity Forum, 4 November 1995, Jakarta, Indonesia. IUCN Environmental Law Centre, Bonn. 15 p.

GLOWKA (L.), BURHENNE-GUILMIN (F.), SYNGE (H.), MCNEELY (J.A.), GÜNDLING (L.), 1994 —
A guide to the Convention on Biological Diversity. IUCN Gland and Cambridge. xii + 161 p.

GRAIN, 1995 —
The directive is dead. *Seedling.*, 12 (1): 4-9.

HARVEY (B.), 1996 —
Banking fish genetic resources: the art of the possible. In DI CASTRI (F.), YOUNES (T.), eds. : *Biodiversity, Science and Development: Towards a New Partnership.* CAB International, Wallingford, UK : 439-445

Iclarm, 1992 —
Recommendations of the meeting on international concerns in the use of aquatic germplasm. 1-5 June, 1992, Ternate, Cavite, Philippines. Iclarm, Manila, Philippines. 19 p.

IDAD-FAO, 1996 —
Farm animal genetic resources. Why are they so important? A call to action. Initiative for Domestic Animal Diversity. FAO. CD-ROM. FAO, Rome.

Inga, 1997 —
Manila Resolution. Strengthening partnerships to advance the science of fish breeding and genetics and development of national fish breeding programs. International Network on Genetics in Aquaculture, Iclarm, Manila, Philippines.

MACARANAS (J.M.), AGUSTIN (L.Q.), EKNATH (A.E.), 1996 —
Multiple hemoglobins in three tilapiine species of the genus Oreochromis and eight strains of O. niloticus (L.).
Aquaculture Research., 27: 597-601.

MACLEAN (R.H), JONES (R.W.), 1995 —
Aquatic biodiversity conservation: a review of current issues and efforts. Strategy for International Fisheries Research, Ottawa, Canada. 59 p.

MCANDREW (B.J.), RANA (K.J.), PENMAN (D.J.), 1993 —
Conservation and preservation of genetic variation in aquatic organisms. In MUIR (J.F.), ROBERTS (R.J.), eds. : *Recent Advances in Aquaculture IV.* Blackwell Scientific Publications, Oxford, 236-295.

PULLIN (R.S.V.), 1988 —
Tilapia genetic resources for aquaculture. Iclarm Conf. Proc. 16. 108 p.

PULLIN (R.S.V.), 1996 —
Biodiversity and aquaculture. In DI CASTRI (F.), YOUNES (T.), eds.: *Biodiversity, Science and Development: Towards a New Partnership.* CAB International, Wallingford, UK : 409-423.

PULLIN (R.S.V.), *in press* —
Tilapia genetic resources in Asia. Paper presented at the Final Workshop of the Dissemination and Evaluation

of Genetically Improved Tilapia in Asia (DEGITA) Project, 18-19 February 1997, Iclarm, Manila, Philippines (to be published by Iclarm and the Asian Development Bank, Manila).

PULLIN (R.S.V.), CASAL (C.V.), eds., 1996 —
Consultation on fish genetic resources. Iclarm Conf. Proc. 51. 61 p.

PULLIN (R.S.V.), 1997 —
Conservation of genetic resources for aquaculture. In HANCOK (D. A), SMITH (D.C.), GRANT (A.), BEUMER (J.P.), eds. Developing and sustaining world fisheries resources: the state of science and management. Second World Fisheries Conference proceeding, CSIRO publishing, Collingwood, Victoria, Australia: 478-484

ROSENDAL (K.), 1992 —
Blue revolution could avoid failures of green predecessor. Biotechnology and Development Monitor, 12 September 1992: 10.

SGRP, 1996 —
Report of the internationally commissioned external review of the CGIAR genebank operations. System-wide Genetic Resources Programme, International Plant Genetic Resources Institute, Rome, Italy. 105 p. [A 37-page detailed external reviewers' report to Iclarm is also available from Iclarm, Makati, Philippines.]

SGRP, 1997 —
Report of the internally commissioned external review of the CGIAR genebank operations. Annex: Centres' responses to the external review System-wide Genetic Resources Program, International Plant Genetic Resources Institute, Rome. 49 p.

TAC/FAO, 1994 —
Stripe Study of genetic resources in the CGIAR. Technical Advisory Committee Secretariat and FAO, Rome. 33 p.

TINKER (C.), 1995 —
A "new breed" of treaty: the United Nations Convention on Biological Diversity.
PACE Environmental Law Review, 12 (2): 191-218.

Transgenic fish. The future of fish with novel genes

Filip Volckaert
Geneticist

Frans Ollevier
Biologist

Introduction

Making transgenic lines of vertebrates represents a rather new technology. While transgenic fish have been introduced in the laboratory since the mid eighties, it has also become a reality in aquaculture since the early nineties. There are many reasons why people want to create genetically modified organisms (GMO) and there are many strategies to make "fish with novel genes". As such it has generated high expectations in society and directed the interests of researchers, but has also raised concerns about environmental and consumer safety, and generated numerous ethical and legal discussions.

A GMO can be defined as a "new" organism which contains an "intergeneric combination of genetic material" (HALLERMAN and KAPUSCINSKI, 1990a) or as an organism which contains "genetic material which has been modified *in vitro*".

I will first explain the technological aspects of generating transgenic fish. The implications for aquaculture are highlighted with the transgenes promoting growth, lowering freezing temperature and inducing disease resistance. I will also discuss environmental and consumer related aspects. In conclusion the future of GMOs is presented.

Aims of making transgenic fish

The transfer of DNA sequences and genes in fish is being done for three main purposes:
(1) to understand the molecular genetics of development
(2) as a model for mammalian, read human genetics
(3) to enhance aquaculture production
The first reason is motivated by fundamental research and is driven amongst others by the desire to study the molecular biology of the early development of zebrafish (ROSSANT and HOPKINS, 1992) and medaka (ISHIKAWA *et al.*, 1997). Progress is such that a growing number of housekeeping and cell-specific genes are being isolated, mapped, and the resulting phenotype characterised (GAIANO *et al.*, 1996a).

The second reason is influenced by the limitations of mammalian models to study human diseases. The reproductive biology of mice sets serious limits to the experimental study of early development, including the regulation of gene expression. ROSSANT and HOPKINS (1992) nicely review the capabilies of zebrafish in this perspective. In general, fish fertilise their eggs externally, have numerous progeny, are easy to culture, and have short generation times. It is possible to make isogenic lines in a single generation and to screen for mutants in haploid progeny (POWERS, 1989). An example of a promising application in medicine is the targeted study of circulatory and cardiac defects in zebrafish.

Finally, transgenesis offers perspectives for the acceleration of selection. Indeed, the domestication of organisms with an economic interest has been going on for tens of centuries. The process has been achieved through careful observation, directed breeding, culling and strain management. But fish has a rather short domestication history and thus the selection of strains and lines is limited. Common carp has been selected in China since 2000 B.P. (KOMEN, 1990), while the selection of rainbow trout started only last century and Atlantic salmon fourty year ago. Tropical fish such as tilapia and catfish have been submitted to directed selection for less than 10 year. A limiting factor is that selection progress is

strongly dependent on generation time; for example bacteria double every hour or so, while Atlantic salmon has a generation time of at least 3 year. As such, transgenesis could speed up the generation of lines with specific phenotypic traits: disease resistance, enhanced growth, colour mutation, and so on.

Methodology

Regardless whether transgenic research is fundamental or applied, three major steps have to be taken to generate transgenic fish. First a recombinant expression vector has to be made (1), second the construct has to be introduced into the germ line (2) and third, the desired phenotype has to be functioning properly (3) (HACKETT, 1993).

The construction of recombinant expression vectors for fish

Vectors

Expression vectors are recombinant DNAs that carry the transgene of interest and the regulatory sequences that determine where, when and at which level the transgene will be expressed (HACKETT, 1993). They include a vector consisting of a plasmid or viral sequence, regulatory sequences and the gene of interest.

Most vectors used are modified *E. coli* plasmids (*e.g.* AMSTERDAM *et al.*, 1995; GIBBS *et al.*, 1994; VOLCKAERT *et al.*, 1994), which can be replicated in *E. coli* cells such that high amounts of recombinant DNA can be obtained.

Retroviral vectors represent an alternative and promising strategy (BURNS *et al.*, 1993). The genome of the Moloney Murine Leukemia Virus (MLV) was modified with the envelope protein of

Vesicular Stomatitis Virus (VSV). The MLV/VSV pseudotype retroviral vectors showed enhanced success of integration although seriously limited by the low viral titers. A new generation of MLV/VSV pseudoptyped retroviral vectors has viral titers up to $1x10^9$ colony forming units/ml with an integration success of 83 % (GAIANO *et al.*, 1996b).

Recombinant DNA

Regulatory elements, promoters, enhancers and silencers

About 2,000 genes permit each cell to perform its specialised function in a particular tissue. Consequently most of these genes are non-functional most of the time and require precise signals to be activated (HACKETT, 1993). Therefore genetic engineering requires knowledge of the elements which initiate, modify and terminate transcription, splice mRNA and initiate protein synthesis. Regulation of transcriptional initiation is the major control in gene activation and is mediated by a number of regulatory modules each composed of a short DNA sequence and the proteins that specifically interact with them. It is the combination of various regulatory modules, each providing a response (tissue-specific expression, induction by a hormone, by a metal ion, by a heat-shock protein, etc) that cause the fine-tuning of gene expression. All the transcription factors bound to their DNA sequence and stabilised by protein/protein interaction, anchor to the promoter of the RNA polymerase II and are included in a large complex with general transcription factors. Reviews have been written amongst others by HACKETT (1993) and IYENGAR *et al.* (1996).

Numerous fish genes have been isolated, sequenced and a lesser number have been characterised, but the number of regulatory elements isolated and sequenced is rather limited. Therefore the number of piscine sequences tested *in vitro* (in a transiently or stably transfected cell system) or *in vivo* (in a transient or stable transgenic system) is limited.

The ubiquitously regulated metallothionein (MT) promoter contains Metal Responsive Elements (MRE) which are DNA sequences that mediate expression in the presence of Zn^{2+} and Cd^{2+} (ZAFARULLAH

et al., 1989; FRIEDENREICH and SCHARTL, 1990; HONG *et al.*, 1993). The interest of this promoter lies in its putative use as a monitor of heavy metal pollution (KINOSHITA *et al.*, 1994).

The housekeeping gene β-actin has been characterised by LIU *et al.* (1990) and MOAV *et al.* (1993) in carp and TAKAGI *et al.* (1994) in medaka. The latter authors developed an expression vector from the medaka β-actin gene for use in medaka. The medaka β-actin promoter showed variable but sometimes ubiquitous transient expression in medaka fry (TAKAGI *et al.*, 1994). Functional analysis of the carp β-actin promoter in fish cells was used to construct two expression vectors for use in transgenic fish (LIU *et al.*, 1990). Expression is species-specific and interactions were observed between the motifs in the proximal promoter and first intron in zebrafish (MOAV *et al.*, 1993).

The type II and type III-Antifreeze Protein (AFP) promotor has been functionally analysed *in vitro* and transiently *in vivo* (GONG *et al.*, 1991). Multiple positive and negative regulatory elements were detected in this promoter which could be useful in controlling gene expression of temperate species during cold temperature periods or low temperature expression in tropical species.

The hormone prolactin I regulates various functions in fish such as osmoregulation (freshwater adaptation in saltwater fish and calcium homeostasis in both saltwater and freshwater fish), Ca^{2+} metabolism, mucus secretion, hatching, general metabolism and behaviour. *In vitro* analysis of the regulatory elements of the prolactinI gene of tilapia (*Oreochromis mossambicus*) and rainbow trout (*Oncorhynchus mykiss*) include several binding sites for a pituitary-specific protein (called *Pit-1*), as well as positive (enhancers) and negative (repressors) regulatory elements (ARGENTON *et al.*, 1996; PONCELET *et al.*, 1996). A glucocorticoid responsive element-like motif is also present close to the second and third *Pit-1* binding sites (ARGENTON *et al.*, 1996). The transient expression of a fusion gene between the tilapia prolactin promoter and a reporter gene in epithelial carp cells is dependent on the co-expression of the pituitary-specific *Pit-1* factor (PONCELET *et al.*, 1996). A transient *in vivo* study of deletion mutants of the tilapia prolactin promoter revealed the presence of enhancing and

repressing sequences. The location of maximal and minimal expression *in vitro* and *in vivo* coincided to a limited degree.

Transgenes

Transgenes refer to the genes which are expressed in a transgenic system, either *in vitro* or *in vivo*, either transiently or stably. Their proper expression is regulated by homologous or heterologous promoter sequences. Three groups of transgenes can be discerned. (1) Transgenes which represent a gain-of-function lead to expression of a new protein in the cell. Examples are the growth hormone and the anti-freeze protein. (2) The use of selectable markers such as the gene conferring resistance to neomycin facilitate the selection and identification of transgenes. Reporter genes such as those encoding β-galactosidase, luciferase and green fluorescent protein permit the examination of regulatory elements. (3) The expression of loss-of-function transgenes interferes with the normal functioning of endogenous genes (HACKETT, 1993). A gene encoding antisense mRNA that would prevent GnRH protein synthesis is under development.

Reviews on this topic have been published amongst others by GONG and HEW (1995), HACKETT (1993) and IYENGAR *et al.* (1996).

Given the observation that in general "all-fish-constructs" perform the best in transgenic fish (BETANCOURT *et al.*, 1993), the currently used gain-of-function transgenes are virtually all of piscine origin and derive from the gene proper (gDNA) or are cDNA sequences with intron sequences and the polyadenylation signal added. There are a number of well documented cases, of which transgenics for growth hormone and antifreeze protein will be discussed later on.

The Xmrk oncogene encodes a receptor tyrosine kinase which causes melanomas and is located on the sex chromosome (SCHARTL, 1990). Injection of the CMVTk/Xmrk construct into medaka embryos induced embryonic tumour formation in tissues normally expressing the Xmrk proto-oncogene (WINKLER *et al.*, 1994). The system is used as a successful model for oncogenesis.

Gonadotropin releasing hormone (GnRH) has been cloned in a number of fish including zebra fish, tilapia, rainbow trout and catfish. It is responsible for sexual maturation and as such draws a

lot of interest in its potential to induce sterility (ALESTRÖM *et al.*, 1992).

Other transgenes of interest include lysozyme, an antibacterial enzyme found in various tissues of vertebrates. Current interest traces its origin to its isolation from rainbow trout by DAUTIGNY *et al.* (1991). The gene encoding the pituitary expressed hormone prolactin has been cloned amongst others from tilapia, it has been characterised *in vitro* by SWENNEN *et al.* (1992) and stably transferred to rainbow trout, in which its phenotype is under investigation (BRETON, pers. comm). The insulin-like growth factor-1 (IGF-1) is regulated by GH and is implicated in the growth promoting effects of GH. It has been cloned, sequenced and characterised amongst others in Atlantic salmon (DUGUAY *et al.*, 1992).

The second group of transgenes, the reporter genes, are meant to facilitate the study of regulatory elements because they are relatively easily and reliably detected. They can also be used in large screens of mutants by gene-trapping (identification of unknown gene products) and enhancer-trapping (identification of unknown promoter sequences) (GAIANO *et al.*, 1996a). They are usually of non-fish origin and carry characteristics which makes them relatively easily detected.

lacZ originates from *E. coli* and encodes the enzyme β-galactosidase which is detected *in vivo*, colorimetrically, histochemically, immunohistochemically or luminometrically (MACGREGOR *et al.*, 1990). It is the most often used reporter gene, is highly dependable and can be used under a variety of conditions (see ALAM *et al.*, 1995; LIN *et al.*, 1994; SEKKALI *et al.*, 1994). False positives can be obtained because of staining of the eye lens and endogenous β-galactosidase activity in the stomach.

Chloramphenicol acetyltransferase (*CAT*) is detected radioactively with a high degree of sensitivity in transgenic tissue extracts and immunohistochemically (see STUART *et al.*, 1990). However the need to work radioactively and the long protein half-life of 50 h (VOLCKAERT *et al.*, 1994) represent major drawbacks.

Luciferase (*luc*) originates from *Photinus pyralis* (firefly) and encodes the enzyme luciferase which can be detected with photon

acquisition systems (scintillation counter, luminometer, X-ray film and CCD camera) and immunohistochemically (see GIBBS *et al.*, 1994; MAYERHOFER *et al.*, 1995; VOLCKAERT *et al.*, 1994). It is currently the most sensitive and one of the most simple systems to use. Advantages include the absence of endogenous background activity, the simple polypeptide structure of luciferase, the linearity over a range of 10^6 and the half-life of 3h (SEKKALI *et al.*, 1994).

Green fluorescent protein (GFP) originates from the jellifish *Aequorea victoria* and produces a bright green fluorescent product under UV light (AMSTERDAM *et al.*, 1995). It does not seem to be harmful to the transgenic fish and does not require exogenously added product. Moreover, spectrally shifted mutants have been isolated (DELAGRAVE *et al.*, 1995).

The tyrosinase enzyme which is involved in pigmentation, has been introduced in mutants of medaka (MATSUMOTO *et al.*, 1992). The melanin concentrating hormone (MCM), also involved in pigmentation is another candidate reporter gene (ALESTRÖM *et al.*, 1992).

Selectable markers such as neomycin phosphotransferase (*neo*), an antibiotic resistance gene, can be used to select fully transgenic animals (see attempt by ZELENIN *et al.*, 1991). However, mosaic transgenic fish (the most common case in parental populations) will also be killed despite *neo* expression in a limited number of cells. This makes *neo* less suitable for transgenic research.

The third group of transgenes, called loss-of-function genes, can be divided in two: transgenes whose product interferes with the normal expression of another gene and transgenes that handicap the normal transcription of a gene through DNA insertion (enhancer-trap and gene-trap). Although this approach has not been used that much in fish for various reasons, there are a few cases. Transgenes that produce antisense mRNA of GnRH to block the normal expression of GnRH belong to the first strategy (ALESTRÖM *et al.*, 1994).

BAYER and CAMPOS-ORTEGA (1992) developed a zebrafish line with preferential expression in primary sensory neurons, which showed all the characteristics of an enhancer-trap. More recent is the insertional mutagenesis in chemically mutated zebrafish which facilitates the cloning of genes corresponding to a specific

phenotype (GAIANO *et al.*, 1996a). Although the latter does not fully correspond to the gene-trap qualification, it sets a standard for this kind of research.

Germline transmission

A second important phase in achieving stable transgenic fish is the permanent establishment of the transgene in the host genome. The transgene has to be incorporated in germ cells to be heritable. It has to be mentioned that with current practices transiently transgenic fish are the most likely outcome of any attempt to insert transgenic constructs. In such case plasmid-like structures amplify in the nucleus but outside the chromosomes during early embryonic development and disappear sometimes later during embryonic development (GONG and HEW, 1995; IYENGAR *et al.*, 1996; VOLCKAERT *et al.*, 1994). Reviews on the techniques to induce transgenes have been prepared amongst other by CLOUD (1990), GONG and HEW (1995), HOUDEBINE and CHOURROUT (1991), MACLEAN and RAHMAN (1994) and OZATO *et al.* (1989).

The first developed, most efficient and still most commonly used technique is micro-injection. Essentially, a micropipette is introduced in the oocyte or the newly fertilised zygote and a small amount of a physiological solution containing the DNA construct is injected. In the first case the target is the nucleus (see OZATO *et al.*, 1989); in the second case the target is the cytoplasm because the polar body can not be localised (WINKLER *et al.*, 1991). Usually about 10^4 to 10^7 copies of the construct are introduced; lower amounts jeopardise transformation and higher amounts tend to be toxic (10^7 construct copies is about equivalent to the DNA content of the nucleus). Fish eggs are relatively large ranging from 1 mm (zebra fish) to 7 mm (salmon), and show various degrees of transparency due to the presence of yolk. The cortical reaction influences the penetration of the chorion to various degrees. Therefore injection occurs in the blastodisc or through the micropyle shortly after fertilisation (VOLCKAERT *et al.*, 1994), or in dechorionated eggs (CULP *et al.*, 1991). DNA should ideally be injected into the nucleus or pronucleus of the egg so that integration

in the host genome is facilitated. If integration occurs during early development (blastula), all cells have a chance to be transgenic, although this depends also on the nature of the construct.

Sperm incubation might seem to be the easiest way to transfect organisms, but the results are variable (CHOURROUT and PERROT, 1992; KHOO *et al.*, 1992). It is thought that DNA might adhere to the cell wall and be introduced in the egg during fertilisation. An alternative is the electroporation of the sperm (MÜLLER *et al.*, 1993; SYMONDS *et al.*, 1994). Short electrical pulses permeate the cell membrane temporarily and thus facilitate the entry of macromolecules such as DNA into the cytoplasm. It allows to treat numerous eggs simulataneously, is highly reproducible and eliminates skilled labour.

Gene bombardment involves the bombardment of cells by high-velocity microprojectiles of small tungsten particles coated with a DNA construct (ZELENIN *et al.*, 1991). Although highly succesful in plant tissues, the absence of a impermeable cell wall of cellulose seems to inhibit transformation.

The inclusion of DNA in lipophylic particles (lipofection) which fuse with the cell membrane efficiently transfects bacteria and eukaryotic cells. It is suitable for use with fish sperm and dechorionated embryos. Transient expression of lipofected eggs of African catfish has been documented by SZELEI *et al.* (1994).

Retroviral infection has been reported above. Most remarkable is that its efficiency in transfecting is several orders higher than the previously mentioned methods. A drawback is that the modified retrovirus has to be injected.

However in all these methods, integration of the transgene in the host genome occurs at random. Homologous recombination, mostly aiming at selective disruption of a target gene or insertion of a reporter gene, is routinely achieved in mice because of the availability of totipotent embryonic stem cells (ES). In a first step, a targeting vector containing about 8 kb of the gene of interest interrupted by sequences encoding a selectable marker is introduced into ES cells. Selection and genomic DNA analysis identify ES cell clones with the appropriate integration event: these are injected into blastocysts to generate chimaeric animals.

Method	Ease of protocol	Parental expression	Stable inheritance
Bombardment	++	YES[1]	NO
Electroporation	++	YES[2]	NO
Lipofection	+	YES[3]	NO
Micro-injection	++	YES[4]	YES[5]
Retroviral infection	+++	YES[6]	YES[6]
Sperm incubation	+	YES[7]	NO

Table 1
The efficiency of various methods to deliver DNA as reported in the literature for ease of protocol, parental expression and stable inheritance. [1] ZELENIN *et al.*, 1991; [2] MÜLLER *et al.*, 1993; [3] SZELEI *et al.*, 1994; [4] Numerous reports, including VOLCKAERT *et al.*, 1994 and WINKLER *et al.*, 1992; [5] STUART *et al.*, 1990 and OZATO *et al.*, 1986; [6] GAIANO *et al.*, 1996b; [7] SYMONDS *et al.*, 1994.

If these have germ cells derived from the recombinant ES cells, they will transmit the genotype to their progeny. No totipotent ES cells are available yet in fish systems that would allow homologous recombination, but several laboratories are investigating exactly that. To this purpose cell transplants among zebrafish blastulae and the production of germ-line chimaeras (LIN *et al.*, 1992), embryonic stem cell culture (COLLODI *et al.*, 1992; HONG and SCHARTL, 1996) and suitable vectors for site-specific integration (IZSVAK *et al.*, 1995; BEARZOTTI *et al.*, 1992) are under development.

Expression of the transgene

Correct expression of the transgene is of course the major aim of transgenic integration. However, it is labour intensive to identify stable transgenic fish with reliable transgenic expression because of the high level of chimaeras observed. Several strategies have been developed to facilitate selection: *in vivo* detection of stable integration on genomic DNA extracted from skin tissue of progeny after proteinase K digestion (KAWAKAMI and HOPKINS, 1996). *In vivo* detection of expression by assaying enzymatic activity of Luc

(MAYERHOFER *et al.*, 1995), tyrosinase (MASTUMOTO *et al.*, 1992) and lacZ (LIN *et al.*, 1994) and by assaying fluorescence of GFP (AMSTERDAM *et al.*, 1995). Other screening methods such as fluorescence *in situ* hybridisation and histochemical staining to verify expression are rather time-consuming.

The fate of the DNA construct is remarkable immediately after introduction (transient replication) and corresponds more to the general expectations in the long term (heritable or stable integration).

Transient replication

The fate of the DNA upon introduction in the transgenic fish system behaves differently from mammals. Soon after being introduced in the cell, the circular fusion genes form head-to-tail concatemers while the linearised fusion genes form random arrangements of head-to-tail, tail-to-tail and head-to-head concatemers in the nucleus (IYENGAR *et al.*, 1996; VOLCKAERT *et al.*, 1994). During the rapid cleavage stages of early development the fusion DNA is quickly replicated at the time of rapid DNA synthesis in the developing embryo up to the 10th or 11th cell division. However after the midblastula transition, degradation rates exceed replication rates and DNA fragments of 200 bp long are formed. The pattern is similar to other lower vertebrates such as *Xenopus* (PRIOLEAU *et al.*, 1994).

Transient expression is in some cases tissue-specific (promoter of the carp myosin heavy chain isoform in zebra fish and catfish (IYENGAR *et al.*, 1996)) while not in others (for example the prolactin promoter in catfish (personal data) and the ubiquitous promoters (ALAM *et al.*, 1996)). However, transient expression seems to be highly influenced by the embryonal tissue. The yolk syncitial layer shows the highest level of expression and thus may influence transient expression in studies using quantative comparative analysis (WILLIAMS *et al.*, 1996).

The long term fate of introduced DNA

Exogenous DNA may have integrated into the host's chromosomal DNA after surviving the degradation process of the cell during early development. The chances of parental integration vary from absent, over poor (most cases, *e.g.* INOUE *et al* (1992) and SHEARS *et al.* (1991)) to 80 % (GAIANO *et al.*, 1996a). The site of integration is thought to be aspecific; often multiple sites of integration are observed (IYENGAR *et al.,* 1996). Moreover transgene expression is often impaired by the sequences flanking its integration site (the so called position effect) since most genomic DNA is considered to be transcriptionally inactive heterochromatin. Another important factor silencing expression is *de novo* methylation of DNA (GIBBS *et al.*, 1994; IYENGAR *et al.,* 1996). Methylation of cytosine residues at CpG sites in the promoter seem to be occurring. Genetic background of the host genome, species-specific developmental dynamics and environmental influences on gene expression may also play a role (KAPUSCINSKI and HALLERMAN, 1991). Stable integration is proven by means of F_1 (a 50 % inheritance is expected) and F_2 (a 100 % expected inheritance is expected) test crosses, Southern blotting of transgenic gDNA, selective amplification by the polymerase chain reaction (PCR) of the introduced sequence or fluorescence *in situ* hybridisation of the karyotypes.

Transgenic fish in aquaculture

Sometimes the novelty of the genetic engineering of transgenic fish has generated exaggetrated expectations in regards to its applications among scientists, fish farmers, administrators and the general public. Delayed maturation, enhanced freezing tolerance, disease resistance and especially enhanced growth of transgenic fish have caught the imagination. The following paragraphs will focus on three cases. In the next chapter the environmental, consumer and ethical aspects of transgenic fish are highlighted.

Growth enhancement

The growth hormone (GH) gene has been cloned in at least 17 fish species (WALLIS, 1996). It encodes for the important circulating peptide growth hormone, secreted in the pituitary and influencing amongst others metabolism and seawater adaptation. It draws special interest because of its growth promoting action when provided exogenously or transgenically. Initial attempts were marred by poor inheritance (an exception is INOUE *et al.*, 1990) and expression, which has been related to the nature of the construct, the mammalian origin, methylation or the absence of regulatory intron sequences. Presently an "all-fish" construct of chinook salmon GH under control of the ocean pout AFP promoter has induced dramatic growth increases (DEVLIN *et al.*, 1994b, 1995a, 1995b). On average the transgenic fish were 10 to 30 times heavier than the control group at 15 months of age. At present, it is not clear to what extent the endocrinology (*e.g.* the titer of growth hormone) and growth rate correlate, nor how the excess GH binds to the receptors. Transgenic coho salmon underwent precocious parr-smolt transformation during their first fall, about 6 months in advance to their non-transgenic siblings. It has to be mentioned that the unregulated overproduction of GH induces side effects such as craniofacial abnormalities (DEVLIN *et al.*, 1994a), colour change (DEVLIN *et al.*, 1995b), excessive fat deposition, and so on. Similar but less dramatic effects have been documented with growth hormone driven by the metallothionein promoter in coho salmon (DU *et al.*, 1992) and pike (GROSS *et al.*, 1992).

Cold tolerance

Arctic fishes synthesize Antifreeze Protein (AFP) which lowers the freezing point of the plasma. AFP is synthesised in the liver and then released in the circulatory system. The winter flounder type I AFP gene is seasonally regulated and contains a well characterised promoter (GONG *et al.*, 1991). Commercial fish with the AFP gene would be in a commercially advantageous position if they tolerated water temperatures below -0.7°C. Attempts are under way to transfer this gene into Atlantic salmon (SHEARS *et al.*, 1991) and

goldfish (WANG *et al.*, 1995) such that their tolerance to freezing can be lowered. About 3% of Atlantic salmon embryos injected with the AFP gene expressed AFP, although at low levels (10 to 50 µg/ml instead of 2-5 mg/ml). Inheritance has been shown, but further research is required before a commercial system is available. To the contrary F_2 and F_3 progeny of goldfish had a lower cold tolerance.

Disease resistance

An important aim of transgenic fish could be to create a barrier against viral and bacterial infections. One only has to think of the impact of furunculosis infections by the bacterium *Aeromonas salmonicida* causing high fish mortalities or Infectious Hematopoetic Necrosis Virus (IHNV), a rhabdovirus affecting Northwest Pacific salmonids. Several strategies are possible: either a protein is produced transgenically for vaccination, either genes are incorporated that eliminate viral or bacterial infections. Lysozyme has been shown to be a potent inhibitor of gram negative bacteria. Its antibacterial activity can be enhanced by protein engineering (IBRAHIM *et al.*, 1994). A rainbow trout lysozyme cDNA has been isolated (DAUTIGNY *et al.*, 1991) and spliced to the ocean trout AFP promoter. Integration in the Atlantic salmon genome is in progress (IYENGAR *et al.*, 1996).

Biosafety

Concerns have surfaced in conjunction with the high expectations from fish with novel genes. Researchers, administrations and the public soon discovered that the new opportunities offered by fish with new traits included as well postive as negative elements. Comments in the scientific literature and press focus on environmental issues, ethical issues and consumer concerns.

Central in the environmental concerns is the question whether escaped transgenic fish will transfer their special genes to wild and natural populations and whether these genetically modified organisms (GMOs) will be selected for. Will GMOs establish themselves permanently outside the confined environment of the aquaculture facility? The question is the more crucial as a growing number of wild species, including fish, go extinct due to habitat destruction, pollution, introductions, hunting and fishing. The accidental or conscious release of transgenic organisms may influence this process and pose a real threat to the natural ecosystem.

Evidence for the introgression of transgenics are not available yet and according to some authors the few transgenic fish released are most unlikely to be selectively favoured for (KNIBB, 1997). Transgenic fish have been documented to be released in nature. Well protected land-based hatcheries are operational for transgenic Atlantic salmon in Canada and Scotland. Some of the environmental concerns refer also to accidental escapes since transgenics are usually generated from domesticated fish and thus may be held in various kinds of containment facilities (land-, ocean- or lake-based). Containment may involve different options; biological containment refers to strategies such as sterilisation and monosex populations, chemical containment refers to the treatment of effluents with chlorination/dechlorination while physical containment varies from minimal options (a fish pen in the ocean) to maximal options (indoor research laboratories with discontinuous outflow) (KAPUSCINSKI and HALLERMAN, 1990). However it is and never will be perfect; the weakest link will determine the chances to escape. Escapes of Atlantic salmon off Norway have reached levels where the domesticated stock surpasses several times the wild stock, but legal pressure has curtailed these historic levels of escapees. Hybridisation between native and domesticated Atlantic salmon in a monitored river system in Ireland has revealed that wild animals and crosses between wild females with farmed fish have a high survival and fitness (CROSS, *pers. com.*). Farmed fish are often less adapted to the local situation, although their numeric dominance may make resources unavailable for the local stock. An interesting example of reduced fitness is the higher risk to natural predation of growth hormone treated fish because of the higher levels of activity

(JÖNSSON *et al.*, 1996). If introgression will happen, genes are likely to pass through the domesticated males. In general, escapes of transgenics draws similarity to the introduction of exotics, especially if a non-native species or strain is involved.

Another method to estimate the effect of transgenics is modelling based on established species (GLIDDON and GOUDET, 1995). Fitness traits, gene flow, fixation of alleles and behaviour affect the chances that a transgenic escapee breeds with local stock. It is clear that each transgenic line has its own traits and thus will have to be dealt with case by case. In addition, the stability of recombinant DNA in the natural environment is influenced by selection since in the absence of selection the insert will disappear due to the costs of carriage and expression of the recombinant (LENSKI and NGUYEN, 1988).

There may be other ways of reducing the risks of transgenic fish by switching on the transgenic genes "on demand". Promoters / enhancers are such that transcription is only initiated and terminated in selected conditions. An example is the antifreeze promoter which is activated at low temperatures (GONG *et al.*, 1991).

If recommendatations are made towards genetically modified transgenics (GMO), the following aspects should be considered:

(1) Is the phenotype sufficiently characterised? Is another trait induced than anticipated? Has the molecular behaviour of the promoter/enhancer and the gene been anticipated? How many copies are integrated? Does the DNA construct induce novel gene combinations? Is inheritance stable? Is the retrovirus sufficiently specific?

(2) Are there sufficient physical, chemical and biological barriers present in the experimental facility such that escapes are virtually impossible. Otherwise said, is the risk sufficiently assessed? What is the weakest link in the system (HALLERMAN and KAPUSCINSKI, 1995)?

(3) If sufficient information has been collected on the GMO in step (1), has it been part of a quantitative breeding program such that the novel trait can be further characterised and selected for? Inbreeding of GMO's is of concern.

(4) Does the customer run a risk when eating transgenic fish?

In general policies are under review amongst others in the USA, Canada, the European Union and Norway (HALLERMAN and KAPUSCINSKI, 1995).

Ethical aspects and consumer acceptance of GMO's intertwine and pose a serious pressure on the fish industry. In general the public is not in favour although the response depends on the socio-cultural background of the customers. A British survey of customers views on the acceptability of transgenic fish in fish farming showed that 36.5% had no opinion, 34.8% thought it was not acceptable and 28.7% thought it was acceptable (PENMAN *et al.*, 1995). The Canadian industry has reacted with the utmost care such that only one hatchery on the East Coast has taken the challange to breed transgenic Atlantic salmon. Food safety will have to be addressed to avoid that non-desirable side-effects are propagated (BERKOWITZ and KRYSPIN-SORENSEN, 1994; CHATAKONDI *et al.*, 1995).

Patenting of transgenic fish has been envisaged (HALLERMAN and KAPUSCINSKI, 1990b). If successful patents are developed, the fish food industry will likely be dominated by a few companies able to invest in the considerable R&D costs.

The future

Transgenic fish have entered the international forum and will stay there. It is clear that their value as research tool to study the regulation of gene expression, including the molecular biology of development and molecular endocrinology is extremely valuable. Several genes have been under scruteny thanks to this approach and many more are awaiting. It is only the question how many of the 10,000 housekeeping genes and 2,000 or so regulated genes will be studied to a certain level of detail.

Some of the genes isolated will be suitable for applications, either in a biomedical or an agricultural (including aquaculture) context. GH

has proven some potential, but because of imperfect knowledge of its regulation and action, much more research remains to be done. Other applications such as fish as bioreactor (to produce selected proteins in bulk) have potential, although not much has been proven at the moment. The current DNA constructs are surely not optimal. Further fine tuning of the promoter/enhancer region to control expression and the flanking regions to enhance specificity of integration are urgently needed. The research community is targeting this topic (IYENGAR *et al.*, 1996).

Another aspect which requires attention is the introduction of the DNA construct. The aim is specificity, but the lack of fish embryonic stem cells for homologous recombination has meant a serious drawback. Recent progress with modified proviruses looks promising (GAIANO *et al.*, 1996b) but suffers from a poor understanding of fish virology. Future research will likely be redirected towards this field.

An issue which has been somewhat neglected is the introduction of GMOs in current selection programmes. Producers will surely want to continue to make progress with selection of traits such as growth, food conversion, disease resistance and so on. How the transgene will be introduced in the population and maintained remains to be proven. Hybridisation is one option, the production of pure lines another one. Also, the question of multi-locus interactions of DNA inserts and environment-transgene interactions have not been addressed.

Environmental and consumer issues will likely dominate the public forum in the coming years. GMO's are new and society doesn't necessarily have a clear answer to such new issues surfacing. Legislation is under continuous review (reflecting the dynamic state of GMOs) by national and multinational organisations including the Organisation for Economic Cooperation and Development (OECD), the International Council for the Exploration of the Sea (ICES), the Food and Agriculture Organisation (FAO) and the United Nations educational, scientific and cultural organisation (Unesco).

Most likely economic factors and consumer acceptance of GMOs will determine large scale developments. At the moment progress is mostly driven by a scientific push, but interest by the economic community is growing steadily.

Acknowledgements
F.V. is a research associate at the Fund for Scientific Research (FWO Vlaanderen). Research reported in this review has been funded by the European Union (grant BIOT CT90-0188), FWO Vlaanderen (grant G2014.94) and theKatholieke Universiteit Leuven (grant OT/90/16).
A. Belayew (K.U.Leuven) kindly made useful comments.

References

ALAM (M.S.), POPPLEWELL (A.), MACLEAN (N.), 1996 — Germ line transmission and expression of *lacZ* containing transgene in tilapia (*Oreochromis niloticus*). *Transgenic Research*, 5: 87-95.

ALESTRÖM (P.), KISEN (G.), KLUNGLAND (H.), ANDERSON (Ø.), 1992 — Fish gonadotropin-releasing hormone gene and molecular approaches for control of sexual maturation: development of a transgenic fish model. *Molecular Marine Biology and Biotechnology*, 1: 376-379.

ALESTRÖM (P.), HUSBYE (H.), KAVUMPURATH (S.), KISEN (G.), 1994 — Zebrafish, a vertebrate model for transgene expression and biological function. *Animal Biotechnology*, 5: 147-154.

AMSTERDAM (A.), LIN (S.), HOPKINS (N.), 1995 — The *Aequora victoria* green fluorescent protein can be used as a reporter in live zebrafish embryos. *Developmental biology*, 171: 123-129.

ARGENTON (F.), RAMOZ (N.), CHARLET (N.), BERNARDINI (Z.), COLOMBO (L.), BORTOLUSSI (M.), 1996 — Mechanisms of transcriptional activation of the promoter of the rainbow trout prolactin gene by GHF1/Pit1 and glucocorticoid. *Biochemical and Biophysical Research Communications*, 224: 57-66.

BAYER (T.A.), CAMPOS-ORTEGA (J.A.), 1992 — A transgene containing *lacZ* is expressed in primary sensory neurons in zebrafish. *Development*, 115: 421-426.

BEARZOTTI (M.), PERROT (E.), MICHARD-VANHEE (C.), JOLIVET (G.), ATTAL (J.), THERON (M.C.), PUISSANT (C.), DREANO (M.), KOPCHICK (J.J.), POWELL (R.), GANNON (F.), HOUDEBINE (L.M.), CHOURROUT (D.), 1992 — Gene expression following transfection of fish cells. *Journal of Biotechnology*, 26: 315-325.

BERKOWITZ (D.B.), KRYSPIN-SØRENSEN (I.), 1994 — Transgenic fish: safe to eat? *Bio/Technology*, 12: 247-251.

BETANCOURT (O.H.), ATTAL (J.), THERON (M.C.), PUISSANT (C.), HOUDEBINE (L.M.), 1993 — Efficiency of introns from various origins in fish cells. *Molecular Marine Biology and Biotechnology*, 2: 181-188.

BURNS (J.C.), FRIEDMANN (T.), DRIEVER (W.), BURRASCANO (M.), YEE (J.), 1993 —
Vesicular stomatistis virus G glycoproteins pseudotyped retroviral vectors : concentration to very high titer and efficient gene transfer into mammalian and non mammalian cells. *Proceedings of the National Academy of Sciences of the USA*, 90: 8033-8037.

CHATAKONDI (N.), LOVELL (R.T.), DUNCAN (P.L.), HAYAT (M.), CHEN (T.T.), POWERS (D.A.), WEETE (J.D.), CUMMINS (K.), DUNHAM (R.A.), 1995 —
Body composition of transgenic common carp, *Cyprinus carpio*, containing rainbow trout growth hormone gene. *Aquaculture*, 138: 99-109.

CHOURROUT (D.), PERROT (E.), 1992 —
No transgenic rainbow trout produced with sperm incubated with linear DNA. *Molecular Marine Biology and Biotechnology*, 1: 282-285.

CLOUD (J.G.), 1990 —
Strategies for introducing foreign DNA into the germ line of fish. *Journal of Reproduction and Fertility*, Supplement 41: 107-116.

COLLODI (P.), KAMEI (Y.), ERNST (T.), MIRANDA (C.), BUHLER (D.R.), BARNES (D.), 1992 —
Culture of cells from zebrafish (*Brachydanio rerio*) embryo and adult tissues. *Cell Biology and Toxicology*, 8: 43-61.

CULP (P.), NÜSSLEIN-VOLHARD (C.), HOPKINS (N.), 1991 —
High-frequency germ-line transmission of plasmid DNA sequences injected into fertilized zebrafish eggs. *Proceedings of the National Academy of Sciences of the USA*, 88, 7953-7957.

DAUTIGNY (A.), PRAGER (E.M.), PHAM-DINH (D.), JOLLES (J.), PAKDEL (F.), GRINDE (B.), JOLLES (P.), 1991 —
cDNA and amino acid sequences of rainbow trout (*Oncorhynchus mykiss*) lysozymes and their implications for the evolution of lysozyme and lactalbumin. *Journal of Molecular Evolution*, 32: 187-198.

DELAGRAVE (S.), HAXTIN (R.E.), SILVA (C.M.), YANG 5M.M.), YOUVAN (D.C.), 1995 —
Red-shifted exicitation mutants of the green fluoresecent protein. *Bio/Technology*, 13: 151-154.

DEVLIN (R.H.), YESAKI (T.Y.), BIAGI (C.A.), DONALDSON (E.M.), SWANSON (P.), CHAN (W.), 1994 —
Extraordinary salmon growth. *Nature*, 371: 209-210.

DEVLIN (R.H.), YESAKI (T.Y.), DONALDSON (E.M.), DU (S.J.), HEW (C.L.), 1995a —
Production of germline transgenic Pacific salmonids with dramatically increased growth performance. *Canadian Journal of Fisheries and Aquatic Sciences*, 52: 1376-1384.

DEVLIN (R.H.), YESAKI (T.Y.), DONALDSON (E.M.), HEW (C.L.), 1995b —
Transmission and phenotypic effects of an antifreeze/GH gene construct in coho salmon (*Oncorhynchus kisutch*). *Aquaculture*, 137: 161-169.

DU (S.J.), GONG (Z.), FLETCHER (G.L.), SHEARS (M.A.), KING (M.J.), IDLER (D.R.), HEW (C.L.), 1992 —
Growth enhancement in transgenic Atlantic salmon by the use of "an all fish" chimeric growth hormone gene construct. *Bio/Technology*, 10: 176-181.

DUGUAY (S.J.), PARK (L.K.), SAMADPOUR (M.), DICKHOFF (W.W.), 1992 —
Nucleoide sequence and tissue distribution of three insulin-like growth factor I prohormones in salmon. *Molecular Endocrinology*, 6: 1202-1210.

FRIEDENREICH (H.), SCHARTL (M.), 1990 —
Transient expression directed by homologous and heterologous promoter and enhancer sequences in fish cells. *Nucleic Acids Research*, 18: 3299-3305.

GAIANO (N.), AMSTERDAM (A.), KAWAKAMI (K.), ALLENDE (M.), BECKER (T.), HOPKINS (N.), 1996a —
Insertional mutagenesis and rapid cloning of essential genes in zebrafish. *Nature*, 383: 829-832.

GAIANO (N.), ALLENDE (M.), AMSTERDAM (A.), KAWAKAMI (K.), HOPKINS (N.), 1996b —
Highly efficient germ-line transmission of proviral insertions in zebrafish. *Proceedings of the National Academy of Sciences of the USA*, 93: 7777-7782.

GIBBS (P.D.L.), PEEK (A.), THORGAARD (G.), 1994 —
An *in vivo* screen for the luciferase transgene in zebrafish. *Molecular Marine Biology and Biotechnology*, 3: 307-316.

GLIDDON (C.), GOUDET (J.), 1995 —
Gene flow and analysis of structured populations. In *Environmental impacts of aquatic biotechnology*. OECD documents, Paris, pp.32-42.

GONG (Z.), HEW (C.), VIELKIND (J.), 1991 —
Functional analysis and temporal expression of promoter regions from fish antifreeze protein genes in transgenic Japanese medaka embryos. *Molecular Marine Biology and Biotechnology*, 1: 64-72.

GONG (Z.), HEW (C.L.), 1995 —
Transgenic fish in aquaculture and developmental biology. *Current topics in Developmental Biology*, 30: 177-214.

GROSS (M.L.), SCHNEIDER (J.F.), MOAV (N.), MOAV (B.), ALVAREZ (C.), MYSTER (S.H.), LIU (Z.), HALLERMAN (E.M.), HACKETT (P.B.), GUISE (K.S.),

FARAS (A.J.), KAPUSCINSKI (A.R.), 1992 —
Molecular analysis and growth evaluation of northern pike (*Esox lucius*) microinjected with growth hormone genes. *Aquaculture*, 103: 253-273.

HACKETT (P.B.), 1993 —
The molecular biology of transgenic fish. *In* HOCHACHKA (P.), MOMMSEN (T.), ed.: *Biochemistry and molecular biology of fishes*, vol 2: 207-240.

HALLERMAN (E.M.), KAPUSCINSKI (A.R.), 1990a —
Transgenic fish and public policy: regulatory concerns. *Fisheries*, 15: 1-20.

HALLERMAN (E.M.), KAPUSCINSKI (A.R.), 1990b —
Transgenic fish and public policy: patenting of transgenic fish. *Fisheries*, 15: 21-24.

HALLERMAN (E.M.), KAPUSCINSKI (A.R.), 1995 —
Incorporating risk assessment and risk management into public policies on genetically modified finfish and shellfish. *Aquaculture*, 137: 9-17.

HONG (Y.), WINKLER (C.), BREM (G.), SCHARTL (M.), 1993 —
Development of a heavy-metal inducible fish-specific expression vector for gene transfer *in vitro* and *in vivo*. *Aquaculture*, 111: 215-226.

HONG (Y.), SCHARTL (M.), 1996 —
Establishment and growth responses of early medakafish (*Oryzias latipes*) embryonic cells in feeder layer-free cultures. *Molecular Marine Biology and Biotechnology*, 5: 93-104.

HOUDEBINE (L.M.), CHOURROUT (D.), 1991 —
Transgenesis in fish. *Experientia*, 47: 891-897

IBRAHIM (H.R.), YAMADA (M.), MATSUSHITA (K.), KOBAYASHI (K.), KATO (A.), 1994 —
Enhanced bactericidal action of lysozyme to *Escherichia coli*

by inserting a hydrophobic pentapeptide into its C terminus. *Journal of Biological Chemistry*, 269: 5059-5063.

INOUE (K.), OZATO (K.), KONDOH (H.), IWAMATSU (T.), WAKAMATSU (Y.), FUJITA (T.), OKADA (T.S.), 1989 — Stage-dependent expression of the chicken δ-crystallin gene in transgenic fish embryos. *Cell Differentiation and Development*, 27: 57-68.

INOUE (K.), YAMASHITA (S.), AKITA (N.), MITSUBOSHI (T.), NAGAHISA (E.), SHIBA (T.), FUJITA (T.), 1990 — Electroporation as a new technique for producing transgenic fish. *Cell Differentiation and Development*, 29: 123-128.

INOUE (K.), ASADA (S.), KABENO (S.), YAMASHITA (S.), NAGAHISA (E.), SATAKE (M.), 1992 — Specific stages of oocyte maturity in medaka to obtain efficient foreign gene transfer by microinjection. *Nippon Suisan Gakkaishi*, 58: 1251-1255.

ISHIKAWA (Y.), HYODO-TAGUCHI (Y.), TATSUMI (K.), 1997 — Medaka fish for mutant screens. *Nature*, 386: 234.

IYENGAR (A.), MÜLLER (F.), MACLEAN (N.), 1996 — Regulation and expression of transgenes in fish: a review. *Trangenic Research*, 5: 147-166.

IZSVAK (Z.), IVICS (Z.), HACKETT (P.B.), 1995 — Characterization of a *Tc1*-like transposable element in zebrafish (*Danio rerio*). *Molecular General Genetics*, 247: 312-322.

JÖNSSON (E.), JOHNSSON (J.I.), BJÖRNSSON (T.), 1996 — Growth hormone increases predation exposure of rainbow trout. *Proceedings of the Royal Society of London B*, 263: 647-651.

KAPUSCINSKI (A.R.), HALLERMAN (E.M.), 1990 — Transgenic fish and public policy : anticipating environmental impacts of transgenic fish. *Fisheries*, 15: 2-11.

KAPUSCINSKI (A.R.), HALLERMAN (E.M.), 1991 — Implications of introduction of transgenic fish into natural ecosystems. *Journal of Fisheries and Aquatic Sciences*, 48: 99-107.

KAWAKAMI (K.), HOPKINS (N.), 1996 — Rapid identification of transgenic zebrafish. *Trends in Genetics*, 12: 9-10.

KHOO (H.W.), ANG (L.H.), LIM (H.B.), WONG (K.Y.), 1992 — Sperm cells as vectors for introducing foreign DNA into zebrafish. *Aquaculture*, 107: 1-19.

KINOSHITA (M.), TOYOHARA (H.), SAKAGUCHI (M.), KIOKA (N.), KOMANO (T.), INOUE (K.), YAMASHITA (S.), SATAKE (M.), WAKAMATSU (Y.), OZATO (K.), 1994 — Zinc-induced activation of rainbow trout metallothionein-A promoter in transgenic medaka. *Fisheries Science*, 60: 307-309.

KNIBB (W.), 1997— Risk from genetically engineered and modified marine fish. *Transgenic Research*, 6: 59-67.

KOMEN (J.), 1990 — *Clones of common carp (*Cyprinus carpio*). New perspectives in fish research*. Doctoral thesis, Agricultural University, Wageningen, The Netherlands, 169 pp.

LENSKI (R.E.), NGUYEN (T.T.), 1988 — Stability of recombinant DNA and its effects on fitness. *Trends in Ecology and Evolution*, 3: 518-520.

LIN (S.), LONG (W.), CHEN (J.), HOPKINS (N.), 1992 — Production of germ-line chimeras in zebrafish by cell transplants from genetically pigmented to albino embryos. *Proc. Natl. Acad. Sci. USA*, 89: 4519-4523.

LIN (S.), GAIANO (N.), CULP (P.), BURNS (J.), FRIEDMANN (T.), YEE (J.K.), HOPKINS (N.), 1994 — Integration and germ-line transmission of a pseudotyped retroviral vector in zebrafish. *Science*, 265: 666-669.

LIU (Z.), MOAV (B.), FARAS (A.J.), GUISE (K.S.), KAPUSCINSKI (A.R.), HACKETT (P.B.), 1990 — Development of expression vectors for transgenic fish. *Bio/Technology*, 8: 1268-1272.

MACGREGOR (G.R.), NOLAN (G.P.), FIERING (S.), ROEDERER (M.), HERZENBERG (L.A.), 1990 — Use of *E.coli* lacZ (β-galactosidase) as a reporter gene. *In* MURRAY (E.J.), WALKER (J.M.), ed.: *Methods in molecular biology*, Vol. 7.

MACLEAN (N.), RAHMAN (A.), 1994 — Transgenic fish. *In* MACLEAN (N.), ed.: *Animals with novel genes*. Cambridge University Press, Cambridge. 63-105.

MATSUMOTO (J.), AKIYAMA (T.), HIROSE (E.), NAKAMURA (M.), YAMAMOTO (H.), TAKEUCHI (T.), 1992 — Expression and transmission of wild-type pigmentation in the skin of transgenic orange-colored variants of medaka (*Oryzias latipes*) bearing the gene for mouse tyrosinase. *Pigment Cell Research*, 5: 322-327.

MAYERHOFER (R.), ARAKI (K.), SZALAY (A.A.), 1995 — Monitoring of spatial expression of firefly luciferase in transformed zebrafish. *Journal of Bioluminescence and Chemiluminescence*, 10: 271-275.

MOAV (B.), LIU (Z.), CALDOVIC (L.D.), GROSS (M.L.), FARAS (A.J.), HACKETT (P.B.), 1993 — Regulation of expression of transgenes in developing fish. *Transgenic Research*, 2: 153-161.

MÜLLER (F.), LELE (Z.), VARADI (L.), MENCZEL (L.), ORBAN (L.), 1993 — Efficient transient expression system based on square pulse electroporation and *in vivo* luciferase assay of fertilized fish eggs. *FEBS Letters*, 324: 27-32.

OZATO (K.), KONDOH (H.), INOHARA (H.), IWAMATSU (T.), WAKAMATSU (Y.), OKADA (T.S.), 1986 — Production of transgenic fish: introduction and expression of chicken δ-crystallin gene in medaka embryos. *Cell Differentiation*, 19: 237-244.

OZATO (K.), INOUE (K.), WAKAMATSU (Y.), 1989 — Transgenic fish: biological and technical problems. *Zoological Science*, 6: 445-457.

PENMAN (D.J.), WOODWARK (M.), MCANDREW (B.J.), 1995 — Genetically modified fish populations. In: *Environmental impacts of aquatic biotechnology*. OECD documents, 22-27.

PONCELET (A.C.), LEVAVI-SIVAN (B.), MULLER (M.), YARON (S.), MARTIAL (J.A.), BELAYEW (A.), 1996 — The tilapia prolactin I gene: evolutionary conservation of the regulatory elements directing pituitary-specific expression. *DNA and Cell Biology*, 15: 679-692.

POWERS (D.A.), 1989 — Fish as model systems. *Science*, 246: 352-358.

PRIOLEAU (M.N.), HUET (J.), SENTENAC (A.), MECHALI (M.), 1994 — Competition between chromatin and transcription complex assembly regulates gene expression during early development. *Cell*, 77: 439-449.

ROSSANT (J.), HOPKINS (N.), 1992 — Of fin and fur: mutational analysis of vertebrate embryonic development. *Genes and Development*, 6: 1-13.

SCHARTL (M.), 1990 — Homology of melanoma-inducing loci in the genus *Xiphophorus*. *Genetics*, 126: 1083-1091.

SEKKALI (B.), HELLEMANS (B.A.), BELAYEW (A.), MARTIAL (J.A.),

OLLEVIER (F.), VOLCKAERT (F.A.), 1994 —
A comparative study of reporter gene activities in fish cells and embryos. *Mol. Mar. Biol. Biotechnol.*, 3: 30-34.

SHEARS (M.A.), FLETCHER (F.L.), HEW (C.L.), GAUTHIER (S.), DAVIES (P.L.), 1991 —
Transfer, expression and stable inheritance of antifreeze protein genes in Atlantic salmon (*Salmo salar*). *Molecular Marine Biology and Biotechnology*, 1: 58-63.

STUART (G.W.), VIELKIND (J.R.), MCMURRAY (J.V.), WESTERFIELD (M.), 1990 —
Stable lines of transgenic zebrafish exhibit reproducible patterns of transgene expression. *Development*, 109: 577-584.

SWENNEN (D.), PONCELET (A.C.), SEKKALI (B.), RENTIER-DELRUE (F.), MARTIAL (J.A.), BELAYEW (A.), 1992 —
Structure of the tilapia (*Oreochromis mossambicus*) prolactin I gene. *DNA and Cell Biology*, 11: 673-684.

SYMONDS (J.E.), WALKER (S.P.), SIN (F.Y.T.), SIN (I.), 1994 —
Development of a mass gene transfer method in chinook salmon: optimization of gene transfer by electroporated sperm. *Molecular Marine Biology and Biotechnology*, 3: 104-111.

SZELEI (J.), VARADI (L.), MÜLLER (F.), ERDELYI (F.), ORBAN (L.), HORVATH (L.), DUDA (E.), 1994 —
Liposome-mediated gene transfer in fish embryos. *Transgenic research*, 3: 116-119.

TAKAGI (S.), SASADO (T.), TAMIYA (G.), OZATO (K.), WAKAMATSU (Y.), TAKESHITA (A.), KIMURA (M.), 1994 —
An efficient expression vector for transgenic medaka construction. *Molecular Marine Biology and Biotechnology*, 3: 192-199.

VOLCKAERT (F.A.), HELLEMANS (B.A.), GALBUSERA (P.), SEKKALI (B.), BELAYEW (A.), OLLEVIER (F.), 1994 —
Replication, expression, and fate of foreign DNA during embryonic and larval development of the African catfish (*Clarias gariepinus*). *Molecular Marine Biology and Biotechnology*, 3: 57-69.

WALLIS (M.), 1996 —
The molecular evolution of vertebrate growth hormones: a pattern of near-stasis interrupted by sustained bursts of rapid change. *Journal of Molecular Evolution*, 43: 93-100.

WANG (R.), ZHANG (P.), GONG (Z.), HEW (C.L.), 1995 —
Expression of the antifreeze protein gene in transgenic goldfish (*Carassius auratus*) and its implication in cold adaptation. *Molecular Marine Biology and Biotechnology*, 4: 20-26.

WILLIAMS (D.W.), MÜLLER (F.), LAVENDER (F.L.), ORBAN (L.), MACLEAN (N.), 1996 —
High transgene activity in the yolk syncytial layer affects quantitative transient expression assays in zebrafish (*Danio rerio*) embryos. *Transgenic Research*, 5: 433-442.

WINKLER (C.), VIELKIND (J.), SCHARTL (M.), 1991 —
Transient expression of foreign DNA during embryonic and larval development of the medaka fish (*Oryzias latipes*). *Mol. Gen. Genet.*, 226: 129-140.

WINKLER (C.), HONG (Y.), WITTBRODT (J.), SCHARTL (M.), 1992 —
Analysis of heterologous and homologous promoters and enhancers *in vitro* and *in vivo* by gene transfert into Japanese medaka (*Orysias latipes*) and *Xiphophorus*. *Molecular Marine Biology and Biotechnology*, 1: 326-337.

WINKLER (C.), WITTBRODT (J.), LAMMERS (R.), ULLRICH (A.), SCHARTL (M.), 1994 —
Ligand-dependent tumor induction in medakafish embryos by a Xmrk receptor tyrosine kinase transgene; *Oncogene*, 9: 1517-1525.

326-337.ZAFARULLAH (M.), OLSSON (P.E.), GEDAMU (L.), 1989 — Endogenous and heavy-metal-ion-induced metallothionein gene expression in salmonid tissues and cell lines. *Gene*, 83: 85-93.

ZELENIN (A.V.), ALIMOV (A.A.), BARMINTZEV (V.A.), BENIUMOV (A.O.), ZELENINA (I.A.), KRASNOV (A.M.), KOLESNIKOV (V.A.), 1991 — The delivery of foreign genes into fertilized fish eggs using high-velocity microprojectiles. *FEBS Letters*, 287: 118-120.

Genetic mapping of Tilapiine fishes

Thomas David Kocher
Geneticist

Introduction

Selective breeding is a powerful tool for improving the performance of domesticated species, and has been employed with spectacular success to improve production of both animal and plant crops. Selective breeding is also essential to maintain the performance of superior stocks, as these stocks will tend to decline over time. This is particularly true in fish, where opposing selective pressures frequently reduce growth rate, and encourage early reproduction.

Most production traits are not controlled by single Mendelian genes. Rather, they depend on the effects of a number of genes, each of which contributes to the phenotype. The large number of highly polymorphic genetic markers which can now be developed for any species make it possible to identify the genes contributing to particular phenotypic traits. This information can be used to more directly select for gene contributing to high performance.

The goal of our study was to develop a comprehensive map of *O. niloticus* using DNA polymorphisms, which might be suitable for analysis of quantitative traits. Our approach was to study the segregation of these polymorphisms in the haploid progeny of a single female *O. niloticus*.

Materials and methods

Haploid gynogenesis

Milt was collected from, *O.niloticus* into glass capillary tubes and diluted to 2.5x 10^7 sperm/ml (HUSSAIN *et al.*, 1993) in modified fish Ringers solution (0.1M NACl; 40mM KCl; 1.4mM $CaCl_2$ $2H_20$; 2mM NaHCO3, pH adjusted to 8.0). One ml of diluted milt was placed into a small petri dish and irradiated with a UV dose of 290-295 microWatts/cm squared for exactly 2 minutes using a 254 nm UV lamp. 600-1000 eggs were stripped from an *O.niloticus* female and fertilized with the irradiated milt. Fry were collected 2-3 days post-hatching, at which point 75 haploids were recovered.

Genomic DNA extraction

After removal of the yolk-sac, fry were placed into individual sterile 1.5ml microcentrifuge tubes containing 150µl TEN buffer (100mM Tris-HCl, pH8.0; 10mM EDTA; 250mM NACl), 10 microliters 20% SDS and 5µl proteinase K (10mg/ml stock). Tubes were placed in water bath at 37°C overnight or at 55°C for a few hours. Two phenol and one chloroform/IAA (24: 1) extractions were carried out. DNA was precipitated using isopropanol. Approximately 1 µg of DNA was obtained from each embryo.

Microsatellite markers

The majority of microsatellite loci scored consisted of 139 di- and tri-nucleotide repeats isolated from an enriched *O. niloticus* genomic DNA library (LEE and KOCHER, 1996). An additional six loci isolated from *O. shiranus* (AMBALI, 1996) were also tested. Four markers isolated from Lake Malawi haplochromines were examined, including two loci from *Pseudotropheus zebra* (PARKER and KORNFIELD, 1996) and two from *Melanochromis auratus* (KELLOGG *et al.*, 1995).

Typing of microsatellites

Genotypes were obtained by automated sizing of fluorescently-tagged alleles amplified via PCR. We used a 25µl reaction volume containing 50mM KCl, 10mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.4mM $MgCl_2$, 0.16mM each dNTP, and 0.16µM each primer, to which we added 20 ng of haploid genomic DNA. The PCR conditions were as follows: 95°C lmin, 50-58°C 2min, 72°C 2min, and 25-30 thermal cycles according to the efficiency of amplification. For sizing, 1 µl from as many as three different PCR reactions were combined into a new tube, and dried in a speed-vac. The pellet was resuspended with both 0.3 µl of GeneScan 500 Tamra size standard (Applied Biosystems Inc., Foster City, CA) and 2.7 µl of formamide loading buffer. After denaturation at 90°C for 2min, the entire solution was loaded on a 6% acrylamide gel on an ABI 373A automated DNA sequencer. ABI GeneScan software (ver. 2.02) was used to analyze the genotypes of the micro-satellite loci.

AFLP markers

Vos *et al.* (1995) described a new technique for analysis of Anonymous Fragment Length Polymorphisms. We used the Perkin-Elmer AFLP plant mapping kit (Rev. A). A total of 250 ng of haploid genomic DNA was used in the initial ligation step. For the selective amplification step, we tested 22 primer pairs on a panel of six haploid progeny. One µl of each selective amplification product, together with the GeneScan 500 Rox size standard was loaded in each lane of the 6% gel on the automated sequencer. The frequency of variable bands per primer pair ranged from 0 (ACT+CAC and ACC+CAG) to 15 (AGG+CTT). Those primer combinations generating more than 5 variable markers were selected for typing the remaining 35 haploids. These primer pairs were [EcoRI+MseI (number of variable bands)]: ACT+CTA (7), AGG+CTG (10), ACA+CAA (11), AGG+CTT (15), ACA+CAC (10), ACT+CAT (13), AGC+CTA (10), ACC+CAA (6), ACT+CAG (11), AGC+CAT (6), AGG+CTA (7), and AGC+CAG (9).

Linkage analysis

We used the Macintosh porting (ver. 2.0) of Mapmaker (LANDER *et al.*, 1987) to identify linkage groups and determine marker order. An initial grouping of markers was performed with a LOD cutoff of 3.0. Because of the high levels of interference observed, final map distances were calculated using the Kosambi function (OTT, 1991).

Result

Genotypes

The parent female and six haploid progeny were screened for a total of 147 microsatellites. The mother was heterozygous for 62 (42%) of these markers. An additional 36 haploids were scored for these 62 microsatellites. We also scored the 41 haploids for 12 AFLP primer combinations, which identified 112 AFLP polymorphisms. The final data set consisted of genotypes of 62 microsatellites and 112 AFLP polymorphisms for 41 haploid progeny of the single female.

Linkage map

Overall, 93.1 % of the markers tested showed detectable linkage to another marker. 59 of the 62 microsatellites (95%), and 103 of 112 (92%) of the AFLP's were detectably linked to another polymorphism. The final linkage map consists of 30 linkage groups spanning 704cM (Figure 1 shows two of them). A total of 162 polymorphisms are included, for an average spacing of 4.3cM. The size of the linkage groups ranges from 0 to 73.6 cM (mean 23.5cM). The number of markers per linkage group varies from 2 to 28, with an average of 2 microsatellites and 3.4 AFLP markers

per group. Twenty-four linkage groups contain at least one micro-satellite polymorphism.

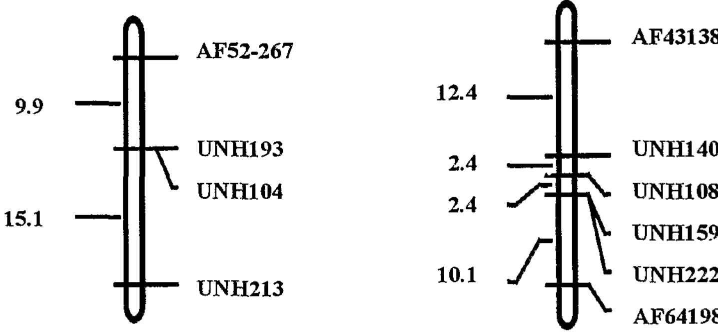


Figure 1
Part of the current linkage map for *Oreochromis niloticus* (two from the thirty linkage groups). The DNA markers fall in 30 linkage groups. Microsatellite loci (in bold) are identified with a combination of letters and numbers to designate the institution which developed the marker (UNH=University of New Hampshire). AFLP markers are designated AF, followed by two digits to indicate the primer combination and three digits to indicate the size of the scored fragment. Numbers to the left of each interval indicate the recombination distance (cM) between the markers.

Estimates of genome size

HULBERT *et al.* (1988) suggest that the ultimate map length can be estimated by observing the proportion of locus pairs linked at specific distances, and comparing this to an expectation based on the assumption that the loci are distributed randomly across the map. We performed these calculations separately for each marker type at four distances. When we analysed the proportion of pairs exhibiting less than 5% recombination, all combinations of marker pairs gave similar estimates of genome size, ranging from 412cM for the AFLP to 668cM for AFLP/micro pairs. These estimates are all smaller than the spanned length of our map. For larger intervals, the estimates are less consistent, and for recombination fractions of

20%, the genome size estimates range from 740 to 1,719cM. Our best estimate is that the genome is about 1,200cM in length.

Discussion

Strategies for QTL mapping

Microsatellites have become the preferred marker for animal gene mapping because of their high heterozygosity and ease of typing via PCR. AFLP is a new approach which offers rapid marker development and typing, but which has a higher error rate, and is less comparable across experiments than microsatellites. It may be possible to use a mixed strategy for mapping quantitative trait loci (QTL). High-density AFLP maps may be anchored with a much smaller set of microsatellite loci. We have already mapped at least one microsatellite on 24 of the 30 linkage groups, and it seems likely that we have mapped at least one 1 microsatellite on each chromosome. The 62 microsatellites we have characterized ensure a 95% probability of uniquely identifying each chromosome with a microsatellite locus in an MS-AFLP map. These anchor loci will allow comparison of AFLP maps produced for QTL analyses in different laboratories.

The Next Step

We have several goals in continuing this line of research. The first is the identification of QTL in different strains of tilapia which might be usefully combined to produce a faster growing tilapia. The map we have constructed is adequate for that purpose. Although we cannot expect that all 62 of these microsatellite markers will be variable in other crosses, we will continue to score the other 84 microsatellites already characterized, and hope eventually to incorporate all of them into the map. Inclusion of 50-60 microsatellites in each experimental cross will be sufficient

to identify homologous chromosomes. Marker density is most conveniently increased in each cross through the typing of AFLP markers.

A second goal is to use these genetic markers to characterize germplasm resources of tilapia. Preliminary work suggests that microsatellites are a useful way to estimate heterozygosity of stocks, and will be very useful for tracking parentage in selection experiments. Preliminary AFLP data suggests that this technique will be useful for classifying tilapia strains to species or identifying their probably hybrid origins.

Finally, we plan to extend our mapping efforts to other groups of cichlids, particularly the species flock of Lake Malawi haplochromines. A large proportion of our tilapia markers also amplify Lake Malawi cichlids, and it will be interesting to determine the extent of synteny with this group. A genetic map for these fish would allow QTL mapping of traits associated with speciation and adaptive radiation in this group.

Acknowledgements

This work is a collaboration with Dr. Woo-Jai Lee (UNH), Halina Sobolewska, David Penman and Brendan McAndrew (Institute of Aquaculture, Stirling) and was supported by grants from the USDA-NRICGP and the University of New Hampshire.

References

AMBALI (A.), 1996 —
The relationship between domestication and genetic diversity of Oreochromis *species in Malawi:* Oreochromis shiranus shiranus *(BOULENGER)* *and* Oreochromis shiranus chilwae *(TREWAVAS)*. Ph.D. thesis, Dalhousie University, Halifax, NS.

HULBERT (S. H.), ILOTT (T. W.), LEGG (E. J.), LINCOLN (S. E.), LANDER (E. S.), MICHELMORE (R. W.), 1988 —
Genetic analysis of the fungus, *Bremia lactucae*, using restriction fragment length polymorphisms. *Genetics*, 120: 947-958.

HUSSAIN (M.G.), PENMAN (D.J), MCANDREW (B.J), JOHNSTONE (R.), 1993 —
Suppression of first cleavage in the Nile tilapia, *O.niloticus* L.- a comparison of the relative effectiveness of pressure and heat shocks. *Aquaculture*, 111: 263-270.

KELLOGG (K.A.), MARKERT (J.A.), STAUFFER JR. (J.R.), KOCHER(T.D.), 1995 — Quantifying multiple paternity in Lake Malawi cichlid fish. *Proc. Roy. Soc. London Ser. B.*, 260: 79-84.

LANDER (E.), GREEN (P.), ABRAHAMSON (J.), BARLOW (A.), DALEY (M.), LINCOLN (S.), NEWBURG (L.), 1987 — MAPMAKER: An Interactive Computer Package for Constructing Primary Genetic Linkage Maps of Experimental and Natural Populations. *Genomics,* 1: 174-181.

LEE (W.J.), KOCHER (T. D), 1996 — Microsatellite DNA markers for genetic mapping in the tilapia, *Oreochromis niloticus. J. Fish Biology*, 49: 169-171.

OTT (J.), 1991 — *Analysis of human genetic linkage.* John Hopkins University Press, Baltimore ND.

PARKER (A.), KORNFIELD (I.), 1996 — Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Env. Biol. Fish.*, 47: 345-352.

VOS (P.), HOGERS (R.), BLEEKER (M.), REIJANS (M.), VAN DE LEE (T.), HORNES (M.), FRIJTERS (A.), POT (J.), PELEMAN (J.), KUIPER (M.), ZABEAU (M.), 1995 — AFLP : a new technique for DNA fingerprinting. *Nucleic Acids Res.*, 23: 4407-4414.

Genetic markers in marine biology and aquaculture research : when to use what

Antonios Magoulas
Geneticist

Giorgos Kotoulas
Geneticist

Kostas Batargias
Geneticist

Eleftherios Zouros
Geneticist

Introduction

Looking thirty years back, one realizes that the word "molecular" in the titles of the 1966 seminal papers by HUBBY and LEWONTIN (1966) and LEWONTIN and HUBBY (1966) was both misleading and visionary. Misleading, because no molecular biologist would ever consider scoring allozyme variation as part of his/her trade. Visionary, because today's population biologists can collect their primary information at the most basic and decisively molecular level: the nucleotide. Indeed, they have a rather wide and diverse collection of tools in their disposal. But as this collection grows, so does the choice of tools become more difficult. It is not always the case that the appearance of a new method renders another method obsolete. Indeed, one may argue that the possibility of simultaneous use of two or more assays of scoring genetic variation may shed light on questions that neither assay could answer alone.

The purpose of this communication is to provide a comparative evaluation of the various techniques now widely used in population genetics. Although the subject of the use of genetic markers for the study of natural populations has been extensively reviewed in the last years (*e.g.* AVISE, 1994), the question of what markers are most appropriate at any given instance has not yet received, to our knowledge, a comprehensive treatment. It is hoped that the elementary and sketchy presentation attempted here will give the impetus for a more formal and complete analysis of the subject. We will first list the various types of markers together with what we consider to be their advantages and disadvantages and then present examples from our own work in a way of illustration.

Tools

Antibody-antigen reaction polymorphism

It may be considered as the first type of "molecular" detection of polymorphism. The technique never received wide application because of sampling difficulties, but also because it became evident from human data that most blood groups are basically monomorphic (HEDRICK and MURRAY, 1978). The HLA polymorphisms in humans have, however, played an important role in the study of human genetics.

Allozymes

The simplicity and general applicability of the technique have made this the most widely studied form of molecular variation. Any source of soluble proteins, from bacterial cultures to animal fluids, is in principle suitable for allozyme analysis and the protocols of electrophoretic separation and staining are easily adjustable from species to species. The genetic interpretation of allozyme profiles (zymograms) is also straightforward. One major drawback has

been the inability to read genotypes from small quantities of tissue, which makes allozymes inapplicable for small organisms or for the immature stages (*e.g.* larvae) of large ones. But the main disadvantage, one that appears to be intrinsically difficult to overcome, is that only a small fraction of enzyme loci appear to be allozymically polymorphic. With small variations, the same set of few loci has been used in allozyme surveys from prokaryotes to fungi, plants and animals. What we have learnt from these studies is that selection is not the sole force that determines the genetic makeup of natural populations and that the theories of Wright, Crow and Kimura have relevance for the real world, beside their mathematical elegance. The present awareness that what we see in natural populations is the result of the interplay between mutation rate, effective population size and selection we owe to the allozyme revolution of the seventies and eighties. The quest to evaluate the relative importance of these forces by looking at coding or regulatory parts of the genome will continue. It may soon become possible to record amino acid polymorphism in large scale surveys by direct sequencing rather than by electrophoretic separation of the peptide product. This will increase enormously the number of protein loci that could be surveyed. Because allozymes cannot be assumed to be selectively neutral and because the amount of their polymorphism is limited (these two aspect are obviously related), they are not the assay of choice for the study of the biogeography of a species or its present population structure, but they might be important for the study of local adaptation. They also appear to be of limited value for phylogenetic studies.

Anonymous nuclear DNA markers

Under this category we include assays that target a segment of DNA of unknown function The segment can be amplified from individual specimens and the polymorphism scored as length difference of the PCR products. Alternatively, the product may be digested by a set of restriction enzymes and the polymorphism scored as restriction fragment length polymorphism (RFLP). The primers are usually designed from sequences originally obtained for other purposes. Another method is to use the PCR product from a reference individual as a probe against digested total DNA from the sampled individuals. This calls for a more cumbersome protocol,

but has the potential to uncover more polymorphism. cDNA probes represent a special version of this technique. Individual clones from a poly-A messanger RNA cDNA library are amplified by using vector primers and used as probes against Southern transfers. The resulting polymorphism could be due to either presence/absence of a restriction site or to variable number of tandem repeats (VNTR). Anonymous DNA markers comprise a large and diverse family. Some reveal a high level of polymorphism, others are mostly monomorphic. In contrast to mini or microsatellites (see below), there is no way of knowing if the assay will produce polymorphism before it is actually used. The interpretation of banding profiles can also be complicated and ambiguous, depending on the nature of the underlying polymorphism. Finally, the assumption of neutrality may not be justifiable in certain cases, as for example in the case of cDNA.

Randomly amplified polymorphic DNA (RAPD)

The method uses single short primers of arbitrary sequence to amplify anonymous regions of genomic DNA. It is a fast and cheap assay, but the penalty for this convenience is poor reproducibility and ambiguity in the interpretation of results. The profiles are usually multibanded and polymorphism is scored as presence/absence of specific bands. Homozygosity for presence of a band cannot be distinguished from heterozygosity. As a result, most population genetics models cannot be applied, and analysis is based on phenotype rather than allelic frequencies. RAPDs are more suitable for species and subspecies comparisons, than for intra-species population studies.

Minisatellites

Discovered serendipitously, this variation forms the basis of DNA fingerprinting. A minisatellite "locus" consists of tandemly repeated "units" each of which contains a "core" sequence of around 12-16 nucleotide bases and two sequences flanking the core. Loci with the same core but different flanking sequences are scattered around the genome. Thus, probing with the core sequence results in

a multibanded profile unique to each individual. If the probe is the repeat unit of a specific minisatellite locus (*i.e.* the core and the two flanking sequences), rather than the core alone, the hybridization may produce two-banded profiles under stringent conditions, so that single-locus allele frequencies can be scored. Minisatellites mutate at a very high rate (as high as 15%), which makes them ideal for individual identification, but reduces their utility for deducing genetic relation among randomly selected individuals from a population (the short-memory concept of identity by descent).

Microsatellites

Although similar by name, this class of markers is quite different from minisatellites. The repeat unit is very simple (mostly two, but also three or more nucleotides), the flunking sequences of each repeat locus are unique and the total length of the "locus" is much smaller than in minisatellites. Most importantly, microsatellites are much more numerous in the genome (particularly of vertebrates) and have a mutation rate between 10^{-3} and 10^{-4}. They are ideal for mapping "causal" genes, whether these are responsible for single factor conditions (*e.g.* muscular distrophy in humans) or for multifactorial traits (quantitative trait loci, QTL). They are also the best markers for determining parenthood in mass-crosses, tracing escapes form contained to wild populations and estimating coefficients of kinship among individuals drawn from a natural population. Their basic drawback remains the high cost and labor-intensiveness of the first phase of the technique, *i.e.* the development of primers. This is to some extent counter-balanced by the usually good crossability of primers in related species.

Mitochondrial DNA (mtDNA) variation

Three properties of mtDNA set it apart from nuclear DNA: it occurs in multiple copies in each cell (in contrast to two copies for a "single copy" nuclear locus), it is transmitted uniparentally, and it does not recombine. Presence of multiple copies does not, however, translate into a large variety of copies within the cell. For reasons not fully understood, the speed with which the maternal lineage of

a heteroplasmically conceived individual becomes homoplasmic is rather high. As a result, we can speak of the "mitotype" of an individual in the same way as we speak of its (nuclear) genotype. In addition (or as a result of this), the number of different mitotypes in a species is not huge, as originally suspected. If heteroplasmy rather than homoplasmy was the rule in nature, mtDNA would have been useless for population genetics. One consequence of uniparental transmission, which applies also to plasmid DNA, is that the effective population size for mtDNA is smaller than that of nuclear DNA, so that mtDNA variation is more exposed to the vagaries of random drift. Sex-specific differences in gene flow could also be revealed by contrasting nuclear with mtDNA. In a species in which mtDNA is maternally transmitted but gene flow occurs mainly or exclusively through males, divergence among populations is expected to be much higher for mtDNA than nuclear DNA. Incidental biparental transmission (when a small percentage of paternal mtDNA "leaks" into progeny) apparently is not a major concern in the use of mtDNA for phylogenetic or biogeographic studies, but biparentally induced heteroplasmy may signal the coexistence of subspecies or of highly diverged conspecific lineages in the population. From the point of view of population genetics, absence of recombination reduces the mtDNA molecule to a single gene. Coupled with uniparental inheritance, this means that the dynamics of mtDNA evolution are similar to those of a locus in a haploid asexually reproducing species. Such systems are prone to strong selective sweeps: an advantageous mutation anywhere in the genome will drive to fixation the type of molecule in which it occurred (or a deleterious mutation will drive it to extinction). At any given time, a population under selective sweeps will have a lower level of mtDNA variation, but viewed over long periods of time the rate of substitution at neutral sites would not be affected. That variation patterns compatible with selective sweeps were observed suggests that many sites in the mtDNA are under purifying selection. The notion that mtDNA evolves faster than nuclear single copy DNA is no longer accepted in its general form. It is evident that the rate varies both among species and among parts of the molecule within the same species. These differences have not reduced the importance of mtDNA as the molecule of choice for biogeographic and phylogenetic studies, but they must be considered when we are in need to provide explanations for observed patterns of variation.

RFLP, SSCP, Sequencing

All types of DNA variation are ultimately viewed as zones (bands). In most cases the different position of bands reflects difference in the length of DNA fragments. When the difference in length is caused by restrictions enzymes, the conventional term for the method of detection is Restriction Fragment Length Polymorphism (RFLP). Clearly, sequencing represents the ultimate level of scoring of DNA variation. But it remains expensive and labor-intense for large scale surveys. Single Strand Conformation Polymorphism (SSCP) is a relatively simple technique whereby the two strands are separated and forced to migrate independently. The speed of migration is affected by the conformation of the single strand. The utility of the technique depends on its discriminatory power. It is not yet known if single nucleotide differences can be detected. Another limitation is that at present it is restricted to small DNA fragments (normally less than 250 nucleotides).

Applications

It is obvious from this short evaluation of the various types of genetic markers that the marker of choice will depend on the particular question one wants to ask. There is no such a thing as an all-purpose marker. In this section we present examples from our own work which illustrate this point.

Heterozygosity and fitness

Even before the advent of genetic markers, evolutionists were divided in two schools. The classical school supports the view that there can be only one best allele at a time and that homozygosity for that allele is the best genotype. The balanced-polymorphism school supports the view that there can be no best allele, but only good or bad combinations of alleles and that most of the time

heterozygosity represents a better combination than homozygosity. The matter remains unsolved to this date. The critical test is to distinguish between homozygote fitness depression caused from homozygosity for the marked locus itself and depression resulting from homozygosity by descent in a linked but unscorable locus. POGSON and ZOUROS (1994) attempted to answer this question by correlating fitness (shell size in individual scallops) with degree of heterozygosity for two sets of loci, allozymes and anonymous cDNA's. These two sets of markers appear to be the best for this purpose. Because both come from transcribed parts of the genome the possibility that they are linked to a hidden deleterious gene is the same. For the classical school this means that allozymes and cDNAs should behave the same way. The balanced school, by placing the emphasis on the scored locus, predicts that allozyme heterozygotes would outperform allozyme homozygotes, but that this difference would not be observed between homozygotes and heterozygotes for cDNA variants which are assumed to be neutral. The results were compatible with the balanced-polymorphism school. It remains to be seen if the same will be observed in other organisms.

Selection versus random drift in natural populations

Typically, ecologists and, particularly, managers of natural biological resources are not interested in the genetic mechanisms of selection. Rather, they want to know if there are populations within a species that can be considered as separate units from the point of view of reproduction and interaction with the environment and the ecosystem. This proved to be an especially difficult issue to resolve by means of populations genetics. If two populations are found to be genetically homogeneous for the markers used, the answer can be that both populations are under the same regime of selection which forcefully maintains the same allele frequencies, or that the two populations are homogeneous because of extensive exchange of migrants. If the populations are found to be different, the explanation can be that they are under different selection pressures or that the populations have been isolated form each other for so long that random drift has established different allele frequencies.

The best way to answer this is to examine two or more types of variation simultaneously. Indeed, allozymes, mtDNA and nuclear markers that can be assumed to be selectively neutral might be the best combination for this purpose. When this was attempted by KARL and AVISE (1992) in the American oyster, the result was unexpected: there was no geographic differentiation for allozymes, but there was a strong dichotomy between Atlantic and Gulf populations for both mtDNA and anonymous nuclear genes. The authors argued that allozymes are under the same type of selection across the distribution of the species. As a result they carry no information about the species past history or its present breeding structure. These could, however, be inferred from the distribution of mtDNA and nuclear variation. Later studies of the same populations (MCDONALD *et al.*, 1996) suggested that the actual situation may not be as simple.

We are currently applying this strategy to the European anchovy and the swordfish, and to a lesser extend to several species of the family Sparidae. A comprehensive mtDNA survey of the anchovy (MAGOULAS *et al.*, 1996) revealed a sharp distinction between Black Sea and the rest of the species distribution that can be best explained by historical events going back to at least the last ice age and by current one-way gene flow from Black Sea into the Aegean. This is one of the best examples of a unidirectional gene flow in the marine environment and how it can preserve the footprints of the population history of the species. As usually happens with observations of special interest, it raises new questions and offers an opportunity to readdress unsettled issues. Is the huge Black Sea anchovy population genetically uniform? Do species that inhabit both the Black and the Aegean Seas exhibit one of three distinct types of genetic heterogeneity determined by the patten of gene flow: those in which migration is restricted in either way, those that experience unidirectional flow and those in which gene flow occurs in both directions? The favored explanation for the uniformity of allozyme frequencies that has been seen in many species distributed over vast and diverse geographic areas is some form of balancing selection that is a characteristic of the species itself rather to the environment of any specific population. If this is true, the Black Sea and the Bay of Biscay populations of anchovy ought to be allozymically similar in spite the fact that the first population has not received genes from the second for the last 10,000 years. If the two populations were found to be different for allozymes, the

selection hypothesis will be considerably weakened and allozyme uniformity, where observed, must be explained by gene flow. Microsatellites are useful here for yet another reason. It is hard to provide direct evidence that an individual with a Black Sea mitotype found in the Northern Aegean is indigenous or an immigrant from the Black Sea. This could be answered if microsatellites turned out to be sufficiently different between the two seas. With a large number of microsatellites it might even be possible to trace immigrants from the Black Sea or their immediate progeny in the Central Aegean and the Ionian Seas.

Unlike anchovy, present day patterns in the genetic differentiation of swordfish populations cannot be attributed to historic accidents, but rather to active migration for feeding or spawning. The observed differences between samples from the Mediterranean and the Gulf of Guinea (KOTOULAS *et al.*, 1995) can be most easily explained by some kind of homing behavior. Again microsatellites are the best genetic markers available to provide support for this hypothesis. If adults from different stocks mix but remain reproductively isolated by returning to different spawning grounds, there must be a strong correlation between mtDNA and microsatellites variants. This correlation, if it exists, could provide the basis for decomposing a sample of individuals caught in a given area into "natives" and "visitors", or for identifying the geographical origin of individuals converging on the same feeding grounds from different parts of the world.

Genetic markers in aquaculture research

We are using microsatellites in two aquacultured species, the gilthead sea bream and the Japanese oyster. In both cases, the primary purpose has been to identify the parents of an offspring from a mass mating. The ability to do this has revolutionized the practice of genetic breeding. It allows for a quick, inexpensive and more accurate estimation of heritabilities, and for evaluating the genetic quality of parents through the performance of their offspring. In the sea bream we have demonstrated additive genetic variance for growth, and identified individual parents that have consistently produced faster growing progeny. The same technique can be used to determine genetic differences in viability and

to estimate inbreeding depression. But in this case the statistical power of the assay is much lower, so that much larger samples are required. Microsatellites are also ideal to trace and verify cytogenetic treatments, such as induction of polyploidy or gynogenesis and have been used for this purpose in the Japanese oyster.

Conclusion

Assuming that obtaining the full nucleotide sequence of long pieces of DNA from thousands of individuals will continue to be an expensive practice for the immediate future, genetic markers will remain the main tool in population genetics. From the variety of markers presently available, it can be said that microsatellites, mtDNA and allozymes will maintain their eminence (more or less in that order). Studies whose the main objective is to trace an individual or deduce the genetic relationship between two individuals may rely exclusively on microsatellites. Similarly, mapping unknown genes affecting a quantitative or qualitative character may need no other markers except microsatellites. One can expect that soon there will be a data bank from which one would be able to retrieve microsatellite primer sequences for an ever increasing number of species.

It is, however, doubtful whether the more elusive questions about the history of natural populations and evolution would be answered by the use of one or the other kind of genetic markers. The most successful studies in this field will be those that would use two or more types of markers. Microsatellites and mtDNA will be part of the arsenal, which must also include a type of variation sensitive to selection. While allozymes serve this purpose at present, there is no reason why they could not be replaced with methods that would be able to survey a wider range of loci more likely to be under selection, such as regulatory, developmental or behavioral genes.

References

AVISE (J.C.), 1994 —
Molecular Markers. Natural History and Evolution. Chapman & Hall, N.Y.

HEDRICK (P.W.), MURRAY (E.), 1978 —
Heterozygosity revisited.
Amer. J. Hum. Gent., 30: 377-382.

HUBBY (J.L.), LEWONTIN (R.C.), 1966 —
A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at defferent loci in *Drosophila pseudoobscura. Genetics*, 54: 577-94.

KARL (S.A), AVICE (J.C.), 1992 —
Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. *Science*, 256: 100-102.

KOTOULAS (G.), MAGOULAS (A.), TSIMENIDES (N.), ZOUROS (E.), 1995 —
Marked mitochondrial DNA differences between Mediterranean and Atlantic populations of the swordfish *Xiphias gladius*.
Mol. Ecol., 4: 201-210.

LEWONTIN (R.C.), HUBBY (J.L.), 1966 —
A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*.
Genetics, 54: 595-609.

MAGOULAS (A.), TSIMENIDES (N.), ZOUROS (E.), 1996 —
Mitochondrial phylogeny and the reconstruction of the population history of a species: the case of the European anchovy (*Engraulis encrasicolus*).
Mol. Biol. Evol., 13: 178-190.

MCDONALD (J.H.), VERRELLI (B.C.), GEYER (L.B.), 1996 —
Lack of geographic variation in anonymous nuclear polymorphisms in the American oyster *Crassostera virginica*.
Mol. Biol. Evol., 13: 1114-1118.

POGSON (G.H.), ZOUROS (E.), 1994 —
Allozyme and RFLP heterozygosities as correlates of growth rate in the scallop *Placopecten magellanicus*: A test of the associative overdominance hypothesis.
Genetics, 37:221-231.

The utilization of ancient DNA to assess fish biodiversity: example of Mormyridae

Sébastien Lavoué
Geneticist

Jean-François Agnèse
Geneticist

Introduction

The zoological and botanical collections of the world's Natural History Museums are an incredible heritage. By their richness, they are supports for important systematic studies. These collections have become even more precious because many species of animals and plants can no longer be harvested either because certain areas cannot be easily accessed (high costs, geopolitical problems) or because of the rarity or disappearance of the species in its natural habitat (LEVEQUE, 1994).

The goal of these collections is to allow the description and the classification of all living and fossil organisms. Classically, the use of these collections was apparent only with morphological, morphometrical or anatomical approaches. Only recently has the molecular exploitation of these collections been envisaged (HIGUCHI *et al.*, 1984; THOMAS *et al.*, 1989), giving them a new dimension. In fact, the *in vitro* chained amplification technique or PCR (MULLIS

and FALOONA, 1987; SAIKI *et al.*, 1988) allows the study of a DNA sequence from a very small quantity of genetic material. The strength of this technique suggests the ability to study rare and/or damaged DNA like that found in fossils or of tissues preserved in museums (ancient DNA, *s.l.*).

After having reviewed the advantages and difficulties of utilizing the DNA of collection specimens during the comparative biology study, and more particularly of those fixed in formaldehyde, we present our results obtained from specimens of the Mormyridae family preserved at the Musée royal d'Afrique centrale (Mrac) in Tervuren and at the Muséum national d'histoire naturelle (MNHN) in Paris.

DNA studies from formaldehyde-fixed tissues

Why use DNA from formaldehyde-fixed tissues ?

The use of DNA from collection specimens may be an answer to the sampling problems encountered in many comparative biology studies, particularly those using molecular biology techniques (LECOINTRE, 1993; WHEELER, 1992). For example, GAUTHIER *et al.* (1988), showed the importance of fossils on the phylogeny of Amniotes and were therefore obliged to modify the phylogeny proposed by GARDINER (1982).

The study of the genetic structures of populations and of the processes of speciation could benefit from a spatial-temporal dimension thanks to the use of ancient individuals whose capture dates and locations are noted in collection registers. Several works (THOMAS *et al.*, 1990; WAYNE *et al.*, 1991; HARDY *et al.*, 1994) have shown the interest of such an approach.

Likewise, the introduction of individuals from exogenous populations of a species into an area could lead to a genetic modification of a local population by introgression. The molecular analysis of specimens captured prior to the introduction and preserved in collections would allow the determination of the degree of introgression. This is even more important considering that the introduction of new species or of geographically distinct populations is quite common, especially in the teleosts (WELCOMME, 1988).

Generally speaking, all types of population genetics studies can benefit from the study of this DNA.

Fixation conditions influence DNA extraction and amplification

Studies of molecular biology exploit two types of samples from which ancient DNA can be extracted (*s.l.*), fossils and animals that have been subjected to a preservation treatment after their deaths and stored in zoological collections. HERMANN and HUMMEL (1993) reviewed the entire body of work on fossil DNA. For animals from zoological collections, there are basically two types of preservation techniques: either by drying (naturalized) or preserving in a liquid medium (generally 70% ethanol) after having been fixed in formaldehyde (diluted to 4-10%, buffered or not, from a few hours to several days). Formaldehyde is the most commonly used fixative because of its ease of use (available in large quantities and not expensive).

The preservation technique chosen is based partially on the lifestyle of the animal. Therefore, terrestrial vertebrates and invertebrates are almost always dried, while aquatic vertebrates especially the teleosts, are preserved in liquid, size permitting. Therefore, the use of ichthyological collections in molecular biology must first pass through the development of techniques capable of rendering the DNA of these formalin-fixed specimens usable.

There is a certain number of studies where part of the sampling has been taken from dry tissues found in museums, such as skin,

feathers or bones (HOUDE and BRAUN, 1988; COOPER *et al.*, 1992; HIGUCHI *et al.*, 1984; THOMAS *et al.*, 1989, 1990; WAYNE *et al.*, 1991; PAABO, 1989; PAABO *et al.*, 1988; etc). The difficulty of extracting and amplifying the DNA from formalin-treated samples is reflected in the lack of work on the subject. If we exclude the successes observed in medicine on tissues of human origin preserved after having been fixed for a very short time (a few hours), carefully washed and included in paraffin (TSAI and O'LEARY, 1993; FITZGERALD *et al.*, 1993; SHIBATA *et al.*, 1991), in 1997 and in spite of certain methodological studies (CRISCUOLO, 1992, on reptiles; DE GIORGI *et al.*, 1994, on nematodes; VACHOT and MONNEROT, 1996, on amphibians), no molecular phylogeny whose sampling includes formalin-fixed specimens has been published. In teleosts, to our knowledge, only the work of SHIOZAWA *et al.* (1992) on a subspecies of trout (Salmonidae) has been published. However, the results obtained must be confirmed.

The quality (DNA fragmentation and successful amplification by PCR) and the quantity of DNA extracted are subject to several factors related to the fixation (GOEBEL and SIMMONS, 1993; VACHOT and MONNEROT, 1996). These factors are the formalin concentration, the pH and the temperature of the fixing solution (KOSHIBA *et al.*, 1993), the duration of the fixation (HAMASAKI *et al.*, 1993; KARLSEN *et al.*, 1994), the age of the collections (VACHOT and MONNEROT, 1996). These different parameters act upon the size of fragments recovered (and thus on the size of fragments that can be amplified) but also on the quantity of DNA extracted (GOEBEL and SIMMONS, 1993; VACHOT and MONNEROT, 1996). Also, the action of the formalin causes the creation of covalent bonds between certain proteins and the DNA, especially with certain histones (BRUTLAG *et al.*, 1969; JACKSON, 1978; KOSHIBA *et al.*, 1993), and the creation of bonds within the DNA molecule. The fragmentation of the DNA molecule is an irreversible process, whereas the creation of bonds between proteins and the DNA within the DNA molecule can be a reversible phenomenon. The fixative can also induce modifications in the sequence (PAABO, 1985; DE GIORGI *et al.*, 1994). If the mechanisms of the formation of these bonds are still unknown and under discussion, the

fragmentation of the DNA is caused by the acidification of the medium, induced by the oxidation of the formalin into formic acid, that acidification being greater still if the formalin is not buffered. It should be noted that even a non-oxidized solution of formalin is acidic. These structural modifications of the DNA limit the size of the amplified fragment to a maximum of a few hundred pairs of bases.

Faced with these problems, some authors have proposed new methods of preservation. GOEBEL and SIMMONS (1993) reviewed the different techniques of preservation, and proposed alternative methods to preserve both the morphology of the animal and its DNA. Their works view the problem from the angle of preserving fragments of maximum size. However, no amplification by PCR was made with the fragments to test these methods. Also, they proposed no particular extraction protocol. VACHOT and MONNEROT (1996), proposed both new preservation techniques and an extraction protocol applicable to amphibian specimens already stored in collections.

Because of the poor quality and the small quantity of DNA extracted and of the extreme sensitivity of PCR, the tissue sampling, the extraction and the amplification are all more susceptible to contamination than in the case of work involving fresh DNA. When working with formalin-fixed DNA, appropriate anti-contamination measures must be taken (HUMMEL and HERMANN, 1993). The measures recommended for avoiding all contamination are not specific to formalin-fixed DNA, but are necessary whenever extracting any type of ancient DNA. A dedicated room is necessary, reserved for pre-PCR manipulations of ancient DNA and equipped with a laminar flow hood with a UV sterilization unit. All equipment used there in must be specific to the room. Technicians should not have been at work in any other labs before entering. Containers and solutions must have been autoclaved and then UV treated for 15 minutes. Also, control samples must be used at every step. The extraction controls allow the determination of the extent of contamination at the extraction phase. They undergo the same treatments as the sample to be extracted but contain no tissue. The first control is processed as the first sample and the second control is

processed as the last sample. The first control reveals contamination from the environment, the second reveals contamination from the other samples. The other controls during the amplification are not specific to the DNA. Because of these contamination problems, the use of one or two probes reserved for the group being studied to amplify the desired fragment is indispensable.

Difficulties to obtain preserved tissues

Besides the technical difficulties posed by the use of this type of material in molecular biology, there is also the problem of the availability and accessibility of material from collections which are rare and limited in quantity. THOMAS (1994) list five criteria to help make the decision to destroy a sample from a museum collection for use in molecular studies: 1) Scientific value and feasibility of the study; 2) Qualifications of the researcher and/or laboratory to undertake this research; 3) Availability of samples from living populations (GRAVES and BRAUN, 1992); 4) Volume of material already taken from the collection in relation to the request; and 5) Efforts of museum personnel to satisfy the request. Within the framework of the fourth criterion, it seems obvious in the case of certain rare or reference specimens not to take material for molecular studies, nor to destroy a specimen for anatomical studies. In the case of fishes, collections are often very rich in numbers of individuals; for example, there are almost a million specimens of African fishes at Musée Royal de l'Afrique Centrale at Tervuren. Therefore it is often possible to obtain samples for the majority of species. It is also important to sample in such a way that the specimen is the least damaged to allow future morphological/anatomical studies. A small sampling of dorsal muscle does minimal damage. Lastly, THOMAS (1994) suggests returning the remaining DNA sample to the museum, which for but is not always reasonable, because not all museums are equipped to store DNA samples.

Example: Phylogenetic relationships in family Mormyridae

The Mormyridae (Teleostei; Osteoglossomorpha) are African fishes found only in fresh water. They are found in virtually every freshwater environment from the Sahara to Northern South Africa (including the Nile basin). The Mormyridae family has 17 genera and about 200 species (NELSON, 1994; GOSSE, 1984). They possess the ability to emit (and to receive) weak electrical signals due to the presence of muscular electrical organs in the caudal peduncle (HOPKINS, 1986). Because of this particularity they have been the major subjects of physiological and electrophysiological studies (MOLLER, 1995). To understand the formation and evolution of their electrical organs, there must first be well defined hypotheses on their phylogenetic relationships. From an osteological analysis, TAVERNE (1992) was the first to propose phylogenetic hypotheses for this family. AGNESE and BIGORNE (1992) and VAN DER BANK and KRAMER (1996) studied four and five genera respectively by enzymatic polymorphism. ALVES GOMES and HOPKINS (1997) studied one genus, *Brienomyrus*.

Within this study, we sequenced a fragment of mitochondrial DNA cytochrome *b* from two specimens coming from the Musée Royal de l'Afrique Centrale at Tervuren (MRAC) collection. We also sequenced four genera available fresh in order to be able to discuss the validity of the sequences coming from formalin-fixed specimens based on their phylogenetic positions.

Material and methods

The origin of the fixed specimens used in this study are *Boulengeromyrus knoepffleri* from Makokou, Ivindo, Gabon in 1975, MRAC (75-24-P1-2) ; *Paramormyrops gabonensis* from Makokou, Ivindo, Gabon in 1975, MRAC (75-24-P7-13) ; *Myomyrus pharao* from Kisangani, Zaire, Zaire in 1980, MRAC

(82-25-P32-45) ; *Genyomyrus donnyi* Kisangani, Zaire, Zaire in 1980 MRAC (83-31P-39-40) ; *Stomatorhinus walkeri* from Route Loubomo, Loukénéné, Gabon MRAC (91-79-P-99-113) ; *Ivindomyrus opdenboschi* from Ma'an, Ntem, Cameroon MRAC (93-82-P-2) ; *Myomyrus macrops* from Epula, Epula, Zaire in 1986 MRAC (91-79-P405-416) ; *Mormyrops zanclirostris* from Loa-Loa, Ivindo, Gabon in 1964 MNHN (1987-897) ; *Isichthys henryi* from Marela, mongo, Guinea in 1986 MNHN (1986-525) ; *Stomatorhinus corneti* from Ybiegn, Nyabarélé, Gabon in 1964 MNHN (1987-910). The origin of the fresh specimens used in this study are *Marcusenius senegalensis* from Batamani, Niger, Mali in 1994 ; *Petrocephalus bovei* from Batamani, Niger, Mali in 1994 ; *Gnathonemus petersii* from Aquarium import ; *Mormyrops zanclirostris* Makokou, Ivindo, Gabon in 1997 and *Heterotis niloticus*, Bia, Côte d'ivoire in 1996.

For each individual, 1 cm^3 of muscle was taken from the dorsal position, and placed in 70°C ethanol. For the specimens coming from collections, no information about preservation techniques could be supplied. The extraction method used on samples from fresh fish was that of WINNPENNINCKX *et al.* (1993). The extraction method used for collection specimens was similar to that developed by VACHOT and MONNEROT (1996) for amphibians. The muscle sample was washed four times with TE pH=8 (SAMBROOK *et al.*, 1989) and left to incubate in the extraction solution (proteinase K 0.8 mg/ml, SDS 2%, EDTA 10 mM, Tris HCl pH=8 100 mM, 50 mM DTT, 100 mM NaCl (VACHOT and MONNEROT, (1996)) for 72 hours at 50°C with slight agitation. Every 12 hours the concentration was increased by 0.7 mg/ml of proteinase K. After digestion, the DNA is extracted by a classical phenol-chloroform method. The DNA was precipitated with two parts by volume of 100 % ethanol in the presence of 0.2 M NaCl for one night at -20°C. After centrifuging, the pellet was rinsed twice with 70 % ethanol. The extract obtained was collected in a volume of 20 µl of twice-distilled water where 10 µl were used to estimate the quantity and the quality of the DNA extract (migration on 1% agarose gel, visualization using ethidium bromide under ultraviolet light).

Two primers were used to amplify a 495 bp fragment from the 3' terminal part of the cytochrome *b*. One of these primers, L15930 was described by KOCHER *et al.* (1989). The other primer L'195 (5'-GAA-ACC-GGM-TCA-AAC-AAC-CC-3') was developed specifically for this study. It was first tested on DNA extracts from fresh fish tissues, Mormyridae, Perciformes and Siluriformes, to ensure its specificity. Amplifications were made using a « crocodile II » thermocycler (Appligene). They were performed in a volume of 50 µl (34.2 µl of water, 2.5 µl of DMSO, 5 µl of MIX 5 (2.15 mM of each dNTP), 1 µM of each probe, 1 µl of genomic DNA, 5 µl of buffer 10X and 2 U of Taq polymerase (Hitaq, Prolabo)). The first cycle was 1mn at 94°C, 1 mn at 54°C and 2 mn at 72°C. Finally a last cycle of 4 mn at 72°C was performed. Five µl of amplified product were set to migrate on 1% agarose gel in the presence of a length marker. The amplified fragments of satisfactory size were then cloned using a pCR-Script(+) cloning kit (Stratagene). The sequencing was performed using the method of SANGER *et al.* (1977) with the help of a T7 Sequencing™ Kit (Pharmacia Biotech). Sequence recording was performed using the MUST program (PHILLIPE, 1993). Analysis using the Distance Neighbor Joining method (SAITOU and NEI, 1987) was also performed with this program. Parsimony analysis was carried out using the PAUP 3.1.1. program (SWOFFORD, 1993). Tree robustness was estimated by the bootstrap method (FELSENSTEIN, 1985) using PAUP with 100 replicates and branch length.

Results and discussion

Extractions and amplifications DNA from fresh and fixed tissues

During this study a fragment of the 3' part of cytochrome *b* of 589 bp was amplified and sequenced for four species of Mormyridae freshly available: *Mormyrops zanclirostris*, *Petrocephalus bovei*, *Marcusenius senegalensis*, *Gnathonemus petersii*, and an extra group, *Heterotis niloticus*.

We extracted total DNA from ten samples of muscular tissue collected from individuals fixed in formalin and preserved in the collections of the Mrac and the MNHN. From these extracts, the quality of DNA was visualized on 1.5% agarose gel under ultraviolet light with BET. In all cases the DNA was degraded. The size of fragments was between 100 and 2000 bp. There seemed to be no obvious relationship between the mean size of fragments obtained and the age (duration) of the specimens in the collection.

From these extracts we tried to amplify using PCR a fragment of 495 bp of the mitochondrial gene coding for cytochrome *b* in the 3' part. One of the two primers used was specific to Mormyidae, in order to avoid some of the threat of contamination. The second primer was universal (KOCHER *et al.*, 1989). The PCR program was slightly modified from that used for samples of fresh tissue (JACKSON *et al.*, 1991). The time of hybridation and elongation were both extended (1 mn 30 instead of 1 mn). Two positive amplifications were obtained from 10 extracts. These two specimens were of two different species, *Genyomyrus donnyi* and *Myomyrus pharao*, preserved in the MRAC collection since 1980.

The two positive amplifications come from these two specimens which were collected during the same mission. Their fixation conditions were similar, and no doubt conducive to the proper preservation of the DNA. Access to the fixation parameters, knowing which type of formalin was used (buffered or not), its concentration, its fixation period, would allow a selection of suitable specimens whenever possible. Unfortunately, this information is never available with specimens in the collections. It has been shown that the greater the size of the fragment to be amplified, the harder it is to amplify it. Classically, it has been recommended to work with fragments less than 500 bp. In this study, the size of the fragment was fairly large (495 bp), but we have not better results with short fragments.

Authenticity of sequences

After treating the amplified fragment, the two sequences were determined and compared to sequences belonging to different Mormyridae species.

All the controls were negative. For *Myomyrus pharao*, we amplified and sequenced the piece of cytochrome *b* twice from two different extractions. The two sequences were identical. The alignment of the sequences of *Genyomyrus donnyi* and *Myomyrus pharao* to those from fresh tissues posed no difficulties. No insertion or deletion were detected. The majority of substitutions were found in the third positions of the codons, which is in agreement with a degeneration of the genetic code. After translation of the sequences into amino acids, no stop codon was found.

In order to validate the sequences by their respective position within the Mormyridae, the sampling for the comparison was determined on several systematic levels: the extra-Mormyridae level (with *Heterotis niloticus*); the Petrocephalinae versus Mormyridae level (with *Petrocephalus bovei*) and the intra-Mormyrinae level (with *Mormyrops zanclirostris*, *Gnathonemus petersii* and *Marcusenius senegalensis*). Numerous morpho-anatomic and physiological characters derived support the validity of each of these levels. The Mormyridae and the Mormyrinae are considered to be monophyletic, these last are grouped as closely related to the Petrocephalinae (TAVERNE ,1972; VAN DER BANK and KRAMER, 1996 (electrophoretic data); ALVES GOMES and HOPKINS, 1997).
After having eliminated the transitions, we obtained by the research option « Branch and Bound » from PAUP 3.1.1., two trees of a minimal length of 107 steps (CI=0.832, RI=0.635). They differed only by the position of *Genyomyrus donnyi*: 1) either *Genyomyrus donnyi* is closely related to *Gnathonemus petersii* (grouping supported by 2 synapomorphies and a bootstrap value of 51%, figure 1); or *Genyomyrus donnyi* is closely related to *Marcusenius senegalensis* (grouping supported by only one synapomorphy and a bootstrap value of 41%). The group formed by *Genyomyrus donnyi*, *Gnathonemus petersii* and *Marcusenius senegalensis* is confirmed by a very high bootstrap value (94%). *Mormyrops zanclirostris* is closed to this group. *Mormyrus pharao* is in a basal position within the Mormyrinae. The Mormyrinae ensemble is supported by a bootstrap value of 83%. The Petrocephalinae (*Petrocephalus bovei*) are sister group of the Mormyrinae. By the Neighbor-Joining method, the topology of the tree is the same as that where *Genyomyrus donnyi* is closely related to *Gnathonemus petersii* (confirmed by a bootstrap value of 67%).

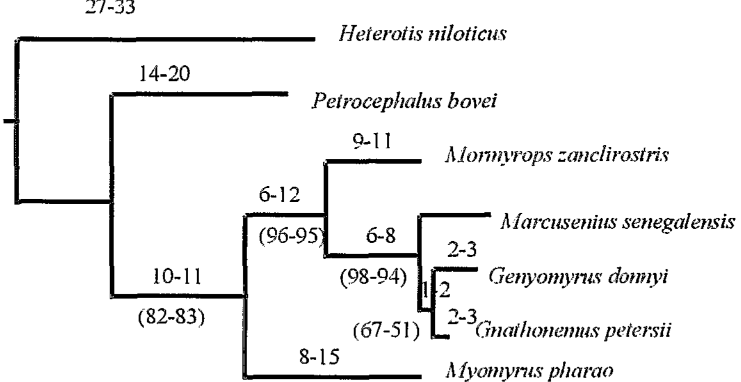


Figure 1
One of two most parsimonious tree obtained from a branch and bound search of PAUP (Swofford, 1993). Transitions are excluded from the analysis. Length = 107 steps. C.I.= 0.822, R.I.= 0.635. Branch lengths are proportional to the number of changes occurring along the branches under Acctran optimization. Numbers above branches are minimal and maximal branch lengths according to the optimization of homoplasies. Numbers below branches are bootstrap proportions obtained from 100 replicates using PAUP and NJ.

The position of *Genyomyrus donnyi*, close to *Gnathonemus senegalensis*, is congruent to that established by osteological data (TAVERNE, 1972). These two genera resemble each other a great deal, showing numerous derived character and therefore naturally very close.

The position of *Myomyrus pharao* is not in total agreement with the morpho-anatomical data. While it is a Mormyrinae, its grouping among them and despite the fact that the sampling was not sufficient, differs from that defined by TAVERNE in 1972 which grouped *Myomyrus*, *Isichthys* and *Mormyrops*. However, Taverne did not support this grouping by derived characters, he merely underlined the similarities between these three genera.

Even if we therefore cannot totally exclude contamination by a close species or the presence of a nuclear pseudo-gene whose divergence time is recent based on its weak argumentation, we still support the authenticity of the sequence. Even if the best proof of the

authenticity of the sequences obtained is not available, that of a comparison to the sequence of a fresh specimen, we are confident of this one. In fact, it is a cytochrome *b* sequence, whose systematic position in our sampling is not inconsistent with data acquired in morpho-anatomy.

Conclusion

To our knowledge, this is the first time that DNA sequences obtained from formaldehyde-fixed Teleost specimens have been used in a phylogenetic analysis. The extraction method used (VACHOT and MONNEROT, 1996, slightly modified) allows extraction of the DNA which is not always amplifiable. We believe that the fixation conditions play an important role in the results obtained. We confirm that the Petrocephalinae are sister group of the other Mormyrinae. Within the Mormyrinae and despite the fact that our sampling was incomplete, *Myomyrus pharao* is closely to the rest of the Mormyrinae. *Genyomyrus* is sister group to *Gnathonemus*.

Acknowledgement
Authors thanks Guy Teugels (Mrac) Guy Duhamel (MNHN) and Rémy Bigorne for samples collection and Anne-Marie Vachot and Véronique Barriel for critical review of this manuscript. This work was made possible thanks to funding from Orstom l'Institut français de recherche scientifique pour le développement en coopération)

Reference

AGNESE (J.F.), BIGORNE (R.), 1992 — Premières données sur les relations génétiques entre onze espèces ouest-africaines de Mormyridae (Teleostei, Osteichthyes). *Rev. Hydrobiol. trop.*, 25 : 253-261.

ALVES-GOMES (J.), HOPKINS (C.D.), 1997 — Molecular insights into the phylogeny of mormyriform fishes and the Evolution of their electric organs. *Brain, Behav. Evol.*, 49 : 324-351.

BRUTLAG (D.), SCHLEHUBER (C.), BONNER (J.), 1969 — Properties of formaldehyde-related nucleohistone. *Biochemistery*, 8 (8) : 3214-3218.

COOPER (A.), MOURER-CHAUVIRE (C.), CHAMBERS (G.K.), ARNDT VON HAESELER, WILSON (A.C.), PÄÄBO (S.), 1992 — Independent origins of New Zealand moas and kiwis. *Proc. Natl. Acad. Sci. USA.*, 89 : 8741-8744.

CRISCUOLO (G.), 1992 — Extraction and amplification of DNA from wet museum collections. *Ancient DNA Newsletter*, 1 : 12-13.

DE GIORGI (C.), SIALER (M.F.), LAMBERTI (F.), 1994 — Formalin-induced infidelity in PCR-amplified DNA fragments. *Mol. Cell. Probes*, 8 : 459-462.

FELSENSTEIN (J.), 1985 — Confidence limits on phylogenies : An approach using the bootstrap. *Evolution*. 39(4) : 783-791.

FITZGERALD (L.M.), YIN (H-Z.), KIESSLING (A.A.), 1993 — PCR amplification of HIV and cellular DNA sequences in formaldehyde-fixed, Immunoreactive white blood cells. *Biotech.*, 15 (1) : 128-133.

GARDINER (B.G.), 1982 — Tetrapod classification. *Zool. J. Linnean Soc.*, 74 : 207-232.

GAUTHIER (J.), KLUGE (A.G.), ROWE (T.), 1988 — Amniote phylogeny and the importance of fossils. *Cladistics*, 4 : 105-209.

GOEBEL (A.M.), SIMMONS (J.E.), 1993 — Preserving DNA in liquid-preserved museum specimens *In* SNYDER (A.M.), ed., *The 1992 American Society of Ichthyologists and Herpetologists Workshop on Collections Care and Management Issues*, 52 : 34-52.

GOSSE (J.P.), 1984 — Mormyridae. *In* DAGET (J.), GOSSE (J.P.), THYS VAN DEN AUDENAERDE (D.F.E.), éd, *Cloffa: Catalogue of the Freshwater Fishes of Africa.* Orstom et Mrac. 63-124.

GRAVES (G.R.), BRAUN (M.J.), 1992 — Museums: storehouse of DNA? *Sciences*, 255 : 1335-1336.

HAMASAKI (S.), KOSHIBA (M.), HABUCHI (T.), TAKAHASHI (R.), SUGIYAMA (T.), 1993 — The effect of formalin fixation on restriction endonuclease digestion of DNA and PCR Amplification. *Path. Res. Pract.*, 189 : 553-557.

HARDY (C.), VIGNE (J.D.), CASANE (D.), DENNEBOUY (N.), MOUNOLOU (N.), MONNEROT (M.), 1994 — Origin of european rabbit (*Oryctolagus cunniculus*) in a mediterranean island: zooarchaeology and ancient DNA examination. *J. Evol. Biol.* 7 : 217-226.

HERMANN (B.), HUMMEL (S.), éds, 1993 — Ancient DNA. pp263.

HIGUCHI (R.), BOWMAN (B.), FREIBERGER (M.), RYDER (O.A.), WILSON (A.C.), 1984 — DNA sequences from the quagga, an extinct member of the horse family. *Nature*, 312 : 282-284.

HOPKINS (C.D.), 1986 — Behavior of Mormyridae. *In* BULLOCK (T.H.), HEILIGENBERG (W.), eds : Electroreception. John WILEY and Sons. 527-576.

HOUDE (P.), BRAUN (M.J.), 1988 — Museum collections as a source of DNA for studies of avian phylogeny. *Auk*, 105 : 773-776.

HUMMEL (S.), HERMANN (B.), 1993 — General aspects of samples preparation. In HERMANN (B.), HUMMEL (S.), eds : *Ancient DNA.*

JACKSON (D.P.), HAYDEN (J.D.), QUIRKE (P.), 1991 — Extraction of nucleic acid from fresh and archival material. *In* MC PHERSON

(M.J.), QUIRKE (P.), TAYLOR (G.R.), eds, *PCR, a pratical approach*. 29-50.

JACKSON (D.P.), 1978 —
Studies on histone organization in the nucleosome using formaldehyde as a reversible cross-linking agent. *cell*, 15 : 945-954.

KARLSEN (F.), KALANTARI (M.), CHITEMERERE (M.), JOHANSSON (B.), HAGMAR (B.), 1994 —
Modifications of human and viral deoxyribonucleic acid by formaldehyde fixation. *Lab. Invest.* 71 (4) : 604.

KOCHER (T.D.), THOMAS (W.K.), MEYER (A.), EDWARDS (S.V.), PÄÄBO (S.), VILLABLANCA (F.X.), WILSON (A.C.), 1989 —
Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA*, 86 : 6196-6200.

KOSHIBA (M.), OGAWA (K.), HAMASAKI (S.), SUGIYAMA (T.), OGAWA (O.), KITAJIMA (T.), 1993 —
The effect of formalin fixation on DNA and the extraction of high-molecular-weight DNA from fixed embedded tissues. *Path. Res. Pract.*, 189 : 66-72.

LECOINTRE (G.), 1993 —
Etude de l'impact de l'échantillonnage des espèces et de la longueur des séquences sur la robustesse des phylogénies moléculaires. Implications sur la phylogénie des Téléostéens. Thèse de doctorat, université Paris VII.

LEVEQUE (C.), 1994 —
Introduction générale : Biodiversité des poissons africains. In Biological diversity in african fresh- and brackish water fishes. Geographical overviews-Symposium PARADI. TEUGELS (G.G.), GUEGAN (J.F.), ALBARET (J.J.). *Ann. Mus. R. Afr. Centr.*, Zool., 275 :7-16.

MOLLER (P.), 1995 —
Electric Fishes: History and Behavior. Chapman and Hall, London. p 584.

MULLIS (K.B.), FALOONA (F.A.), 1987 —
Specific synthesis of DNA *in vitro via* a polymerase catalysed chain reaction. *Methods Enzymol.*, 155 : 335-350.

NELSON (J.S.), 1994 —
Fishes of the world. Third edition. John Wiley and Sons, Chichester, New york. p 600.

PÄÄBO (S.), 1985 —
Molecular cloning of ancient egyptian mummy DNA. *Nature*, 314 : 644-645.

PÄÄBO (S.), GIFFORD (J.A.), WILSON (AC.), 1988 —
Mitochondrial DNA sequences from a 7000-year old brain. *Nucl. Acids Res.*, 16 : 9775-9787.

PÄÄBO (S.), 1989 —
Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. *Proc. Natl. Acad. Sci. USA*, 86 : 1939-1943.

PHILIPPE (H.), 1993 —
MUST : A computer package of Management Utilities for Sequences and Trees. *Nucl. Acids. Res.*, 21 (22) : 5264-5272.

SAIKI (R.K.), GELFAND (D.H.), STOFFEL (S.), SCHARF (S.J.), HIGUCHI (R.), HORN (G.T.), MULLIS (K.B.), ERLICH (H.A.), 1988 —
Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 239 : 487-491.

SAITOU (N.), NEI (M.), 1987 —
The Neighbor-joining Method: A New method for reconstruting phylogenetic trees. *Mol. Biol. Evol.*, 4 (4) : 406-425.

SAMBROOK (J.), FITSCH (E.F.), MANIATIS (T.), 1989 —
Molecular Cloning: A laboratory manual, 2d Edition. 3 vol. Cold Spring Harbor Laboratory, New-York.

SANGER (F.), NICKLEN (S.), COULSON (A.R.), 1977 —
DNA sequencing with chain-

terminating inhibitors. *Proc. Natl. Acad. Sci. USA*, 74 (12) : 5463-5467.

SHIBATA (D.), KUROSU (M.), NOGUSHI (T.T.), 1991 —
Fixed Human Tissues: A Resource for the Identification of individuals. *J. Forensic Sci.*, 36 (4) : 1204-1212.

SHIOZAWA (D.K.), KUDO (J.), EVANS (R.P.), WOODWARD (S.R.), WILLIAMS (R.N.), 1992 —
DNA extraction from preserved trout tissues. *Great Basin Naturalist*, 52 (1) : 29-34.

SWOFFORD (D.L.), 1993 —
PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1.1. Illinois Nat. Hist. Surv., Champaign, Illinois

TAVERNE (L.), 1972 —
Ostéologie des genres *Mormyrus* Linné, *Mormyrops* Müller, *Hyperopisus* Gill, *Myomyrus* Boulenger, *Stomatorhinus* Boulenger et *Gymnarchus* Cuvier. Considérations générales sur la systématique des Poissons de l'ordre des Mormyriformes. *Annales du Musée royal de l'Afrique centrale. Série in-8°. Sci. Zool.* 200, 194.

THOMAS (R.H.), 1994 —
Molecules, museums and vouchers. *Tree*, 9 (11) : 413-414.

THOMAS (R.H.), SCHAFFNER (W.), WILSON (A.C.), PÄÄBO (S.), 1989 —
DNA phylogeny of the extinct marsupial wolf. *Nature*, 340 : 465-467.

THOMAS (W.K.), PÄÄBO (S.), VILLABLANCA (F.X.), WILSON (A.C.), 1990 —
Spatial and temporal continuity of kangaroo rat populations shown by sequencing mitochondrial DNA from museum specimens. *J. Mol. Evol.*, 31 : 101-112.

TSAI (M.), O'LEARY (T.J.), 1993 —
Identification of *Toxoplasma gondii* in formalin-fixed, paraffin-embedded tissue by polymerase chain reaction. *Methods in Pathology*, 6 (2) : 185-188.

VACHOT (A.M.), MONNEROT (M.), 1996 —
Extraction, Amplification and sequencing of DNA from formaldehyde-fixed specimens. *Ancient biomolecules*, 1 : 1-20.

VAN DER BANK (F.H.), KRAMER (B.), 1996 —
Phylogenetic relationships between eight African species of mormyriform fish (Teleostei, Osteichthyes): resolution of a cryptic species and reinstatement of *Cyphomyrus* Myers, 1960. *Biochem. Syst. Ecol.* (In press).

WAYNE (R.K.), GILBERT (D.A.), LEHMAN (N.), HANSEN (K.), EISENHOWER (A.), GIRMAN (D.), PETERSON (R.O.), MECH (L.D.), GOGAN (P.J.P.), SEAL (U.S.), KRUMENAKER (R.J.), 1991 —
Conservation genetics of the endangered Isle Royal Gray wolf. *Conservation Biology*, 5 : 41-51.

WELCOMME (R.L.), 1988 —
International introductions of inland aquatic species. FAO Fisheries Technical Paper No. 294, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy, 318 pp.

WHEELER (W.C.), 1992 —
Extinction, sampling, and Molecular Phylogenetics. In NOVACEK (M.J.), WHEELER (Q.D.), eds, *Extinction and phylogeny.* Columbia University Press, New York. p 205-215.

WINNPENNINCKX (B.), BACKELJAU (T.), DE WACHTER (R.), 1993 —
Extraction of high molecular weight DNA from molluscs. *T. I. G.* 9 (12) : 407.

Natural hybridization in tilapias

Jean-François Agnèse
Geneticist

Béatrice Adépo-Gourène
Geneticist

Laurent Pouyaud
Geneticist

Introduction

Tilapia species are well known for their abilitiy to hybridize in captivity, and a large number of crosses have been carried out. Quite often, the F1 are viable but the sex ratio of the descendants is unbalanced. For example, the crossing of a female *Tilapia tholloni* and a male *Oreochromis mossambicus* yields 100% females where the crossing of a female *O. spilurus* and a male *O. leucostictus* yields 98% males. Sometimes the F1 are sterile but they can also be equally fertile. Under natural conditions there are numerous cases of hybridization. However, these hybridizations are not always easily observed and proven. Since the development of genetic techniques, the characterization of tilapia species has not ceased to advance and it is easier today to prove the existence of natural tilapia hybrids.
These natural tilapia hybrids can be classified in three categories: those following species' introduction, those following manmade perturbations of the environment and those which are truly natural
This classification is no doubt purely artificial because the mechanisms occurring during hybridization are probably always the same. One or two factors leads to the rupture of the ethological

barriers between species (when they existed), then competition phenomena between the parent species and their hybrids help the situation to evolve, often, but not always in favor of one of the forms of the parental species.

Hybridization following species' introduction

The majority of wild hybridization cases reported belong to this first category. In certain cases an introduced species hybridizes with a local species, in others, two introduced species hybridize in a new environment.

In Lake Naivasha, *O. spilurus nigra* and *O. leucostictus* were probably introduced in the 1950's. In the beginning of the 1960's, many hybrids were observed with meristic and anatomic characteristics somewhere between those of the parent species. In the 1970's, a few rare hybrids were observed (SIDDIQUI, 1977, 1979) and *O. spilurus nigra* seemed to have disappeared from the lake.

In Lake Bunyoni in Uganda, *O. niloticus* was introduced in 1927 from Lake Edward, *O. spilurus nigra* in 1932 from Lake Naivasha. In 1937 hybrids between the two species were harvested and from 1947 onwards, *O. spilurus nigra* as a pure species had disappeared (LOWE, 1958).

In Lake naivasha in Kenya, *O. spilurus nigra* was introduced in 1925 and later *O. leucostictus* was also introduced (ELDER *et al.*, 1971). In 1959, the first hybrids between the two species were harvested. In 1972, *O. spilurus* have disappeared from the lake (SIDDIQUI, 1979).

In Lake Itasy, in Madagascar, *O. macrochir* was introduced in 1958 and *O. niloticus* in 1961. In 1965 and 1966 intermediate specimens between these two species were harvested and named 3/4

tilapia (DAGET and MOREAU, 1981). These hybrid individuals had a noticeable pharyngial bone resembling that of *O. niloticus* but a morphology closer to that of *O. macrochir*. Between 1963 and 1969, the hybrid population in the captures went from 5% to 74%. *O. macrochir* was considered a vanished species in 1971. Finally, the *O. niloticus* became predominant.

On the contrary, in Lake Ihema in Rwanda, *O. macrochir* was introduced near the end of the 1960's, after the introduction of *O. niloticus* in the 1940's. Hybrids were observed in the 1970's. From 1983 to 1987, the proportion of *O. niloticus* decreased from 30 to 20%, that of hybrids increased from 10 to 20 %, the *O. macrochir* population remained stable at 60% (MICHA *et al.*, 1996).

In the 1950's, *O. niloticus* was introduced into Lake Victoria several times (TREWAVAS 1983). The introduction occurred in Uganda with individuals from Lake Edward (BALIRWA, 1992) and numerous stockings took place later. TREWAVAS (1983) believes that even fish from Lake Turkana raised in Uganda were introduced into the lake. During the 1960's, we started to find this species in the fisheries statistics (WELCOMME, 1967; BALIRWA, 1992). WELCOMME (1967), in the mid-1960's described the existence of hybrids between *O. niloticus* and *O. variabilis*. These hybrids were all males. Hybridization with *O. esculentus* were also suspected by other authors. Since then these two species have disappeared from Lake Victoria and *O. niloticus* is suspected of being the cause of these disappearances (WELCOMME, 1967; OGUTU-OHWAYO, 1990). In this particular lake we have begun to compile some genetic data.

Hybridizations following environmental disturbances

Tilapia zillii and *T. guineensis* are two fish species of the subgenus *Coptodon* (THYS VAN DEN AUDENAERDE, 1970) and are genetically very close (POUYAUD and AGNESE, 1995). *Tilapia zillii* and

T. guineensis are present in West African rivers and streams but, while *T. zillii* frequents the upper waters, we find *T. guineensis* in the lower parts of rivers and in the lagoons. Nevertheless, *T. guineensis* is capable of climbing the rivers, sometimes over hundreds of kilometers. Therefore these two species can be considered as sharing a common range. For these two species, we are fortunate to have the genetic analysis of a great many populations: Among the 33 populations studied by POUYAUD (1993), mostly in rivers, no hybrids or gene introgressions were observed in the 25 West African basins studied, except in two places in Côte d'Ivoire. As a result of this work, a more in-depth study was begun (AGNESE and col.) covering the entire territory of Côte d'Ivoire.

Lake Ayamé is an artificial lake built in the 1950's on the Bia river. Observation of captures taken from the lake shows the rarefaction, even the disappearance of two tilapia species: *T. busumana* and *T. discolor*. Another tilapia species, *Sarotherodon melanotheron* was introduced a few years ago. It now represents the largest part of the total biomass harvested. All these phenomena result in a certain number of disturbances which need to be identified and the origins of which can be assumed to be manmade for the most part.

To the samples from the Bia River were added samples from Adzopé, the Aby Lagoon, the Ebrié Lagoon, Bouaké, Lake Kossou, Lake Taabo, Man, Lake Buyo, Lake Dagou, the Valoa River, and from Sassandra (fig. 1).

Concerning Lake Ayamé, the rivers which flow into the lake (including the Bia), are dried up in the dry season (at least in March and April). We must assume that the fish populations of the rivers are temporary and come from individuals from the lake. The situation below the dam is somewhat similar. When the hydroelectric plant does not function for a period of time, the river dries up for several kilometers. Among the loci studied, two are diagnostic loci, which means that the alleles present in one species are all different from those present in the other. These are PGI2 (*T. guineensis* is characterized by allele 100 and *T. zillii* by allele 108) and LDH1 (100 in *T. guineensis* and 250 in *T. zillii*).

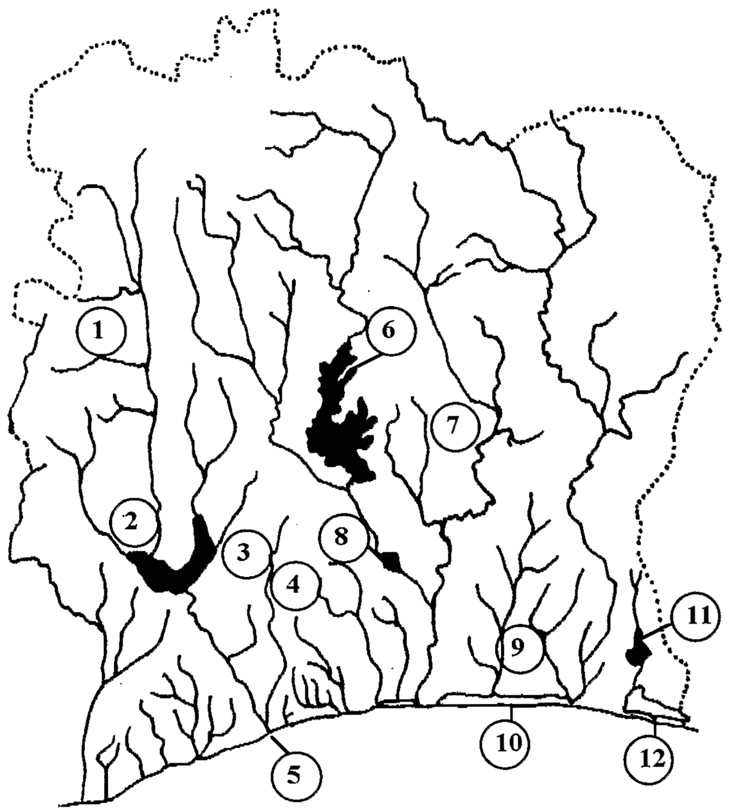


Figure 1
Map showing the collecting sites of tilapias in Côte d'Ivoire : 1, Man, 2, Lake Buyo, 3, Lake Davo, 4, Valoa River, 5, Sassandra River, 6, Lake Kossou, 7, Bouaké, 8, Lake Taabo, 9, Adzopé, 10, Ebrié Lagoon, 11, Lake Ayamé, 12, Aby Lagoon.

For three other loci, we find some alleles only in *T. guineensis*. These are AAT-2 (alleles 50 and 160), ADH (allele 100), and PT1 (allele 70). The samples from Bouaké (7) of *T. zillii* and from Layo (10) of *T. guineensis* were used as controls. We observe the existence of hybrid individuals in all the lake populations with all possible combinations of hybrid genotypes, which seems to indicate that these hybrids are perfectly fertile and have a non-negligible adaptive value.Works are in progress to determine if this observed

structure will remain static or if it will evolve over time. Elsewhere in Côte d'Ivoire other cases of hybridization have been found. In Lake Taabo, of 42 individuals, we found 30 *T. zillii*, 7 *T. guineensis* and 5 hybrids, in Lake Kossou, further upstream, of 130 individuals, 67 were *T. zillii* and 63 were *T. guineensis*, no hybrids were found. In the Sassandra basin, the situation is a little different because *T. zillii* naturally possesses allele PGI 100, only the locus LDH-1 is diagnostic in this basin. If in Man all individuals can be identified as *T. zillii*, in Sassandra, the 9 specimens studied were all *T. guineensis*, confirming the preferential distribution of these two species. In Lake Buyo, we observe a new allele PGI 112 which probably belongs to *T. guineensis* because it is always observed with allele LDH-1 250, typical of this species. Therefore we have 1 *T. zillii*, 24 *T. guineensis* and 30 hybrids. Further South, in Lake Dagou which is a very small lake of a few square kilometers, of the 29 specimens analyzed, we found 5 *T. zillii* 6 whose status is undetermined, 18 hybrids and no *T. guineensis*. In the river a few kilometers below the lake we find 20 *T. zillii*, 9 undetermined, 15 hybrids and no *T. guineensis*. It seems therefore, that in Côte d'Ivoire, the cases of hybridization of *T. zillii* and *T. guineensis* are numerous and closely related to the presence f dams. *T. zillii* is a riverine species and *T. guineensis* is more a lagoon species. The creation of artificial lakes seems to destroy or decrease the ethological barriers between these two species. However, in the largest lake in Côte d'Ivoire, Lake Kossou, these two species do not hybridize, which implies that the mere presence of a dam is not enough to explain this hybridization.

Natural hybridizations

In this last case, hybridization did not occur between two species but between three species: *Tilapia zillii*, *T. guineensis* and *T. dageti* (POUYAUD, 1993). *T. zillii* is found throughout the Comoé basin while *T. dageti* is limited to the regions above the Koroboué Falls

and *T. guineensis* to the regions below, the falls acting as the barrier between these two species.

In order to genetically characterize each species, 21 *T. guineensis* from Ebrié Lagoon, as well as 27 *T. zillii* and 22 *T. dageti* from the Abengourou region were analyzed. Among the 23 loci studied, 3 were shown to be diagnostic. *T. guineensis* individuals were characterized by alleles LDH-1 (100), LDH-3 (100) and GPI-2 (100), *T. zillii* individuals were characterized by alleles LDH-1 (250), LDH-3 (100) and GPI-2 (108) and *T. dageti* individuals were characterized by alleles LDH-1 (110), LDH-3 (95) and GPI-2 (112).

Among the 49 individuals which have been analysed below the Koroboué Falls, 24 possessed alleles characteristic of *T. guineensis*, 5 of *T. zillii* and 13 of *T. dageti*. The 7 remaining specimens possessed a combination of the different diagnostic alleles: 4 could be considered as hybrids between *T. guineensis* and *T. dageti*, 2 between *T. dageti* and *T. zillii* and 1 possessed alleles from the three species. After localization of each captured specimen, it was possible to observe the progressive disappearance of the alleles characteristic of *T. dageti* beginning with the second sector below the falls. Therefore these falls truly do play the role of barriers keeping fish from climbing the river. For the moment, the hypothesis retained is of a historical-biogeographical type. *T. guineensis* and *T. dageti* are two very close species, most likely sister species coming from a common ancestral species (POUYAUD and AGNESE, 1995). Other than the fact that the ethological barriers are not well established, it seems that the two species are mutually exclusive in a basin where they may be in cohabitation. *T. dageti* would have colonized the Comoé River from the upper basin thanks to contact with the Volta where *T. guineensis* would have colonized the Comoé from the mouth because it is a species more often found in lagoons. From the river mouth *T. guineensis* would have colonized upriver, stopping at the impassable falls at Koroboué. There, a hybridization phenomenon occurred with individuals of the species *T. zillii*. Of course, this study is only preliminary. More work will be necessary to confirm this hypothesis and so that we can better understand the mechanisms that are engaged during this hybridization.

Conclusions

The numerous hybridizations observed in tilapias show just how unfinished the phenomenon of speciation is in this group. In certain cases, the barriers between species are purely ethological, such as those between *T. zillii* and *T. guineensis*. Therefore species' integrity is only maintained in places where they are the only ones to fill a certain ecological niche, lagoons for *T. guineensis* and rivers for *T. zillii* for example. If the environment is modified, as in the creation of an artificial lake for example, then these barriers may disappear. However, in the majority of cases there are post-zygotic barriers. If sometimes they are very strong and lead to non-viability of the hybrids, quite often the F1 are viable and in part fertile. In most cases, these hybrids in fact have a lower adaptive value than either of the pure species and these hybridizations usually lead to the disappearance of one of the two pure species and eventually of the hybrids as well. The double experiment of the introduction of *O. niloticus* and *O. mossambicus* in Lakes Itasy in Madagascar and Ihema in Rwanda shows that we cannot predict which species will win the competition. We often also don't know the consequences of the elimination of a species after hybridization with another species. In particular, the vanished species may have left some of its genes in the established species. Such introgression has not yet been established in natural tilapia populations in Africa, certainly because of the low number of genetic studies, but which has been observed in reservoirs in Sri Lanka where the feral *O. niloticus* population has *O. mossambicus* genes (DESILVA and RANASINGHE, 1989). With the development of genetics techniques, the characterization of the tilapia genome is progressing rapidly. Proof of hybridization and introgression is now possible for a great number of natural species and populations.

References

BALIRWA (J.S.), 1992 —
The evolution of the fishery of *Oreochromis niloticus* (Pisces, Cichlidae) in Lake Victoria. *Hydrobiol.*, 232(1): 85-90.

DAGET (J.), MOREAU (J.), 1981 —
Hybridation introgressive entre deux espèces de sarotherodon (Pisces, Cichlidae) dans un lac de Madagascar. *Bull. Mus. Nat. Hist. Nat.*, 4e sér. 3, section A, no2: 689-703.

DESILVA (C.D.), RANASINGHE (J.), 1989 —
Biochemical evidence for hybrid gene introgression in some reservoir populations of tilapia in southern Sri lanka. *Aquaculture and Fisheries Management*, 20: 269-277.

ELDER (H.Y.), GARROD (D.J.), WHITEHEAD (P.J.P.), 1971 —
Natural hybrids of the African Cichlid fishes *Tilapia spilurus nigra* and *Tilapia leucosticta*: a case of hybrid introgression. *Biol. J. linn. Soc.*, 3 (2): 103-146.

LOWE (R.H.), 1958 —
Observations on the biolgy of Tilapia nilotica (Lin.) in East African waters. *Rev. Zool. Bot. afr.*, 57 (1-2): 129-170.

MICHA (J.C.), CUVELIER (R.), TILQUIN (C.), MURAILLE (B.), BOURGEOIS (M.), FALTER (U.), 1996 —
Comparative growth of hybrids (F1, F2 anf F3) of *Oreochromis niloticus* (L.) and *O. macrochir* (Blgr). p 354-360. *In* PULLIN (R.S.V.), LAZARD (J.), LEGENDRE (M.), AMON KOTHIAS (J.B.), PAULY (D.), ed.: *the third international Symposium on Tilapia in Aquaculture.* Iclarm conf. Proc. 41, 575p.

OGUTU-OHWAYO (R.), 1990 —
The decline of the native fishes of Lake Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile tilapia, *Oreochromis niloticus*. *Env. Biol. Fish.* 27: 81-96.

POUYAUD (L.), 1993 —
Génétique des populations de tilapias d'intérêt aquacole en Afrique de l'Ouest. Relations phylogénétiques et structuration populationnelles. Thèse de l'université de Montpellier-II

POUYAUD (L.), AGNESE (J.-F.), 1995 —
Phylogenetic relationships between 21 species of three tilapiine genera *Tilapia*, *Sarotherodon* and *Oreochromis* using allozyme data. *Journal of Fish Biology* 47: 26-38.

SIDDIQUI (A.Q.), 1977 —
Lake Naivasha (East Africa, Kenya) fishery and its management together with a note on the food habits of fishes. *Biol. Conserv.* 12: 217-228.

SIDDIQUI (A.Q.), 1979 —
Reproductive biology of *Tilapia zillii* (Gervais) in Lake Naivasha, Kenya. *Env. Biol. Fish.*, 4(3): 257-262.

THYS VAN DEN AUDENAERDE (D.F.E.), 1970 —
An annoted bibliography of Tilapia (Pisces : Cichlidae). Docum. Zool. Mus. R. Afr. Centr., 14: 406 pp.

TREWAVAS (E.), 1983 —
Tilapiine fishes of the genera Sarotherodon, Oreochromis and Danakilia. British Museum (Natural History), London.

WELCOMME (R.L.), 1967 —
Observation on the biology of the introduced species of tilapia in Lake victoria. *Rev. Zool. Bot. Afr.*, 124 (3-4): 249-279.

A database approach to illustrate genetic trends in fishes

Maria Lourdes D. Palomares
Hydrobiologist

Christine Marie V. Casal
Hydrobiologist

Introduction

Data on finfish are available in a wide range of sources such as books, journals, proceedings, reports and computerized databases. However, these sources are often not readily available to users, be they policy-makers, scientists or students seeking information on the genetic characteristics of fishes for varied purposes. Such lack of information materials is especially felt in developing countries where meager resources do not allow for institutional or individual subscription to important journals. Thus, assembling available information into one document and making old and current data available is a significant support to scientists, researchers, policy-makers, educators and students.

Encoding data into well-structured databases is a recent approach in facilitating information gathering, dissemination and exchange. The technological advances brought about by the computer industry have improved the feasibility of working with large information systems and databases. These systems not only facilitate the dissemination of data but also provide tools for their easy manipulation and analysis. This is an important step, especially in

the field of biological science (*e.g.* in the study of biodiversity and genetics) in order to transform data into information which can then be used as a basis to educate natural resource managers and policy-makers on the status of living resources. Such information thus facilitates the definition and implementation of management measures to conserve these resources.

The question then is how to turn data into useful information. One method that can be implemented post-entry is the use of graphics in depicting relationships between any two (or more) parameters. An example of efficient and easy to implement graphical tool are scatter plots. Drawing scatter plots, however, must be preceded by a process of hypothesis building.

Some postulations involving genetic data and which were later tested using scatter plots are enumerated below. The data from the biological database known as FishBase, developed by Iclarm with support from FAO and many other collaborators and supported by the European commission, were used. The hypotheses centered on heterozygosity, polymorphism, DNA content and chromosome numbers, viz:

1. Heterozygous organisms have a higher degree of polymorphic loci than homozygous organisms and a direct (possibly linear) relationship between values of heterozygosity and polymorphism can be postulated.
2. Plotting observed *vs.* expected values of a parameter tests the predictive value of empirical formulae (SOKAL and ROHLF, 1995). Ideally, an observed value should be equal to the expected value, resulting in a scatter plot that shows a "direct" correspondence between expected and observed values, *i.e.*, a linear progression at a 45° angle from the origin of the XY axis. If a line is traced to join these points, a straight line results. This is called the 1:1 correspondence line. However, variation is intrinsic in natural processes, hence observed actual values deviate from the 1:1 line, but should remain close to it. Outliers merit scrutiny.
3. HINEGARDNER and ROSEN (1972) presented haploid DNA content data for almost 300 teleost species and showed that more specialized (or evolutionally advanced) fishes have less DNA than more generalized forms. This trend was further verified by CUI *et*

al., (1991) who determined the cellular DNA content of 42 species of Chinese freshwater fishes.

4. Since chromosomes carry the genetic material, *i.e.* DNA, then it can be postulated that the number of chromosomes is directly related to the DNA content.

Materials and methods

Three types of genetic data (continuous numeric variables) were investigated here, through FishBase (FROESE and PAULY 1996). The first study used heterozygosity and polymorphism values derived from allele frequency data obtained from the literature on electrophoretic studies for 195 species (*i.e.* 10,900 records), extracted from about 40 references. The other study compared the DNA content of over 350 species and the chromosome numbers of over 1300 species, extracted from over 400 references.

Heterozygosity and Polymorphism

Two graphs were created: one to plot heterozygosity against polymorphism and the other to plot observed heterozygosity against expected heterozygosity. Data points for a chosen species were overlaid against all other species for which data existed.

Heterozygosity is defined as the proportion of heterozygotes for a given locus in a population. Heterozygous individuals are diploid organisms that have inherited different alleles from each parent, *i.e.* they carry different alleles at the corresponding places on paired chromosomes. Heterozygosity for each locus was computed from allele frequency data using the Hardy-Weinberg equation (CARPENA *et al.*, 1993) expressed as: $p^2+2pq+q^2=1$.

Where p is the frequency of the dominant allele and where q is the frequency of the recessive allele. The expected heterozygosity was

computed by averaging values of heterozygosity per locus over all loci studied for a particular population.

A polymorphic locus hosts two or more different alleles (LAWRENCE 1995). The percentage of polymorphic loci was calculated as:

% polymorphism = number of polymorphic loci/total number of loci studied * 100

Figure 1 was created to test this hypothesis for finfish. Note that the plot was made using expected and not observed heterozygosity, because not all of the sources used in FishBase provided estimates for this variable.

Expected and observed heterozygosity values were plotted following these above assumptions using only populations for which both values were available. Data points which deviated from the 1:1 line, *i.e.* beyond an imaginary 95% confidence interval, were verified. The original data source was inspected for possible discrepancies arising from encoding errors.

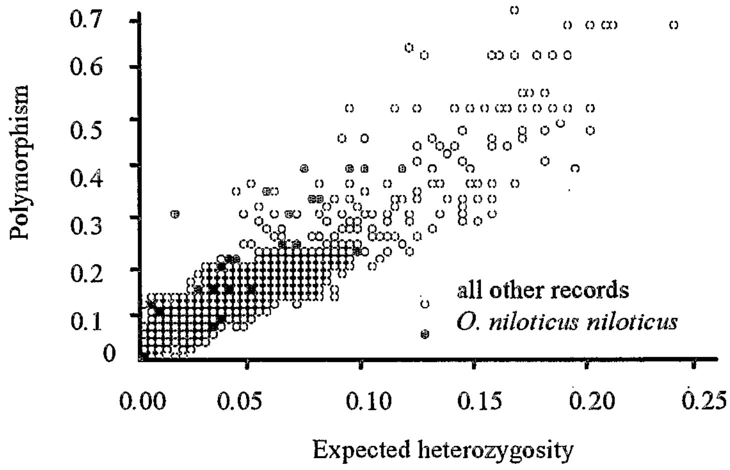


Figure 1
Scatter plot of polymorphism *vs.* expected heterozygosity for *Oreochromis niloticus niloticus* printed from FishBase (Froese and Pauly 1996). Note outlier standing out from the group of black dots with polymorphism = 0.3.

DNA content, chromosome number and phylogenetic order

To test this hypothesis, a scatter plot was created for the DNA content (expressed as the haploid value, in picograms) against the phylogenetic order of families presented by NELSON (1994). The DNA content data was obtained from the Genetics table of FishBase. Assuming that this relationship is also true for chromosome numbers, diploid chromosome numbers were plotted against the phylogenetic order of families. The chromosome data was also obtained from the Genetics table of FishBase.

Results and discussion

Heterozygosity and Polymorphism

The scatter plots for expected heterozygosity vs. polymorphism are presented in Figure 1 for *Oreochromis niloticus niloticus* (Linneaus, 1758). A strong increasing trend is evident with data points concentrated in the center of the graph and around an imaginary 1:1 correspondence line. Note that one (in parenthesis) of the 27 data points for *O. n. niloticus* deviates from the general trend. Verification of the record entered against the original source confirmed that an error was made in encoding the allele frequency of one locus. Scatter plots for observed *vs.* expected heterozygosity are presented in Figure 2. It is evident that one particular population of *O. n. niloticus* (in parenthesis) showed a very large difference between expected and observed heterozygosity. Verification of the record entered showed that the necessary linkage between allele frequencies and the publication used for some of the loci recorded for this species in this specific study were erroneous, *i.e.* the allele frequency records were linked to the wrong population. This resulted in erroneous computations of expected heterozygosity.

These results confirm, as expected, a direct relationship between heterozygosity and polymorphism and showed the importance of investigating outliers.

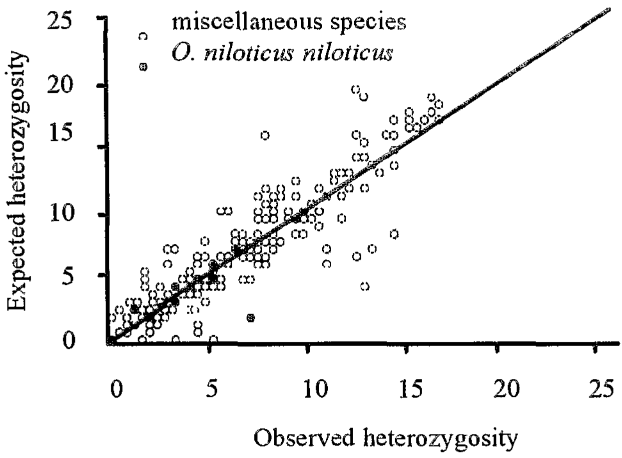


Figure 2
Scatter plot of expected vs. observed heterozygosity for *Oreochromis niloticus niloticus* printed from FishBase (Froese and Pauly 1996). Note outlier standing out from the group of black dots close to the 1:1 correspondence line.

DNA content, chromosome number and phylogenetic order

Scatter plots of the phylogenetic order of family against DNA contents and chromosome numbers are presented in Figures 3 and 4. These figures show two apparent trends: (1) a decrease in DNA content/chromosome number in the course of evolution; and (2) a decrease in variability of DNA content/chromosome numbers of species within orders of increasing phylogenetic order. Some very high values of DNA content (4.0-4.5) and chromosome number (250-300) identified in these graphs belong to polyploid groups

(*e.g.* Gasterosteidae). The following studies support the observed trends, viz.:

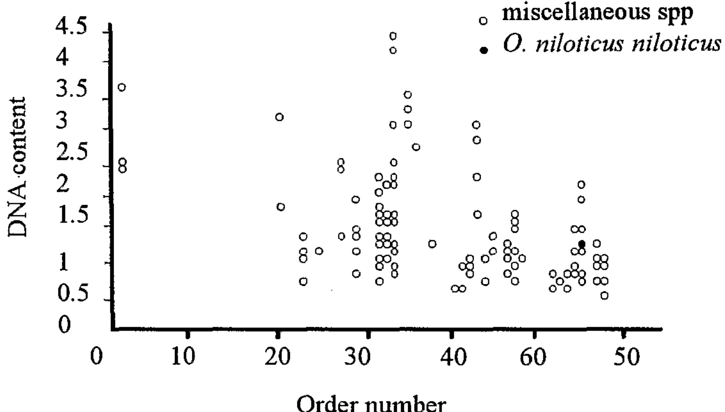


Figure 3
Scatter plot of DNA content *vs* phylogenetic order printed from FishBase (FROESE and PAULY 1996).

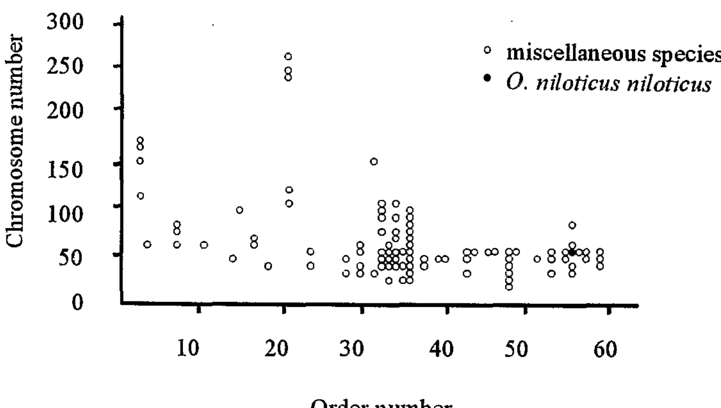


Figure 4
Scatter plot of chromosome number *vs* phylogenetic order printed from FishBase (FROESE and PAULY 1996).

- karyotypes with large numbers of chromosomes (HINEGARDNER and ROSEN, 1972) and including a large proportion of telocentric chromosomes are more representative of a primitive elasmobranch genome than are other karyotypes (SCHWARTZ and MADDOCK, 1985);
- selachians have high chromosome numbers (2n=50-100) which decrease in more specialized species through a loss of acrocentrics and microchromosomes (STINGO and CAPRIGLIONE, 1985);
- within a taxonomic group, an increase followed by a gradual decrease in DNA, which is associated with specialization, appears to have accompanied fish evolution (HINEGARDNER and ROSEN, 1972).

Data quality control

As was shown above, the task of compiling biological databases is large and complex. The quality of the information being entered (and therefore to be disseminated) must be assured. The most common method is to ask collaborators or experts to verify the data entered. This is a time consuming work, because the amount of information to be verified can comprise tens of thousands of records. It is thus difficult to ask "volunteer" collaborators to put their own work aside and to spend days or weeks verifying rows and rows of encoded data.

The outliers, *i.e.*, data points outside the general (expected) pattern of a relationship identified here resulted from (a) an encoding (human) error (Figure 1); (b) a source code level (programming) error (Figure 2). Errors in the original data source might also occur, but no example could be identified with the data set used. Note that only very rarely would outliers indicate a "way-out" fish species because fish are all "built" according to a common design pattern, dictated by the laws of thermodynamics, etc. Scatter plots permit the efficient verification of large volumes of data in a short period of time (usually a few minutes depending on computer speed) and provide a picture of how the encoded data, taken together, behave.

Conclusions

Genetic data compiled to date in FishBase allowed to test and verify the relationship between heterozygosity and polymorphism. The equation to estimate expected heterozygosity was confirmed in a 1:1 plot over observed heterozygosity. A plot of DNA content and chromosome number of the phylogenetic rank of fish orders showed a decreasing trend and confirmed some predictions from recent literature. Furthermore, scatter plots turned out to be also useful tools in identifying errors, which can thus be verified and repaired. Such functions, if habitually incorporated in databases, permit the automatic and regular verification of data being encoded, thus improving the quality of the data stored.

References

CARPENA (A.L.), ESPINO (R.R.C.), ROSARIO (T.L.), LAUDE (R.P.), 1993 — *Genetics at the population level.* SEARCA, UPLB, Laguna, Philippines, 244 p.

CUI (J. X.), YU (R.Q.), 1991 — Nuclear DNA content variation in fishes. *Cytologia*, 56(3): 425-429.

FAN (Z.), FOX (D.P.), 1991 — Robertsonian polymorphism in plaice, *Pleuronectes platessa* L. and cod, *Gadus morhua* L. (Pisces, Pleuronectiformes and Gadiformes). *J. Fish Biol.*, 38(5): 635-640.

FROESE (R.), PAULY (D.), 1996 — (eds). *FishBase 96. Concepts, design and data sources.* Iclarm, Manila, Philippines, 179 p.

HINEGARDNER, (R.) and (D.E.) ROSEN. 1972 — Cellular DNA content and the evolution of teleostean fishes. *American Naturalist*, 106(951):621-644.

KLINKHART (M. M.), TESCHE (H.) GREVEN, 1995 — (eds.) *Database of fish chromosomes.* Westarp Wissenschaften, 237 p.

LAWRENCE (E.), 1995 — (ed.) *Henderson's dictionary of biological terms.* 11th Edition. Longman Singapore Publishers (Pte) Ltd., Singapore. 693 p.

SOKAL (R.R.), ROHLF (F.J.), 1995 — *Biometry. The principles and practice of statistics in biological research.* Third Edition. W.H. Freeman and Company, New York, 887 p.

STINGO (V.), CAPRIGLIONE (T.), 1985 — DNA and chromosomal evolution in cartilaginous fish. In UYENO (T.), ARAI (R.), TANIGUCHI (T.), MATSUURA (K.), (eds.) *Indo-Pacific Fish Biology.*

Proceedings of the second international conference on Indo Pacific Fishes, Tokyo National Museum, Ueno Park, Tokyo, July 29-Aug. 3, 1985. p140-147

SCHWARTZ (F.J.), MADDOCK (M.B.), 1985 —
Comparisons of karyotypes and cellular DNA contents within and between major lines of elasmobranchs.
In UYENO (T.), ARAI (R.), TANIGUCHI (T.), MATSUURA (K.), (eds.) *Indo-Pacific Fish Biology. Proceedings of the second international conference on Indo Pacific Fishes*, Tokyo National Museum, Ueno Park, Tokyo, July 29-Aug. 3, 1985. p148-157

WARD (R.D.), WOODWARK (M.), SKIBINSKI (D.O.F.), 1994 —
Comparison of genetic diversity levels in marine, freshwater and anadromous fishes.
J. Fish Biol., 44: 213-232.

The paradox of international introductions of aquatic organisms in Africa

Benedict P. Satia
Biologist

Davin M. Bartley
Biologist

Introduction and methods

Introductions and Transfers (briefly introductions) of aquatic organisms into and out of Africa are an old practice. Compared to other continents, the phenomenon is recent, approximately 150 years old. This time factor may, however, be a reflection of the absence of records. There are few major rivers and lakes in Africa which have not been subjected to deliberate or inadvertent introductions.

In the early 1980's FAO started a database on international introductions of inland aquatic fishes (WELCOMME, 1988). Recently, this database has been expanded, by distributing internationally a questionnaire and by performing a literature search, to include marine organisms and other aquatic taxa, such as molluscs and crustaceans (BARTLEY and SUBASINGHE, 1996), Coverage in the database is still uneven, probably being most complete for freshwater fish and most incomplete for aquatic plants. Although some introductions that have resulted from ballast water and fouling organisms are included, no effort has been made to include these inadvertent introductions.

The information in this report is derived from the FAO database. The data base indicates that over 2,800 introductions have been performed world-wide. Of these, 430 introductions were performed into Africa; about 30 out of Africa and about 140 among African countries.

The fundamental premise of this paper is that international introductions of aquatic organisms in and out of Africa is a paradox. These introductions reflect prevailing attitudes and values by the public and private sectors in which the primary concerns are socio-economic benefits. There is very little evidence that conservation, protection and the long term sustainable use of humans of components of biodiversity were central considerations.

Results and discussion

Species Introductions

One hundred and thirty-nine (139) species from 87 genera and from 46 families have been introduced into 42 countries. The majority of these organisms are finfishes (79%) with relatively small percent of molluscs (7%) and crustaceans (9%). The five most often introduced species were common carp (28 records), rainbow trout (19), large mouth bass (19), Nile tilapia (17) and grass carp (15). Thus, 1% of the species account for 23% of the introductions. By family, the most often introduced were Cichlidae (116), Cyprinidae (81), Centrarchidae (50) and Salmonidae (40). Similarly, 9% of the families account for 67% of the introductions. The large and varied number of species from tropical to temperate environments implies efforts to exploit almost all the aquacultural zones of the continent.

Three main waves of introductions are identified: before 1949 (93 records); 1950-1989 (226) and after 1990, 22 records. There are also 77 introductions of unknown dates. Figure 1 shows introductions per decade. The relatively high number of intro-

ductions between 1950-1959 (78) and 1960-69 (60) is a reflection of the search for the "appropriate" species for aquaculture development, for the stocking of man-made lakes and for the control of disease vectors and weeds. The subsequent reduction per decade after 1980 is apparently related to the growing awareness of the possible negative effects of species introductions and legislation, particularly in developed countries prohibiting such introductions.

Africa has received introductions from all continents except Oceania and Antarctica. The source of parent stock seemed to be linked to "colonial affinities". At the same time, Africa has also given to the other continents. The most remarkable of these exports was the Nile tilapia (*O. niloticus*) from four countries (Ghana, Egypt, Kenya and Senegal) to the Philippines). This parent material has been improved genetically under the Genetic improvement of farmed tilapia (Gift) Project by Iclarm researchers to become what is known as the "super tilapia" (EKNATH *et al.*, 1993). There have also been about 140 intra-African introductions of species. The intensities of introductions that is, number of introductions into and out of a country, for the ten countries that have had the most introductions is summarized in Table 1.

Country	No. introduced	Imported from Africa	Imported from Other	Exported to Africa	Exported to Other
South Africa	41	2	30	42	2
Morocco	37	1	34	2	1
Kenya	26	14	7	14	3
Zambia	26	18	6	5	0
Zimbabwe	25	18	2	5	0
Madagascar	24	9	13	3	0
Mauritius	23	7	14	1	0
Congo	20	15	4	10	1
Egypt	16	5	8	1	7
Tunisia	15	3	10	0	0

Table 1
Intensities of introductions for 10 principal countries.

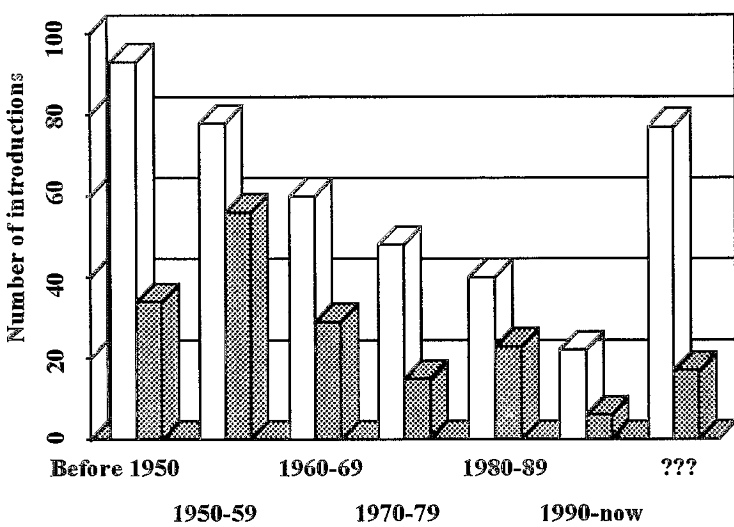


Figure 1
Numbers of fish species introductions per decades
(white, into African countries; black, out of African countries).

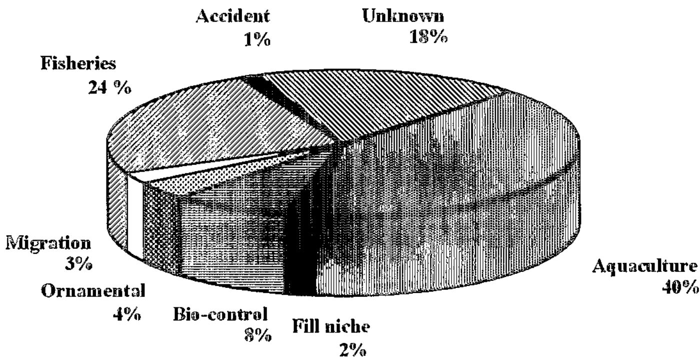


Figure 2
FAO data bank analysis of the reasons
for introductions of species.

Reasons for the Introductions

In many developed countries, species are often introduced to freshwater bodies to create sport fisheries. In most African countries, introductions have been promoted to produce high quality fish protein, alleviate poverty and hunger, as well as provide employment, control disease vectors and weeds. In the FAO data bank these different purposes have been grouped into three main classes: Aquaculture development, biological control and capture and sports fisheries.

Analysis of the data bank provides the results given in Figure 2. In addition to the above reasons there were five reported cases of accidents, 10 cases of migration to the wild and eight instances to fill vacant so called "niches". There were also 71 cases of unknown reasons of introductions.

Status and Impact of the Introductions

The status, impact and benefits of these introductions are summarized in Table 2. The objectives of some introductions could not be met but several species have become widespread in both rivers and lakes. In a number of particular instances, success has followed the introductions of species as a foundation for capture fisheries. This has been the case with the introduction of the voracious Nile perch (*Lates niloticus*) into Lake Victoria. This predatory species is reported to have contributed to the elimination of over 300 species of haplochromine cichlid and change the primarily small-scale artisanal fishery on the lake into a multi-dollar commercial fishery that supports industrialized processing and exportation ventures (PITCHER and BUNDY, 1996; MANN, 1970; OGUTU OHWAYO, 1990).

Another example is the pelagic Clupeid *Limnothrissa Miodon* into Lakes Kivu and Kariba and its accidental diffusion downstream to Lake Cahora Bassa, leading to the establishment of substantial stocks of fish that has formed the basis of important Kapenta/Sardine fisheries in these lakes and reservoir. The sardines have, however, altered the zoo plankton composition and possibly other aspects of the ecosystem (MARSHALL, 1991; MAYABE 1987; JACKSON, 1960). Yet a third important fishery that has been established through introductions is the *Heterotis niloticus* fishery

on the Nyong River in Cameroon (DEPIERE and VIVIEN, 1977). In the three cases cited, the current fishery provides two to three times as much fish before the introduction, and fish consumption in the areas of such fisheries have remained high despite significant increases in human population. However, these changes have also introduced a series of socio-economic problems from deforestation to provide fuelwood for processing to a shift in the rural economy of the locality (DEPIERE and VIVIEN, 1977; REYNOLD and GREBOVAL, 1989). However, overcapitalization of the various sub-sectors of the fishery could contribute to the collapse of the fisheries. With regard to aquaculture species, *Cyprinus Carpio* have become well established in many countries but the *Oreochromis sp.* remain the principal aquaculture candidate. It is reported (LAZARD, 1990) that the introduction of *O. niloticus* into Côte d'Ivoire has led to a significant development of fish culture in the country. It is, however, a paradox that, while Africa was/is scouting for the best fish species to be introduced for aquaculture, *O. niloticus* introduced into the Philippines from Africa was genetically improved under the GIFT Project.

Reason for introduction	Ecological effects	Socio-economic effects
Aquaculture - of the 153 reported introductions, 74 became established in the wild.	adverse 4	adverse 3
	beneficial 3	beneficial 6
	undecided 12	undecided 12
	blank 134	blank 132
Fisheries (sport and commercial)	adverse 7	adverse 0
	beneficial 5	beneficial 11
	undecided 2	undecided 2
	blank 80	blank 81
Biological control - of 32 reported introductions, 22 became established in the wild.	adverse 0	adverse 0
	beneficial 2	beneficial 3
	undecided 2	undecided 2
	blank 28	blank 27

Table 2
Status and impact of African introductions for the three most common reasons for introducing aquatic organisms.

Regrettably, very few broad spectrum analysis that take into account ecological as well as socio-economic parameters have been done on the introductions in Africa (BARTLEY, 1993; REINTHAL, 1993; COATES, 1995). Where analysis have been undertaken (Lake Victoria and River Nyong) it is reported that the fish fauna has been drastically altered; native species have been eliminated; the situation is virtually irreversible and the introduced fauna is established and cannot be removed easily in economic or practical terms. OGUTU-OHWAYO and HECKY (1990) report, however, that while the introduction of *L. Miodon* into Lake Kivu and Kariba reservoir has established highly successful fisheries, the effect on the pre-existing fish community or trophic ecology is very small.

It is important to note also that the effects of introductions could take a long time to manifest. In the three flourishing fisheries cited in this study the timeframe was 15 to 20 years. In general terms, the negative effects of introductions include the degradation of the host environment, the disruption of the host community through competition and displacement, stunting and predation as well as nuisance to the fisheries (LEVEQUE and QUENSIERE, 1988; OGUTU-OHWAGO and HECKY, 1990; MOREAU *et al.*, 1988). It is quite probable that impacts of species introductions are irreversible and unpredictable. Hence, recognizing the necessity, in the interest of present and future generations of humans, to protect the environment and its biota from any potential negative impacts, fisheries professional societies, governments, intergovernmental organizations, non-governmental organizations, etc. have contributed to the enactment/adoption of regulations, biosafety protocols and codes of practice on the responsible use of exotic species (FAO, 1995a,b; PULLIN, 1994). Foremost in this regard are the ICES/EIFAC Codes of Practices and Manual of Procedures for consideration of Introductions and Transfers of Marine and Freshwater Organisms (TURNER, 1988). The code has been adopted for use by the Committee for Inland fisheries in Africa (CIFA). These instruments emphasize a precautionary approach to species introductions in order to reduce the risk of adverse impacts on the introductions on capture fisheries and aquaculture; to establish corrective or mitigating procedures in advance of actual adverse effects; and to minimize unintended introductions to wild ecosystems and associated capture fisheries (FAO, 1997).

Conclusion

Africa faces a major challenge. There is, on the one hand, public outcry at the undesirable ecological consequences of some introductions. On the other hand, the contribution of some introductions to increase fish protein is undeniable. What mechanisms would permit the sacrifice of short-term gains to the present generation in order to realize long term gains for future generations. What strategies are appropriate in promoting conservation and sustainable use of biodiversity in the face of hunger and poverty.

References

BARTLEY (D.M.), 1993 —
An application of international codes of practice on introductions of aquatic organisms: Assessment of a project on the use of Chinese Carps in Mozambique. FAO Fisheries Circular, Rome, Italy, No. 863. 21p.

BARTLEY (D.M.), SUBASINGHE (R.P.), 1996 —
Historical aspects of international movements of living aquatic species. *Rev. Sci. Tech. Off. Int. Epiz.*, 15(2): 387-400.

COATES (D.), 1995 —
Implementation of the ICES (International Council for the Exploration of the Sea/EIFAC European Inland Fisheries Advisory Commission) Codes of Practice; experience with the evaluation of international fish transfers in the Sepik River Basin, Papua New Guinea. In Protection of Aquatic Biodiversity Proc. World Fisheries Congress, Theme 3, Athens, Greece, May 1992, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India.

DEPIERRE (D.), VIVIEN (J.), 1977 —
Une réussite du service forestier du Cameroun. L'introduction d'*Heterotis niloticus* dans le Nyong - *Bois et Forêts Tropicaux* : 59-68.

EKNATH (A.E.), TAYAMEN (M.M.), PALADA DE VERA (M.), DANTING (J.C.), REYES (R.A.), DIONISIO (E.E.), CAPILI (J.B.), BOLIVAR (A.V.), BENTSEN (H.B.), GJERDE (B.), GJEDREM (T.), PULLIN (R.S.V.), 1993 —
Genetic Improvement of Farmed Tilapias: the growth performance of eight trains of *Oreochromis niloticus* tested in different farm environments, *Aquaculture*, 3: 171-188.

FAO, 1995a —
Precautionary approach to fisheries Part I - Guidelines on the precautionary approach to capture fisheries and species

introductions.
FAO Fisheries Technical Paper No. 350/1, Food and Agriculture Organization of the United Nations, Rome, Italy, 52p.

FAO, 1995b —
Code of Conduct for Responsible Fisheries. Food and Agriculture of the United Nations, Rome, Italy, 41pp.

FAO, 1997 —
FAO Technical Guidelines for Responsible Fisheries. No. 5, Food and Agriculture of the United Nations, Rome, Italy, 40pp.

JACKSON (P.B.N.), 1960 —
On the desirability or otherwise of introducing fishes to waters that are foreign to them. CCTA/CSA, Third Symposium on Hydrobiology and Inland Fisheries. Problems of major lakes, Lusaka, 18-24 Augut 1960. Publ. Cons. Sci. Afr. South Sahara 63: 157-164.

LAZARD (J.), 1990 —
Transferts de poissons et développement de la production piscicole. Exemple de trois pays d'Afrique Sub-Saharienne. *Rev. Hydrobiol. trop.*, 23(3): 251-265.

LEVEQUE (C.), QUENSIERE (J.), 1988 —
Fish Communities in Shallow Lakes. *In* Lêvéque (C.), Bruton (M.N.), Ssentongo (G.W.), ed.: *Biology and Ecology of African Freshwater Fishes.* Orstom, Paris, 216: 303-324.

MANN (M.J.), 1970 —
A *resume of the Tilapia fisheries of Lake Victoria up to the year 1960.* EAFFRO Annual Report 1969, Append. B: 21-27.

MARSHALL (B.E.), 1991 —
The impact of the introduced sardine *Limnothirissa miodon* on the ecology of Lake Kariba. *Biol. Conserv.*, 55(2): 151-165.

MAYABE (A.B.E.), 1987 —
Some management aspects and constraints in the Lake Kariba Fishery, Naga, Oct. 1987: 10-12.

MOREAU (J.), AVIGNON (J.), JUBB (R.A.), 1988 —
Les introductions d'espèces étrangères dans les eaux continentales africaines: Intérêts et limites. *In* LEVEQUE (C.), BRUTON (M.N.), SENTONGO (G.W.), ed.: *Biology and Ecology of African Freshwater Fishes.* Orstom, Paris, 216: 395-425.

OGUTU-OHWAYO (R.), 1990 —
The decline of the native fishes of Lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile Perch, *Lates niloticus* and the Nile tilapia, *Oreochromis niloticus. Envir. Biol. Fishes*, 27: 81-96.

OGUTU-OHWAYO (R.), HECKY (R.E.), 1990 —
Fish introductions in Africa and some of their implications. *Can. J. Fish and Aquat. Sci.*, 48, (Suppl.1): 8-12.

PITCHER (T.J.), BUNDY (A.), 1996 —
Successful species introductions in the African Lakes: Assessment, Uncertainties and Strategies for Fishery Management. *In* GORDON (K.), DOUGLAS (M.), EGGERS (J.R.), MARASCO (P.C.), TERRANCE (J.), QUINN, ed.: *Proceedings of the International Symposium on Management Strategies for exploited fish populations.* University of Alaska Sea Grant College Program Report No. 93-03: 545-570.

PULLIN (R.S.V.), 1994 —
Exotic species and genetically modified organisms in aquaculture and enhanced fisheries: Iclarm's position. Naga (International Centre for Living Aquatic Resources Management), Iclarm Quarterly, 17 Oct. 19-24.

REINTHAL (P.), 1993 —
Evaluating biodiversity and conserving Lake Malawi's cichlid fish fauna. *Conserv. Biol.*, 7(3): 712-718.

REYNOLDS (J.E.), GREBOVAL (D.F.), 1989 —
Socio-economic effects of the evolution of Nile perch fisheries in Lake Victoria: a review. Committee on the Inland Fisheries of Africa Technical Paper No. 17, FAO, Rome, Italy, 148 pp.

TURNER (G.E.), ed., (1988) —
Codes of practice and manual of procedures for consideration of introductions and transfer of marine and freshwater organisms. EIFAC Occasional Paper No. 23 - European Inland Fisheries Advisory commission (EIFAC), FAO, Rome, Italy, 46 pp.

WELCOMME (R.L.), 1988 —
International introductions of inland aquatic species. FAO Fisheries Technical Paper No. 294, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy, 318 pp.

Genetic impacts of some fish species introductions in African freshwaters

Jean François Agnèse
Geneticist

Introduction

Africa is a continent that has suffered relatively few fish species' introductions (WELCOMME, 1988). However, certain cases are famous such as the Nile perch *Lates niloticus* introduced to Lake Victoria in the 1950's. The consecutive disappearance of several hundred native haplochromine species brought to light the problems that species introductions may cause. The elimination of native species is with no doubt, the most spectacular and the most important long-term impact. It is also an impact that is sometimes difficult to evaluate separately from other manmade effects such as the environmental modifications brought about by man. Although there is a large body of literature on the introduction of exotic species, there is much less works which evaluate the genetic consequences of these introductions.

Genetic impacts can be defined not only as changes to the gene pool of native species but also as changes suffered by the introduced species themselves. We can consider that there are two types of genetic effects. On one hand, the alterations to the gene pool of a species can be direct by hybridization between a native species and an introduced species, or by crossing between a native population and an introduced population of the same species, or even between

two introduced species or populations. On the other hand, the genetic effects can also be indirect; in this case, they result from a serious decrease in the effective size of the population (native or not) which is to say, a decrease in the number of individuals participating in the establishment of the next generation. Such decreases, if they are important enough can cause an increase in genetic drift and in the consanguinity of the population. This loss of variability naturally threatens the adaptive value of these populations. The works of FERGUSSON and DRAHUSHCHAK (1990) showed that in rainbow trout, *Oncorhynchus mykiss*, those individuals which were the most heterozygous had a resistance to disease greater than that of less heterozygous individuals.

Another indirect genetic effect more difficult to show is the displacement of the selective forces acting upon the population. We can assume that this modification of the selective forces leads to a modification of the gene pool of the population.

Hybridizations

As soon as the reproductive isolation of two species or populations is maintained by only geographic or ecological barriers, any of man's actions which may affect these barriers will have hybridizations and or introgressions as a consequence.

These hybridizations can have an effect on the adaptive value of the individuals produced. If in certain cases we can expect an increase in this adaptive value, heterosis or hybrid vigor, in most cases there are genetic incompatibilities between the two parental species and a decrease in the adaptive value of hybrid individuals results.

In the case of hybrid vigor, if in the first generation (F1) each individual have a complete copy of the parental genome, the following generations, because of recombinations during meiosis will have different combinations of the parental genomes which will

have as a consequence a decrease in the adaptive value below that of both parents almost every time.
In certain cases, hybridizations can give rise to new populations with different potentials. ARTHINGTON (1991) and MATHER and ARTHINGTON (1991), showed that the tilapia population from North East Australia results from a hybridization between several species including *O. mossambicus*, *O. hornorum* and *O. niloticus*. These same authors showed that the hybridization between two strains of carp *Cyprinus carpio* also in Australia gave birth to a new strain that spread very quickly and has posed some ecological problems.

Generally speaking however, hybridization like introgression often results into a decrease in the fecundity of the hybrids. This decreased fecundity can go as far as total sterility in the first generation's hybrids.

Bottlenecks

Bottlenecks are serious decreases in the effective size of a population, acting both on the number of alleles (loss of rare alleles) and on the allelic frequencies (heterozygosity). However, the heterozygosity rates are less affected in the beginning and much more sensitive in the growth phase of the population. NEI *et al.* (1975) showed that the more slowly the population grew after the introduction, the better chance it had for an important loss in heterozygosity.

The time necessary, in generations, for one allele to be lost and the other to remain (with two alleles at the same locus), is of course a function of the respective frequencies of each allele but also of the effective size of the populations.

The loss in heterozygosity over time expressed in generations is a function of the effective size. It appears that for an effective size of 500, the loss is low after 500 generations. On the contrary, with an effective size of 10, heterozygosity drops very rapidly.

Lastly, the number of alleles which will be transmitted to the following generation is a function of their respective frequencies and the effective size of the populations. For a locus with 4 equally frequent alleles (25%), an effective size less than 10 conserves the four alleles. On the contrary, when we have one very frequent allele and the other three are rare, more than 100 individuals are required to have a high chance of preserving the four alleles.

African examples

If we consider all of the cases of introductions or transfers of species in Africa, there are unfortunately very few studies undertaken from the angle of their genetic impacts.

For example, *O. niloticus* and *O. mossambicus* have been hybridized in Lake Itasy in Madagascar, and in Lake Ihema in Rwanda.

In Lake Itasy, in Madagascar, *O. macrochir* was introduced in 1958 and *O. niloticus* in 1961. In 1965 and 1966 intermediate specimens of these two species were harvested and called tilapia 3/4 (DAGET and MOREAU, 1981). These hybrid individuals possessed notably a pharyngial bone resembling that of *O. niloticus* but having a morphology closer to that of *O. macrochir*. Between 1963 and 1969, the hybrid population in the captures went from 5% to 74%. *O. macrochir* was considered a vanished species in 1971. Finally the *O. niloticus* population became predominant.

Inversely, in Lake Ihema in Rwanda, *O. macrochir* was introduced around the end of the 1960's, after the introduction of *O. niloticus* around the end of the 1940's. Hybrids were observed around the end of the 1970's. From 1983 to 1987, the proportion of *O. niloticus* decreased from 30 to 20%, that of hybrids increased from 10 to 20%, while the population of *O. macrochir* has remained stable at 60% (MICHA *et al.*, 1996).

In both these cases, no genetic studies could be done. Of course it's too late to follow the progression of these hybridizations but it would still be interesting to study the tilapias which form the actual populations of these lakes in order to determine if introgressions have occurred.

The situation is somewhat similar in Lake Victoria, where *O. niloticus* was introduced forty years ago in 1967, WELCOMME notes the existence of hybrids between *O. niloticus* and *O. variabilis*. These hybrids could all be males. Hybridizations with *O. esculentus* were also suspected by other authors. These two native species *O. variabilis* and *O. esculentus* have since disappeared from Lake Victoria and *O. niloticus* is suspected of being at the origin of these disappearances (WELCOMME, 1967; OGUTU-OHWAYO, 1990). Here also, hybridizations between *O. niloticus* and *O. variabilis* or *O. esculentus* were not studied genetically. Today, we are beginning to have a few data on the genetics of these populations. The works of AGNESE and collaborators recently targeted the *O. niloticus* population of Lake Victoria and *O. esculentus* from one of the satellite lakes, Lake Kanyaboli.

Two investigations dealt with the *O. niloticus* population of Lake Victoria: One on its origin and the other on its purity in regard to phenomes passed by hybridization with native tilapias. The genetic analyses showed that this introduced population no doubt had multiple origins, composed in part of individuals from the Nile basin (probably Lake Edward) and in part of individuals from Kenya (probably Lake Turkana). These works also showed that the *O. niloticus* population of the lake, considering its multiple origins, had lost a great deal of its genetic variability. We can assume that the effective sizes of the introduced populations, that is, the number of individuals which effectively contributed to the next generation, was low. WAPLES (1991) showed that the mortality following the release of cultured individuals can be very high. In this case, *O. niloticus* was very likely introduced from cultured stocks. We also have no idea of the speed of expansion of the introduced population which may have been low from the beginning. This speed as shown by NEI *et al.* (1975) can also have played a role in the observed loss of variability.

We also have observations on the genetic variability of the *O. esculentus* population of Lake Kanyaboli. It seems almost certain that this population suffered no introgression of genes from *O. niloticus*. However, its low genetic variability may have been caused by a bottleneck during the colonization of the lake.

These early data lead to the assumption that *O. niloticus* must have taken the place of the two native species by competition rather than by hybridization. This hypothesis is yet to be confirmed.

The last two examples do not concern hybridization but are studies of the introduced populations themselves.

The Bouaké strain of *Oreochromis niloticus* was released in a great many countries, but first and foremost in all the waterways of Côte d'Ivoire. ROGNON (1993) studied the genetic variability of this strain at its site of origin, the Idessa aquacultural station in Bouaké (Côte d'Ivoire) and certain feral populations from two large rivers in Côte d'Ivoire: that from Lake Buyo on the Sassandra River and that from Lake Kossou on the Bandama River. In both cases, voluntary seeding with large numbers of fingerlings (to increase fishing activities) was carried out (the exact numbers are not known). ROGNON's study (1993) was based on the observation of thirty enzymatic loci. The Bouaké strain is characterized by a heterozygosity rate of 7.1% and a polymorphism P99% rate of 26.7% (P95%=23.3). The Buyo and Kossou populations possess comparable values to those of the strain they are issued from 8.7 and 5.8% respectively for the H values, 23.3 and 26.7 respectively for the P99% values (23.3 and 16.7 for P95%).

We can therefore state that, concerning the allozymes, there was no notable loss of genetic variability during the transfer and adaptation phases of this strain to the natural environment.

Another fairly well documented case concerns *Limnothrissa miodon*. This Clupeidae originates in Lake Tanganyika. In the hope of increasing fish culture production in Lake Kivu, 57,400 clupeidae (in part *Limnothrissa miodon* but also some *Stolothrissa tanganicae*) were introduced from Lake Tanganyika in 1959. Only *Limnothrissa miodon* succeeded in establishing itself in Lake Kivu. HAUSER *et al.* (1995) recently published a study in which they compare the transplanted population to the original population.

They performed a morphologic study, but also genetics with allozymes and DNAmt. They saw no statistically significant changes in the allozymic diversity: no differences between samples from Lake Tanganyika and those from Lake Kivu for heterozygosity levels, for the number of mean alleles, or for the percentage of polymorphous loci. Ninety-eight percent of the genetic variation can be attributed to sample variation and 0.26% to the differentiation between lakes. For the mtDNA analysis, they amplified a fragment of 2.5 Kb from the ND 5/6 region. They found 85 different haplotypes in 363 analyzed fish. The nucleotidic diversity like the number of haplotypes were significantly lower in samples from Lake Kivu which indicates a decrease in the genetic diversity of the mtDNA in the population of introduced fish.

This decrease in mtDNA variability following the phenomenon of introduction may, as we have already seen, come from a low effective size of the founding population and from the time required by this population to achieve a sufficient effective size (NEI *et al.*, 1975). In this case, it is highly likely that *Limnothrissa miodon* because of its very high fecundity, took very little time to recover its important effective size. It follows that the loss of mitochondrial variability observed in Lake Kivu is probably due entirely to a low effective size in the founding population. We might be surprised if we considered the large number of fish introduced (57,400). However, these fish represented two different species (*Limnothrissa miodon* and *Stolothrissa tanganicae*) and we do not know the proportion of *Limnothrissa miodon* in this first population. A calculation suggests that of the 57,400 fish introduced, only a maximum of 150 could be at the origin of the current population. Considering that 24 different haplotypes were observed in Lake Kivu, we can assume that at least 24 females were in this founding population (if we exclude the apparition of new haplotypes by mutation).

Of course it is difficult to cite an exact number when speaking of the effective size of this founding population, but it is almost certain that this number can be counted by tens, not hundreds.

Conclusion

It is probably too early, considering the small number of studies performed, to draw general conclusions about the genetic impact of fish introductions in Africa. A large number of populations remains to be analyzed. These studies are often difficult to carry out because the founding populations are from far-away countries, because data on introductions are not always available, because several genetic techniques must be used simultaneously to correctly estimate a situation. Certain phenomena like hybridizations are transitory and it is not always possible to be there when you should be. It is probable that concerning this subject, the use of museum collections can sometimes aid in going backwards in time.

Two of the first results presented (*O. niloticus* from Lake Victoria and *L. miodon* from Lake Kivu) show losses in genetic variability in introduced populations. Evidently, even when introducing several thousand individuals, the founding population may be made up of only tens of individuals.

It is obvious that if it is easy enough to theoretically predict the possible repercussions of introductions and transfers of species, it is much more difficult to predict the evolution of any particular case. The different behaviors of two confrontations between *O. niloticus* and *O. mossambicus* in Lakes Itasy and Ihema is an example.

References

ARTHINGTON (A. H.), 1991 — Ecological and Genetic impacts of introduced and translocated freshwater fishes in Australia. *Can. J. Fish. aquat. Sci.*, 48 (suppl. 1): 33-43.

DAGET (J.), MOREAU (J.), 1981 — Hybridation introgressive entre deux espèces de sarotherodon (Pisces, Cichlidae) dans un lac de Madagascar. *Bull. Mus. Natn. Hist. Nat., 4e sér.* 3, section A, 2: 689-703.

FERGUSSON (M.M.), DRAHUSHCHUK (L.R.), 1990 — Disease resistance and enzyme heterozygosity in rainbow trout. *Heredity*, 64: 413-417.

HAUSER (L.), CARVALHO (G.R.), PITCHER (T.J.), 1995 —

Morphological and genetic differentiation of the African clupeid Limnothrissa miodon 34 years after its introduction to lake kivu. *J. Fish Biol.*, 47 (Supp. A): 127-144.

MATHER (P.B.), ARTHINGTON (A.H.), 1991 —
An Assessment of genetic differentiation among feral Australian Tilapia populations. *Aus. J. mar. Freshwat. Res.*, 42: 721-728

MICHA (J.C.), CUVELIER (R.), TILQUIN (C.), MURAILLE (B.), BOURGEOIS (M.), FALTER (U.), 1996 —
Comparative growth of hybrids (F1, F2 anf F3) of *Oreochromis niloticus* (L.) and *O. macrochir* (Blgr). p. 354-360. *In* PULLIN (R.S.V.), LAZARD (J.), LEGENDRE (M.), AMON KOTHIAS (J.B.), PAULY (D.), ed.: *the third international Symposium on Tilapia in Aquaculture.* Iclarm conf. Proc. 41, 575p.

NEI (M.), MARUYAMA (T.), CHAKROBORTY (R.), 1975 —
The bottleneck effect and genetic variability in populations. *Evolution*, 29: 1-10.

OGUTU-OHWAYO (R.), 1990 —
The decline of the native fishes of Lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the nile tilapia, *Oreochromis niloticus. Env. Biol. Fishes*, 27: 81-96.

ROGNON (X.), 1993 —
Diversité génétique et relations phylogénétiques chez les tilapias (Pisces, cichlidae). Comparaison des données du polymorphisme enzymatique et mitochondrial. PhD thesis, université de Paris Sud Orsay, France.

WAPLES (R.S.), 1991 —
Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest.

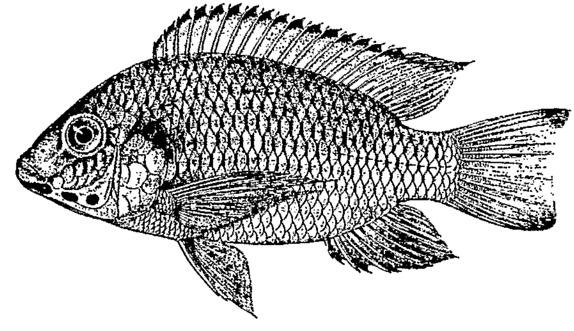
Can. J. Fish. aquat. Sci., 48 (suppl. 1): 124-133.

WELCOMME (R.L.), 1967 —
Observation on the biology of the introduced species of tilapia in Lake victoria. *Rev. Zool. Bot. Afr.*, LXXVI, 3-4: 249-279.

WELCOMME (R.L.), 1988 —
International introductions of inland fish species. FAO Fish. Tech. Pap. No. 294, 120 pp

Cichlids

part 2



An overview of the biological diversity and culture of tilapias (Teleostei, Cichlidae)

Germain Gourène
Ichthyologist

Guy G. Teugels
Ichthyologist

Introduction

Tilapias are African fishes currently used in warm water aquaculture throughout the world. The idea of introducing and developing tilapia culture in Africa arose around the forties, especially in the Belgian Congo (now the Democratic Republic of Congo) because of the difficulties in food supply caused by the Second World War. The first attempts were held at the Kipopo Station of the National Institute for Agronomic Studies of the Belgian Congo (DE BONT, 1950). Since then, and after numerous experiments with many different species (for a review see TEUGELS and THYS VAN DEN AUDENAERDE, 1991 ; TREWAVAS and TEUGELS, 1991a ; TREWAVAS and TEUGELS, 1991b), African tilapia culture has is presently mostly focused on one species, *Oreochromis niloticus* or the Nile tilapia. This herbivorous microphagous species is well suited to the low-technology culture systems used in developing countries (PULLIN, 1988).

Tilapia are ranked fourth in world fishculture, behind carps (Cyprinidae), salmonids and milk fish (*Chanos chanos*, Chanidae) (LAZARD and LEGENDRE, 1994). The estimated production is 500 000 metric tons (GARIBALDI, 1996). Tilapia culture has progressed considerably during the last ten years in certain countries, especially China, Thailand and the Philippines (PULLIN, 1988). In fact, the Philippines imported the first *Oreochromis niloticus* breeders in 1975, and now produce 70 000 metric tons per year (LAZARD *et al.* 1991). However, the culture of these fishes in their native continent has not been developed well, as is the case for African fishculture in general. Africa has produced 56 344 metric tons of cultured fish, in the global total of 11.1 million metric tons (GARIBALDI, 1996). Most of this African production consist of tilapias.

Biological Diversity and Systematic Considerations of Tilapias

Tilapias belong to the Cichlidae which, in number of species, is the third largest family of fishes out of a total of 482 families, after the Cyprinidae and the Gobiidae (NELSON, 1994). The Cichlidae, like the other two families cited, has more than 400 species (NELSON, 1994). According to TEUGELS et THYS VAN DEN AUDENAERDE (1992), the Cichlidae, belonging to the sub-order Labroidei and the order Perciformes, is particularly characterized by a single nostril on each side of the head. The body, of variable form but never very elongated, is rather compact and covered with cycloid or ctenoid scales. All fins are present. The inferior pharyngeal bones are fused, forming a bony, toothed triangle. The Cichlidae, freshwater or sometimes brackish water fishes, are found in Central and South America (one species ranges as far north as northern Texas). They are also found in the West Indies, and in the coastal zone in Madagascar, Sri Lanka, Israel, Syria, and of course, in Africa (NELSON, 1994 ; TEUGELS and THYS VAN DEN AUDERNAERDE,

1992). According to the most recent list, the Cichlidae family has 143 genera on the African continent (DAGET *et al.*, 1991).

Until recently the tilapias formed a single taxonomic unit, a generic rank, in the Cichlidae : *Tilapia* (*sensu lato*). Based on ethological characteristics, later supported by morphological and osteological criteria, TREWAVAS (1983) classified the tilapias in four genera belonging to the sub-family Tilapiinae. These are : *Tilapia* Smith, 1840 *sensu stricto*, *Sarotherodon* Rüppell, 1882 and *Oreochromis* Günther, 1889 and *Danakilia* Thys van den Audenaerde, 1969. The first three are of interest to aquaculture. They are briefly discussed below.

Genus Tilapia *Smith, 1840*

The genus *Tilapia s.s.* only contains the species that fix their eggs on a substrate, contrary to the other genera which perform oral incubation. Besides this ethological characteristic, the *Tilapia* species differ from other tilapias by the inferior pharyngeal bone which is as long as it is wide with the anterior point shorter than the toothed section ; the posterior pharyngeal teeth are bicuspid or tricuspid (sometimes quadricuspid). There are a maximum of 17 gill rakers on the lower part of the first branchial arch *versus* 28 in the other genera. Thirty-eight species are currently recognized (TEUGELS et THYS VAN DEN AUDENAERDE, 1991 ; STIASSNY *et al.*, 1992). Several of these have been tested in aquaculture (see TEUGELS and THYS VAN DEN AUDENAERDE, 1991 for a bibliographic review), but introduction trials have mostly been based on three species : *Tilapia rendalli* (Boulenger, 1896) and *T. zillii* (Gervais, 1848) in freshwater and *T. guineensis* (Bleeker, 1862) in brackish water.

The results obtained were not very satisfactory, in part due to their mode of reproduction. A brief description of the three species is given below. The characteristics used are from THYS VAN DEN AUDENAERDE (1970) and from TEUGELS and THYS VAN DEN AUDENAERDE (1992).

Tilapia rendalli is a deep bodied fish with convex dorsal and ventral surfaces (body depth considerably greater than that of

T. zillii). The caudal fin is clearly truncated (the end seems to have been cut). The eggs are yellow. Adults are colored olive green, darker on the back ; the chest and belly are a dirty white marked with black and cherry-red spots which extend to the cheeks and the lower flanks ; the lower part of the caudal fin is reddish (yellow), the upper part is greenish or marked ; dark vertical bars may appear on the flanks. In young fish (about 5 cm), the pelvic fins are orange in *T. rendalli* and without color in *T. zillii*. The natural distribution of *T. rendalli* includes the Shaba, the Upper Kasai and the Lualaba system in Congo, Lakes Tanganyika and Malawi, the Zambesi, the coastal region from the Zambesi delta to Natal, the Okavango and the Cunene. The estimated production of this species in 1994 was 868 metric tons, of which 803 metric tons were produced in Africa (GARIBALDI, 1996).

Tilapia zillii (Figure 1) is usually marked with two horizontal dark bands (when stressed), one on the lateral line, the other near the back ; these are crossed by vertical bars and spots appear at the intersections ; the caudal fin is covered with a grayish network with pale interstices ; the dorsal formula is XIV-XVI.10-14, mean XV.12 ; sub-truncated rounded caudal fin ; the eggs are green.

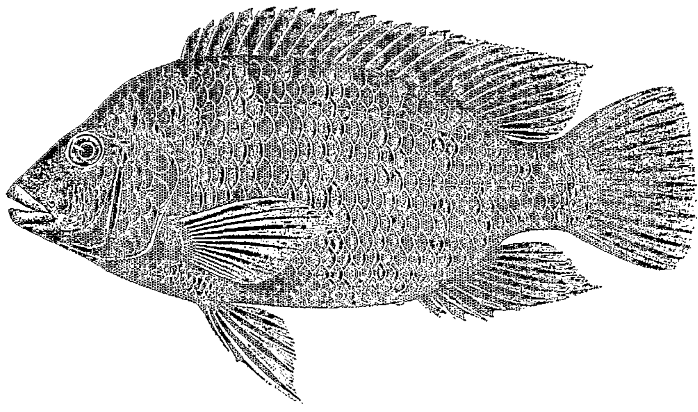


Figure 1
Tilapia zillii (from BOULENGER, 1901).

The natural distribution of *Tilapia zillii* covers southern Morocco, the Sahara, the Nilo-Sudanian basins (Senegal, Niger, including the Benue, Volta, Chad, Nile), the Sassandra, Bandama and Comoe basins in Côte d'Ivoire, part of the Congo basin (Ubangi, Uele, Ituri), and Lakes Albert and Turkana. Eighteen metric tons of *T. zillii* were produced in 1994 on the African continent.

Tilapia guineensis generally has a strongly sloped head profile ; the dorsal profile is convex and the ventral profile is horizontal. The dorsal formula is XVI-XVI. 12-13. Bright and highly visible colors, with mixes of deep blue green, copper green, deep black and zones of bright cherry red on the lower parts of the head and body ; these colors change considerably according to the physiological state and the sexual maturity of the individual. The natural distribution of *Tilapia guineensis* covers the coastal zones (fresh and brackish waters) from the Senegal River to the mouth of the Cuanza in Angola. No data were found on the production of this species.

Genus Oreochromis *Günther, 1889*

The genus *Oreochromis* contains the species where oral incubation is exclusively practiced by the females. Added to this is the reduced size of the belly scales compared to the size of the scales on the flanks ; the genital papilla is well developed in both sexes ; the inferior pharyngeal bone is longer than or as long as it is wide ; its toothed part is as long as or a little longer than its anterior part ; the posterior pharyngeal teeth are bicuspid, or with a reduced inferior cusp or without clear cusp (TEUGELS and THYS VAN DEN AUDENAERDE, 1992).

Thirty-three species of *Oreochromis* exist in Africa. Several of these have been tested in aquaculture (see TREWAVAS and TEUGELS, 1991a, for the bibliographic review). Note that *O. niloticus* is the most polymorphic species and the species most used in aquaculture. It is distinguished from the other taxa in the same genus by a caudal fin with regular black vertical bands all along its length. The species contains 8 subspecies, of which three, according to PULLIN (1988), have been cultured (*O. niloticus niloticus*, *O. n. eduardianus* and *O. n. vulcanis)*. *O. niloticus* (Figure 2) is found naturally in the

coastal basins of Israel, the Nilo-Sudanian basins and in numerous East African lakes. Its global production is estimated at 426 773 metric tons of which 27 162 are produced on the African continent(GARIBALDI, 1996).

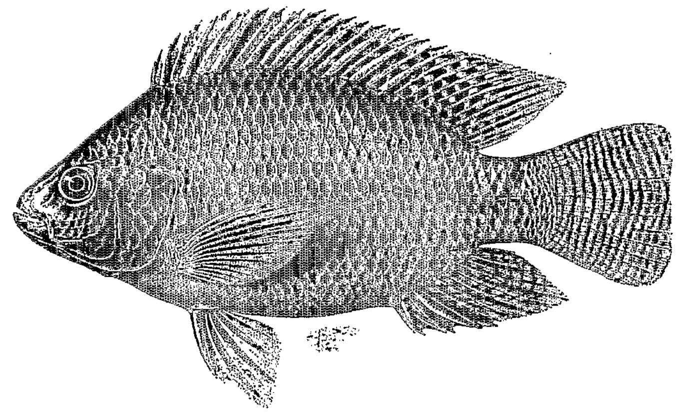


Figure 2
Oreochromis niloticus (from BOULENGER, 1907).

GARIBALDI (1996) cites six other species used in aquaculture : *Oreochromis mossambicus* (51 870 metric tons of which 55 in Africa), *O. aureus* (11 871 metric tons of which 74 in Africa), *O. andersonii* (2 200 metric tons produced in Africa), *O.macrochir* (350 metric tons produced in Africa), *O. spilurus* and *O. urolepis* (no production data).

Genus Sarotherodon *Rüppell, 1852*

The genus *Sarotherodon* contains the species where both males and females practice oral incubation. Beside this ethological characteristic, they are distinguished by belly scales which are of

almost the same size as those of the flanks ; the genital papilla of the male is smaller ; the inferior pharyngeal bone is longer than or as long as it is wide and its toothed part is shorter than the anterior part ; the posterior pharyngeal teeth are bicuspid or with a reduced inferior cusp or without clear cusp. Ten species belong to this genus. Two of these have been used in numerous aquacultural studies : *S. galilaeus* and *S. melanotheron* (Figure 3). The bibliographic review is given by TREWAVAS and TEUGELS (1991b). They are distinguished from each other by the number of gill rakers on the inferior part of the first branchial arch : (18) 19-27 for *S. galilaeus*, 12-19 (20) for *S. melanotheron*. They are also the most polymorphic with 5 subspecies each. The natural distribution of *S. galilaeus* extends from Jordan to the Congo basin. *Sarotherodon melanotheron* is a brackish water species, found from the mouth of the Senegal to the mouth of the Congo.

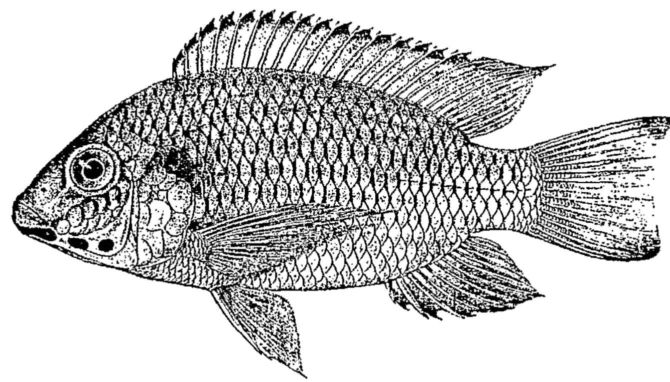


Figure 3
Sarotherodon melanotheron (from BOULENGER, 1915).

In total, 82 species of tilapia are present on the African continent. They are split among the genera *Sarotherodon* (12.2%), *Oreochromis* (40.2%), *Danakilia* (1.2%) and *Tilapia* (46.3%). When referring to the mode of reproduction of these fishes, we see that 54% of the tilapia species practice oral incubation against 46%

substrate spawners. A review of the geographical distribution of different tilapia species (DAGET *et al.*, 1991) shows clearly that the species with the greatest natural distribution are the ones that are currently used in aquaculture. In fact, a wide distribution confers to a species, without any doubt, a greater capacity to adapt to various types of environmental conditions. These adaptations, expressed in an aquaculture situation in terms of zootechnical performance, are an additional advantage.

References

BOULENGER (G.A.), 1901 — *Les poissons du bassin du Congo.* Bruxelles: xii + 532 p.

BOULENGER (G.A.), 1907 — Zoology of Egypt. The fishes of the Nile published for the Egyptian Government. 2 vol., London: 578 p.

BOULENGER (G.A.), 1915 — Catalogue of the fresh-water fishes of Africa in the British Museum (Natural History). London, vol.3: 526 p.

DAGET (J.), GOSSE (J.P.), TEUGELS (G.G.), THYS VAN DEN AUDENAERDE (D.F.E.), (Eds), 1991 — Check-List of the Freshwater Fishes of Africa. Cloffa IV. ISNB (Bruxelles), Mrac (Tervuren), Orstom (Paris), 740 p.

DE BONT (A.), 1950 — Rapport annuel 1947-1948 de la station de recherches piscicoles. *Bull. agr. Congo belge.* 41 (2): 473-538.

GARIBALDI (L.), 1996 — List of animal species used in aquaculture. *FAO, Fish. Circul.*, 914: 1-38.

LAZARD (J.), LECOMTE (Y.), STOMAL (B.), WEIGEL (J.Y.), 1991 — Pisciculture en Afrique subsaharienne. *Rapport d'étude, Ministère de la Coopération et du Développement*, 129 p.

LAZARD (J.), LEGENDRE (M.), 1994 — La pisciculture africaine: enjeux et problèmes de recherches. *Cahiers Agric.*, 3: 83-92.

NELSON (J.S.), 1994 — Fishes of the World. 3rd édition.J. Wiley et Sons, Inc. (New York), 541 p.

PULLIN (R.S.V.), 1988 — Ressources génétiques en tilapias pour l'agriculture. Atelier, 23 - 24 Mars 1987. Bangkok, Thaïlande, 129 p.

STIASSNY (M.L.J.), SCHLIEUWEN (U.K.), DOMINY (W.J.), 1992 — A new species flock of cichlid fishes from Lake Bermin, Cameroon with a description of eight new species of *Tilapia* (Labroidei: Cichlidae). *Ichth. Explor. Freshwaters*, 3 (4): 311-346.

TEUGELS (G.G.), THYS VAN DEN AUDENAERDE (D.F.E.), 1991 — *Tilapia. In*: Check-List of the Freshwater Fishes of Africa. Cloffa IV. DAGET (J.), GOSSE (J.P.), TEUGELS (G.G.), THYS VAN DEN AUDENAERDE (D.F.E.), (Eds).ISNB (Bruxelles),

Mrac (Tervuren), Orstom (Paris), pp 482 - 508.

TEUGELS (G.G.), THYS VAN DEN AUDENAERDE (D.F.E.), 1992 — Cichlidae. *In* : Faune des poissons d'eaux douces et saumâtres d'Afrique de l'Ouest. LEVEQUE (C.), PAUGY (D.), TEUGELS (G.G.), (Eds). Mrac (Tervuren)/Orstom (Paris). Tome II, pp 714 - 779.

THYS VAN DEN AUDENAERDE (D.F.E.), 1970 — Bijdrage tot een systematische en bibliografische monografie van het genus *Tilapia* (Pisces, Cichlidae). Unpublished Thesis, Rijksuniversiteit Gent.

TREWAVAS (E.), 1983 — Tilapiine fishes of the genera *Sarotherodon*, *Oreochromis* and *Danakilia*. British Museum (Natural History), London 583 p.

TREWAVAS (E.), TEUGELS (G.G.), 1991a — *Oreochromis*. *In:* Check-List of the Freshwater Fishes of Africa. Cloffa IV. DAGET (J.), GOSSE (J.P.), TEUGELS (G.G.), THYS VAN DEN AUDENAERDE (D.F.E.), (Eds). ISNB (Bruxelles), Mrac (Tervuren), Orstom (Paris), pp 307 - 346.

TREWAVAS (E.), TEUGELS (G.G.), 1991b — *Sarotherodon*. *In*: Check-List of the Freshwater Fishes of Africa. Cloffa IV. DAGET (J.), GOSSE (J.P.), TEUGELS (G.G.), THYS VAN DEN AUDENAERDE (D.F.E.), (Eds). ISNB (Bruxelles), Mrac (Tervuren), Orstom (Paris), pp 425 - 437.

Hemoglobin variations in some tilapiine species (Teleostei, Cichlidae) of the genera *Oreochromis* and *Sarotherodon*

Thomas M. Falk
Geneticist

Eddie K. Abban
Geneticist

Wolfgang Villwock
Evolutionary Biologist

Lothar Renwrantz
Immunologist

Hemoglobin multiplicity in its broad sense has been well demonstrated in freshwater and marine teleost fishes including tilapias (CHEN and TSUYUKI, 1970; HINES *et al.*, 1971; FYHN *et al.*, 1979; PEREZ and RYLANDER, 1985; VAL *et al.*,1987; OBERST *et al.*, 1989; WEBER, 1990; MACARANAS *et al.*, 1996; FALK *et al.*, 1996, FALK *et al.*, in press). Our present studies on these oxygen binding molecules of 5 tilapiine species of the genera *Oreochromis* and *Sarotherodon* (*Oreochromis andersonii*, *O. aureus*, *O. niloticus*, *Sarotherodon galileus*, *S. melanotheron*) provide evidence indicating the occurrence of hemoglobin and globin chain variations within and among the species under study. In addition, some basic biochemical characteristics of these molecules, like their molecular weights (mws), isoelectric points (pIs), N-terminal globin chain sequence data and subunit compositions are presented.

Characteristically, all species investigated have been found to reveal highly heterogeneous hemoglobin phenotypes. On average about 23 distinguishable hemoglobins were detected per species by thin layer isoelectric focusing indicating the presence of multiple globin chain variants involved in the composition of different tetrameric hemoglobin types. Their estimated pIs ranged between pH 5.94 and pH 8.06. Moreover, variations of hemoglobin types among and within tilapia species have been observed resulting in the identification of species (*Oreochromis andersonii*, *O. aureus*, *O. niloticus*, *Sarotherodon galileus*, *S. melanotheron*), subspecies (*O. niloticus sugutae*) and hybrid (*O. niloticus* x *O. andersonii*) characteristic hemoglobin patterns.

This diversity of tilapia hemoglobins was shown to result from the occurrence of different types of globin chains. By acidic urea PAGE a total of seven major α-chains and five major β-chains could be identified and characteristic globin chain variants were found to occur. The species involved each showed two major α-chains and four (*O. andersonii*, *S. galileus*, *S. melanotheron*) or five (*O. aureus*, *O. niloticus*) β-chain variants. The mws of these monomeric hemoglobin types were estimated to range between 16.3 and 17.6 kDa indicating a molecular mass of about 65 to 70 kDa in their originally tetrameric form. These estimates were in agreement with results obtained by gel filtration chromatography for the tetrameric molecules (67 and 69 kDa).

According to MIED and POWERS (1978) the theorical number of hemoglobin tetramers of a given species may be calculated by combination of possible α/β-dimers, although some of them may be expected to exhibit unstable quaternary structures. Considering the relatively high number of postulated hemoglobin tetramers in the tilapiine species studied here one could expect the occurrence of pairs of identical α/β-dimers (symmetric tetramers) as well as associations of differently composed α/β-units (asymmetric tetramers) comprising a maximum of 4 different globin chains. This assumption was supported by extraction of particular hemoglobins obtained by isoelectric focusing of hemolysates and their subsequent analysis by acidic urea PAGE. Tetrameric hemoglobin variants were found to consist of doublets of identical α- and β-chains ($\alpha_2\beta_2$, symmetric tetramers), or combinations of three

($\alpha_2\beta\beta^*$; $\alpha\alpha^*\beta_2$) or four ($\alpha\alpha^*\beta\beta^*$) distinct chains (asymmetric tetramers). It is interesting to note that the majority of tilapia hemoglobins analysed could be considered to be asymmetrically composed.

Finally, globin chains of *O. niloticus* were isolated and subjected to partial N-terminal amino acid sequencing (pos. 1-40). The N-termini of both major α-chains were found to be blocked, a known charasteristic feature for a-chains of teleost fishes (HILSE and BRAUNITZER, 1968; POWERS and EDMUNDSON, 1972a,b; BOSSA *et al.*, 1976; D'AVINO *et al.*, 1990). Among the three major β-chains investigated (β_{2ONi}, β_{4ONi}, β_{5ONi}) amino acid replacements have been observed in positions 9, 12, 21 and 29 and a micro-heterogeneity has been found at amino acid position 12 of the β_{4ONi}-chain where Thr and Ala were detected in equal amounts. In comparison to known β-globin chain sequences of different fish species (GRUJIC-INJAC *et al.*, 1980; RODEWALD and BRAUNITZER, 1984; PETRUZELLI *et al.*, 1984) our data obtained for the β-chains of *O. niloticus* confirmed their β-chain identity and thus, supported our α/β chain notation for the globin chain variants of tilapias.

Our findings outlined here also demonstrate some interesting applications for hemoglobin and globin chain studies. First of all, the basic taxonomic issue of morphological species characterization and discrimination (THYS VAN DEN AUDENAERDE, 1970; TREWAVAS, 1983; TEUGELS and THYS VAN DEN AUDENAERDE, 1992) could be supported or refined by standardized hemoglobin and globin chain analysis techniques, in particular in case of morphologically similar species. Secondly, natural (POUYAUD, 1994) or artificial (TREWAVAS, 1983) interspecific hybridization events could be recognized and verified by hemoglobin and globin chain studies, an important application for fisheries management, aquaculture and the protection of small endangered tilapia populations. Finally, a further completion of population genetic studies and researches on aquacultural strains by hemoglobin and globin chain data could be of great future interest, since population characteristic differences have recently been described in tilapias and striking divergent functional properties among individual hemoglobin components have already been demonstrated in fish (HASHIMOTO *et al.*, 1960; BINOTTI *et al.*, 1971; POWERS, 1972;

POWERS and EDMUNDSON, 1972a,b; GILLEN and RIGGS, 1973b; BRUNORI, 1975; WEBER and DE WILDE, 1976; DI PRISCO and TAMBURRINI, 1992). This probably adaptive feature should be considered important especially, in regard to the variety of tilapia culture systems used to date. Moreover, it remains to be elucitated if these multiple hemoglobin types are an expression of optimum physiological adaptation to varying environmental conditions. As pointed out by PEREZ and MACLEAN (1976) it has to be considered that in tropical aereas water temperature and oxygen tensions are very variable and often fishes have to compete with an increasing osmotic pressure.

References

BINOTTI (I.), GIOVENCO (S.), GIARDINA (B.), ANTONINI (E.), BRUNORI (M.), WYMAN (J.), 1971 —
Studies on the functional properties of fish hemoglobins. 2. The oxygen equilibrium of the isolated hemoglobin components from trout blood.
Arch. Biochem. Biophys. 142. 274-280.

BOSSA (F.), BARRA (F.), COLETTA (M.), MARTINI (F.), LIVERZANI (A.), PETRUZZELLI (R.), BONAVENTURA (J.), BRUNORI (M.), 1976 —
Primery structure of hemoglobins from trout (*Salmo irideus*). Partial determination of amino acid sequence of Hb trout IV.
FEBS Lett., 64: 76-80

BRUNORI (M.), 1975 —
Molecular adaptations to physiological requirement: The hemoglobin system of trout.
Curr. Top. Cell. Regul. 9, 1-39.

CHEN (F.Y.), TSUYUKI (H.), 1970 —
Zone electrophoretic studies on the proteins of *T. mossambica* and *T. hornorum* and their F1 hybrids, *T. zillii* and *T. melanopleura*.
J. Fish Res. Board Can., 27: 2167-2174

D'AVINO (R.), CAMARDELLA (L.), CARRATORE (V.), DI PRISCO (G.), 1990 —
The amino acid sequence of the α-chain of Hb 2 completes the primary structure of the hemoglobins of the antarctic fish *Nothothenia coriiceps neglecta*.
Comp. Biochem. Physiol., 97B: 803-807.

DI PRISCO (G.), TAMBURRINI (M.), 1992 —
Mini review: The hemoglobins of marine and freshwater fish: The search for correlations with physiological adaptation.
Comp. Biochem. Physiol., 102B: 661-671.

FALK (T.M.), ABBAN (E.K.), VILLWOCK (W.), RENWRANTZ (L.), *in press* —
Species and subspecies characteristic hemoglobins in some tilapiine fishes and a comparative study on their globin chains. *Aquaculture International.*

FALK (T.M.), ABBAN (E.K.), OBERST (S.), VILLWOCK (W.), PULLIN (R.S.V.), RENWRANTZ (L.), 1996 — *A biochemical laboratory manual for species characterization of some tilapiine fishes.* Iclarm Educ. Ser. 17, Manila, Phillippines, 93p.

FYHN (U.E.H.), FYHN (H.J.), DAVIS (B.J.), POWERS (D.A.), FINK (W.L.), GARLICK (R.L.), 1979 — Hemoglobin heterogeneity in Amazonian fishes. *Comp. Biochem. Physiol.*, 62A: 39-66.

GILLEN (R.G.), RIGGS (A.), 1973 — Structure and function of the isolated hemoglobins of the american eel, *Anguilla rostrata. J. Biol. Chem.* 248: 1961-1969.

GRUJIC-INJAC (B.), BRAUNITZER (G.), STANGL (A.), 1980 — Hemoglobins, XXXV: the sequence of the β_A- and β_B-chains of the hemoglobins of the carp (*Cyprinus carpio*). *Hoppe-Seyler's Z physiol. Chem.*, 361: 1629-1639.

HASHIMOTO (K.), YAMAGUSHI (Y.), MATSUURA (F.), 1960 — Comparative studies on two hemoglobins of salmon. IV. Oxygen dissociation curve. *Bull. Jpn. Soc. Sci. Fish.* 26: 827-834.

HILSE (K.), BRAUNITZER (G.), 1968 — Die Aminosäuresequenz der a-Ketten der beiden Hauptkomponenten des Karpfenhämoglobins. *Hoppe-Seyler's Z physiol. Chem.*, 349: 433-450.

HINES (R.), YASHOUV (A.), WILAMOVSKI (A.), 1971 — Differences in the electrophoretic mobility of the hemoglobins of *T. aurea*, *T. vulcani* and their hybrid cross. *Bamidgeh,* 23: 53-55.

MACARANAS (J.M.), AGUSTIN (L.Q.), EKNATH (A.E.), 1996 — Multiple haemoglobins in three tilapiine species of the genus *Oreochromis* and in eight strains of *O. niloticus* (L.). *Aquaculture Research*, 27: 597-601.

MIED (P.A.), POWERS (D.A.),1978 — Hemoglobins of the killifish *Fundulus heteroclitus*. Separation, characterization and a model for subunit compositions. *J. Biol. Chem.*, 253: 3521-3528.

OBERST (S.), VILLWOCK (W.), RENWRANTZ (L.), 1989 — Antisera from Tilapia species to differentiate among erythrocytes from *T. aurea*, *T. galilaea* and *T. nilotica* by agglutination assays, and a comparative analysis of hemoglobins. *J. Appl. Ichthyol.*, 1: 18-27.

PEREZ (J.E.), MACLEAN (N.), 1976 — Multiple globins and haemoglobins in four species of grey mullet (Mugilidae, Teleostei). *Comp. Biochem. Physiol.*, 53B: 465-468.

PEREZ (J.E.), RYLANDER (M.K.), 1985 — Hemoglobin heterogeneity in Venezuelan fishes. *Comp. Biochem. Physiol.*, 80B: 641-646.

PETRUZZELLI (R.), BARRA (D.), GOFFREDO (B.M.), BOSSA (F.), COLETTA (M.), BRUNORI (M.), 1984 — Amino acid sequence of ß-chain of hemoglobin IV from trout (*Salmo irideus*). *Biochim. Biophys. Acta*, 789: 69-73.

POUYAUD (L.), 1994 — *Génétique des populations de tilapias d'intérêt aquacole en Afrique de l'Ouest. Relations phylogénétiques et structuration populationnelles.* Ph. D. thesis, université de Montpellier-II, France.

POWERS (D.A.), 1972 — Hemoglobin adaptation for fast and slow habitats in sympatric catostomid fishes. *Science*, 177: 360-362.

POWERS (D.A.), EDMUNDSON (A.B.), 1972a —
Multiple hemoglobins in Catostomid fish. 1. Isolation and characterization of the isohemoglobins from *Catostomus clarkii*.
J. Biol. Chem., 247: 6686-6693.

POWERS (D.A.), EDMUNDSON (A.B.), 1972b —
Multiple hemoglobins in Catostomid fish. 2. The amino acid sequence of the major α-chain from *Catostomus clarkii* hemoglobins. *J. Biol. Chem.*, 247: 6694-6707.

RODEWALD (K.), BRAUNITZER (G.), 1984 —
Die Primärstruktur des Hämoglobins vom Goldfisch (*Carassius auratus*). *Hoppe-Seyler's Z Physiol. Chem.*, 365: 95-104.

TEUGELS (G.G.), THYS VAN DEN AUDENAERDE (D.F.E.), 1992 —
Cichlidae. *In: Faune des poissons d'eau douces et saumâtres de l'Afrique de l'Ouest.* LEVEQUE (C.), PAUGY (D.), TEUGELS (G.G.), eds. Collection Faune Tropicale 28. Orstom Editions/Mrac, 714-779.

THYS VAN DEN AUDENAERDE (D.E.F), 1970 —
An annoted bibliography of tilapia (Pisces: Cichlidae). Documentation Zoologique, Musée Royal d'Afrique Centrale 14.

TREWAVAS (E.), 1983 —
Tilapiine fishes of the genera Sarotherodon, Oreochromis *and* Danakilia. British Museum (Natural History), London.

VAL (A.L.), AIMEIDA-VAL (V.M.F.), MONTEIRO (P.J.C.), 1987 —
Aspectos biologicos de peixes amazonicos. IV. Padroes eletroforeticos de hemoglobinas de 22 especies coletadas na Ilha da Marchantaria (Manaaus-AM).
Acta Amazonica, 16/17: 125-134.

WEBER (R.E.), 1990 —
Functional significance and structural basis of multiple hemoglobins with special reference to ectothermic vertebrates. In: TRUCHOT (J.P.), LAHLOU (B.), ed.: *Animal nutrition and transport processes. 2. Transport, respiration and excretion: comparative and environmental aspects.* Comparative Physiology 6. Karger, Basel, pp 58-75.

WEBER (R.E.), DE WILDE (J.), 1976 —
Multiple hemoglobins in plaice and flounder and their functional properties.
Comp. *Biochem. Physiol.* 54B: 433-437.

Genetic differentiation among natural populations of the Nile tilapia *Oreochromis niloticus* (Teleostei, Cichlidae)

Jean-François Agnèse
Geneticist

Béatrice Adépo-Gourène
Geneticist

Laurent Pouyaud
Geneticist

Introduction

Among all tilapia species, the Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), originating in West and East Africa (TREWAVAS, 1983), is commercially the most important. It has been introduced into many Asian and South American countries.

The natural range of *O. niloticus* includes the Senegal, Gambia, Niger, Volta, Benue, Chari, Nile, and Awash Rivers and many lakes like those of the Rift Valley: Edward, George, Albert, Kivu, Tanganyika, Baringo, Turkana. TREWAVAS (1983) described seven subspecies, using morphometrical analysis: *O. niloticus niloticus* from West Africa and the Nile, *O. n. eduardianus* from Lake George, Edward and Tanganyika, *O. n. cancellatus* from the Awash River system in Ethiopia, *O. n. filoa* from the hot springs of

the Awash River, *O. n. vulcani* from Lake Turkana, *O. n. baringoensis* from Lake Baringo, *and O. n. sugutae* from River Suguta in Kenya. SEYOUM and KORNFIELD (1992a) described a new subspecies, *O. n. tana* from lake Tana in Ethiopia, using genetical (mitochondrial DNA) characteristics.

Even though *O. niloticus* has a wide distribution and a real economic importance, little is known about the genetic characterization of natural populations. This could be of great importance for the future development of aquacultural strains, for the protection of small endangered populations (like those of small lakes such as Baringo or the Suguta River), and for biogeographical inferences. In this study, investigations have been done using standardised techniques on natural populations from the major basins (the Senegal, Niger, Volta, Nile, Awash, and Suguta Rivers; Lakes Chad, Tana, Turkana, Edward, Baringo) and representing all the described subspecies.

Materials and methods

Sampling and allozyme study

Specimens of *O. niloticus* were collected from August 1993 to December 1994, in 17 locations : River Senegal at Dagana, River Niger at Selingue, River Niger at Bamako, Lake Volta at Akosumbo, Lake Chad near Karal, River Chari at N'Jamena, Lake Manzalla at Manzalla, River Nile at Cairo, Lake Tana at Bahar Dar, Hot springs of the Awash system at Sodore, Lake Koka at Koka, Lake Zyway at Meki, Lake Awasa at Awasa, Lake Turkana at Loyangalani, River Suguta at Kapedo, Lake Baringo at Kampi ya Samaki, Lake Edward at Mweya.

They were kept at -20°c for a few days and then maintained at -80°c for later analysis except for specimens from Lake Tana which were immediately preserved in alcohol. Specimens of *O. aureus*

come from a farmed strain (Lake Manzalla; Egypt). Standard horizontal starch gel (12%) electrophoresis was carried out to investigate the products of 25 loci. The stain recipes and buffer used were those described in POUYAUD and AGNESE (1995) and PASTEUR *et al.* (1987). The nomenclature is that proposed by SHAKLEE *et al.* (1990).

Amplification of the control region of mitochondrial DNA.

To amplify a 1 Kb fragment in the control region of mtDNA, HN20 and LN20 primers (BERNATCHEZ and DANZMANN, 1993) were used.

Digestion of the amplified products

Five to eight µl of the PCR-amplified control region was digested by 5 units of one of the restriction enzymes in a final volume of 20 µl containing the appropriate buffer.

Microsatellites

A total of 4 different primer sets for *Sarotherodon melanotheron* described by POUYAUD *et al.* (submited) have been used : SMEL1 (Gene Bank number U69153), SMEL2 (X99799), SMEL3 ((X99800), SMEL4 (X99801).

Analysis of the data

To analyze allozymic, microsatellites or RFLP data, different programs from Phylip (Phylip software package, Felsenstein, v. 3.5) were used: Consense, Mix, Genedist, Neighbor, Seqboot.

Results

Allozymes

Sixteen of the 25 loci studied were polymorphic. The rate of observed heterozygosity (H) was between 0.000 (Lake Baringo) and 0.045 (Volta River) and the rate of observed polymorphism (P95%) between 0.00 (Lake Baringo) and 0.08 (all West African and Nile populations except the Niger River at Selingue). These values are comparable to those obtained in previous studies of natural *O. niloticus* populations (SEYOUM and KORNFIELD, 1992b; ROGNON *et al.*, 1996) even if the loci analyzed were not the same as in the present study.

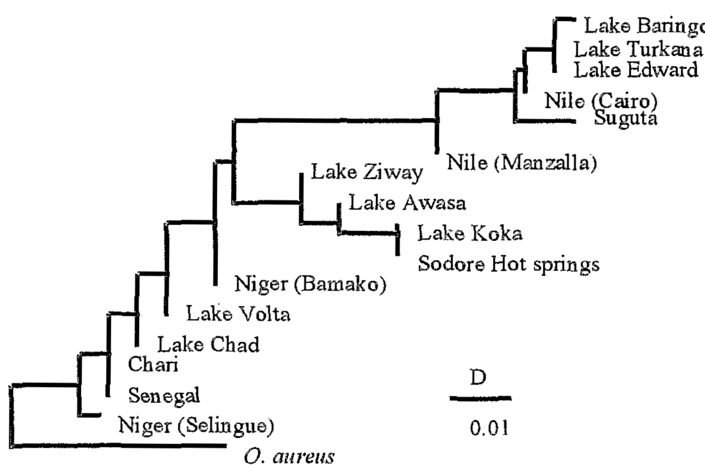


Figure 1
Network produced by Phylip on the 16 populations representing seven subspecies of *O. niloticus*. This is a consensus tree produced using Consense from 1000 trees produced using Seqboot and Neighbor. The number at each junction represent the frequency of its occurrence

To build a genetic network, a total of 1000 randomly modified frequency matrices were obtained using the program Seqboot. These matrices were then transformed into NEI's (1972) genetic distance matrices using the Genedist program. The corresponding trees were built by the programme neighbor and summarized into a single tree using with Consense (Fig. 1).

Populations are clustered in three major groups. One is composed of the Nile drainage (the Nile and Lake Edward), and the Kenyan Rift Valley populations (Lake Turkana, Lake Baringo and the Suguta River). The second major group is composed of the Ethiopian Rift Valley populations (Sodore and Lakes Koka, Awasa, Ziway) and the third group of the West African populations (the Senegal, Niger, Volta, Chari Rivers and Lake Chad).

Microsatellites

The four microsatellites loci were polymorphic with 3 to 27 alleles. West African populations are less polymorphic than East African population (H=0% Bamako and Selingue population and H=52% lake Turkana).

To build a genetic network (Fig. 2), the same procedure as previously described for allozymes was done using Mix program (a parcimony algorithm). Populations from Ethiopia are clusetered together, while populations from West Africa and Nile are on the other side of the network. Populations from Kenyan and ugandan Rift valley are clustered between these two groups.

RFLP mtDNA

Six enzymes (AsnI, HinfI, RsaI, AvaII, MspI, TaqI) gave 13 phenotypes corresponding to nine different haplotypes (Fig. 3). In Sodore, Lake Edward and the Nile, more than one haplotype was found (2, 2 and 4 respectively). Individuals from Suguta and Baringo have private haplotypes. On the contrary, Lake Chad, Volta and the Niger share the same haplotype which is also present in the Nile population and in *O. aureus*. Specimens from Lakes

Turkana, Edward and Tana also shared the same haplotype found in the Nile. Fig. 3 shows the consensus tree calculated from the 18 most parsimonious networks obtained with the MIX program. The mtDNA haplotypes are geographically distributed. At one side of the network, all populations from West Africa and *O. aureus* are clustered (they share the same haplotype), on the other side there are the two Ethiopian Rift Valley populations and between these two groups are the Kenyan and Ugandan Rift Valley populations. Nile population shows affinities with West African populations and with specimens from Lake Tana and Turkana.

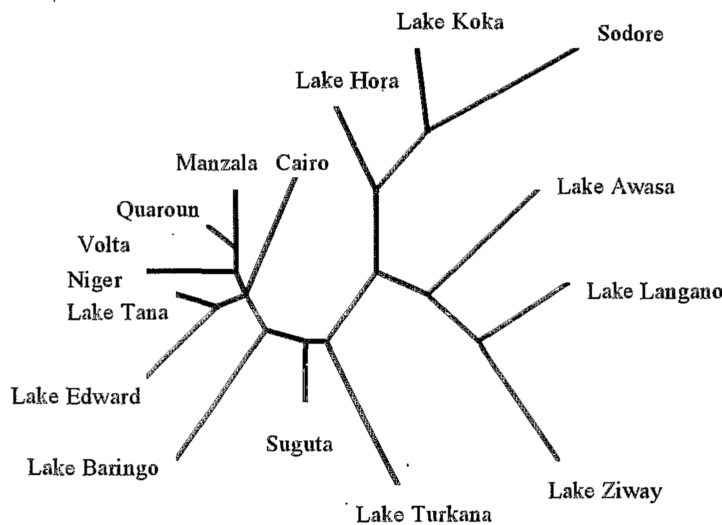


Figure 2
Network produced by Phylip using microsatellites data, on the 16 populations representing seven subspecies of *O. niloticus*. This is a consensus tree produced using Consense from 1000 trees produced using Seqboot and Neighbor.

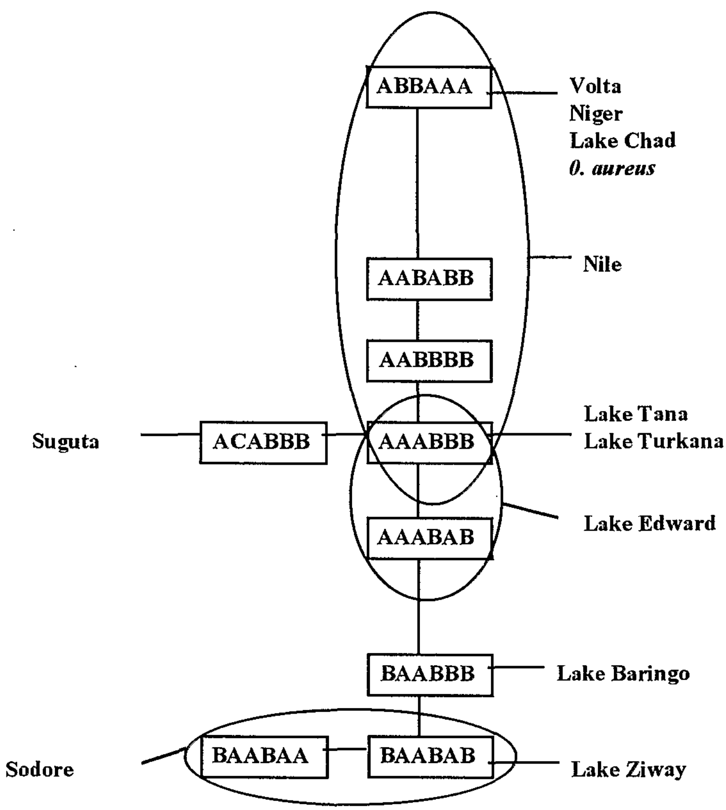


Figure 3
Network produced by Phylip on the 9 mtDNA haplotypes observed. This is a consensus tree produced using Consense from the 18 most parsimonious trees produced using Mix

Discussion

These last results showed that West African *O. niloticus* mtDNA cannot be distinguished from *O. aureus* mtDNA. Two hypotheses can explain why *O. aureus* and *O. niloticus* can share the same

mtDNA. First, this haplotype can be an ancestral one which existed before the two species were isolated. Second, the mtDNA of one species could have been established in the other without nuclear contamination. This phenomenon has already been observed in fishes (DUVERNELL and ASPINWALL, 1995). Tilapia species are well known for their ability to hybridize in captivity (CRAPON DE CRAPONA and FRITZSCH, 1984), in the case of introduced species (DAGET and MOREAU, 1963; ELDER *et al.*, 1971), or in natural conditions (POUYAUD, 1994). TREWAVAS (1983) reported some experiments of hybridization between *O. aureus* and *O. niloticus*. The crosses *O. aureus* male with *O. niloticus* female and *vice versa*, gave a high proportion of males (90 to 100%). In these conditions, transfer of a mtDNA haplotype from one species to the other by natural hybridization is difficult. These observations seem to favour the ancestral DNA hypothesis.

The results obtained by SEYOUM and KORNFIELD (1992a, 1992b), with total mtDNA digestions suggested that *O. n. cancellatus* and *O. n. filoa* form a group independent of all other subspecies. The population of *O. n. tana* also showed, in these studies, a large divergence from other populations. Accordingly, they decided to consider *O. n. cancellatus* and *O. n. filoa* as a new taxon with two subspecies : *O. cancellatus cancellatus* and *O. cancellatus filoa*, respectively. the Population from Lake Tana was then considered as a new subspecies, *O. n. tana*. Our results suggest that the conclusions of SEYOUM and KORNFIELD (1992a, 1992b) have to be considered with reserve.

There are also some differences observed between our results and TREWAVAS' (1983) nomenclature. TREWAVAS (1983) assigned population from Lake Tana to *O. n. cancellatus*, not on a firm morphological basis but because the non-cichlid fishes of Lake Tana were all assigned to Ethiopian species (SEYOUM and KORNFIELD, 1992a). In our study, the Lake Tana mtDNA haplotype is similar to one observed in the Nile population and different from the one observed in the Ethiopian populations. Microsatellites also revealed that population from Lake Tana is closed to *O. n. niloticus* populations. These results could allow to modify the subspecific status of this population (*O. n. niloticus* instead of *O. n. cancellatus*). Another difference between our results and

TREWAVAS (1983) nomenclature is the genetic differentiation observed in *O. n. niloticus*. All West African populations (Senegal, Niger, Volta, Chad basins) are closely related whereas populations from Nile are closer to East African populations (Lake Edward, Turkana, Baringo and River Suguta). Morphological differentiation on which the subspecific nomenclature is based, is then different of genetical differentiation. For the genetic point of view, natural populations of *O. niloticus* are clustered in three groups: 1) the West African populations (Senegal, Niger, Volta, Chad drainages), 2) the Ethiopian Rift Valley populations (Lake Ziway, Awasa, Koka and Sodore hot springs in the Awash River), 3) The Nile drainage populations (Nile, Lake Tana, Edward) and the Kenyan Rift Valley populations (lake Turkana, Baringo and River Suguta).

These results and a better knowledge of the morphological differentiation of *O. niloticus* populations will be essential to modify the subspecific taxonomy of natural populations of this species.

Acknowledgements

This work was supported by the European Community (contract n° ERBTS3 *CT920079) and Orstom (L'institut français de recherche scientifique pour le développement en coopération). Authors wish to thank Mrs. E. K. Abban, Y. Fermon, S. Gilles, R. Berthonnet, J. Lemoalle,. O. Mikolasek, A. Pariselle, J. C. Thouvenel, J. L. Zeddam, P. Golbutsov and W. Dimmick for their precious help in collection of material.

References

AGNESE (J.-F.), 1989 — *Différenciation génétique de plusieurs espèces de Siluriformes Ouest-Africains ayant un intérêt pour l'aquaculture*. Ph.D. Thesis, university of Montpellier, France.

BARDAKCI (F.), SKIBINSKI (D.O.F.), 1994 — Application of the RAPD technique in tilapia fish: species and subspecies identification. *Heredity*, 73: 117-123.

BASIO (Z.U.), TANIGUCHI (N.) 1983 — An investigation of enzyme and other protein polymorphism in Japanese stocks of the tilapias *Oreochromis niloticus* and *Tilapia zillii*. *Aquaculture*, 38: 335-345.

BERNATCHEZ (L.), DANZMANN (R.G.) 1993 —
Congruence in Control-Region Sequence and Restriction-site variation in Mitochondrial DNA of Brook Charr (*Salvelinus fontinalis* Mitchill). *Mol. Biol. Evol.* 10(5): 1002-1014.

CRAPON DE CRAPONA (M.D.), FRITZSCH (B.), 1984 —
Interspecific fertile hybrids of haplochromine Cichlidae (teleostei) and their possible importance for speciation. *Neth. J. Zool.*, 34: 503-538.

DAGET (J.), MOREAU (J.), 1963 —
Hybridation introgressive entre deux espèces de *Sarotherodon* (Pisces, Cichlidae) dans un lac de Madagascar. *Bull. nat. hist. nat. Paris* (4) 3A(2): 689-703.

DUVERNELL (D.D.), ASPINWALL (N.) 1995 —
Introgression of *Luxilus cornutus* mtDNA into allopatric populations of *Luxilus chrysocephalus* (Teleostei: Cyprinidae) in Missouri and Arkansas. *Molecular Ecology*, 4: 173-181.

ELDER (H.Y.), GARROD (D.J.), WHITEHEAD (P.J.P.), 1971 —
Natural hybrids of the African Cichlid fishes *Tilapia spilurus* and *T. leucosticta* : a case of hybrid introgression. *Biol. J. Linn. Soc. Lond.* 3: 103-146.

FRYER (G.), ILES (T.D.) 1972 —
The Cichlid fishes of the great lakes of Africa: their biology and evolution. Oliver and Boyd, Edimburgh.

HAFFER (J.), 1982 —
General aspects of the refuge theory. *In Biological diversification in the tropics.* Edited by G. T. Prance New York, 6-24.

KAUFMAN (L.), 1992 —
Catastrophic change in species-rich freshwater ecosystems. The lessons of Lake Victoria. *Bioscience* 42 (11): 846-858.

LIVINGSTONE (D.A.), 1982 —
Quaternary geography of Africa and the refuge theory. *In Biological diversification in the tropics.* Edited by G. T. Prance New York, 523-536.

MALEY (J.), 1991 —
The African rain forest vegetation and paleoenvironments during late quaternary. *Clim. Change*, 19: 79-98

MARACANAS (J.M.), AGUSTIN (L.Q.), ABLAN (C.K.), PANTE (J.R.), EKNATH (A.A.), PULLIN (R.S.V.), 1995 —
Genetic improvement of farmed tilapias: biochemical characterization of strain differences in Nile tilapia. *Aquaculture International*, 3: 43-54.

MC ANDREW (B.J.), MAJUMDAR (K.C.), 1983 —
Tilapia stock identification using electrophoretic markers. *Aquaculture*, 30: 249-261.

NAISH (K.A.), WARREN (M.), BARDAKCI (F.), SKIBINSKI (D.O.F.), CARVALHO (G.R.), MAIR (G.C.) 1995 —
Multilocus DNA fingerprinting and RAPD reveal similar genetic relationships between strains of *Oreochromis niloticus* (Pisces: Cichlidae). *Molecular Ecology*, 4: 271-274.

NEI (M.), 1972 —
Genetic distances between populations. *Am. Nat.* 106: 283-292.

OGUTU-OHWAYO (R.), 1990 —
The decline of the native fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile tilapia, *Oreochromis niloticus. Environ. Biol. Fish.* 27: 81-96.

PASTEUR (N.), PASTEUR (G.), BONHOMME (F.), CATALAN (J.), BRITTON-DAVIDIAN (J.), 1987 —
Practical Isosyme genetics. Hellis Horwood Ltd., Chichester, UK, 215 p.

POUYAUD (L.), 1994 —
Génétique des populations de tilapias d'intérêt aquacole en Afrique de l'Ouest. Relations phylogénétiques et structuration populationnelle. Ph.D.

Thesis Université de Montpellier-II, France.

POUYAUD (L.), AGNESE (J.F.), 1995 — Phylogenetic relationships between 21 species of three tilapiine genera *Tilapia*, *Sarotherodon* and *Oreochromis* using allozyme data. *J. Fish Biol.*, 47: 26-38.

ROGNON (X.), 1993 — *Diversité génétique et relations phylogénétiques chez les tilapias (Pisces, Cichlidae). Comparaison des données du polymorphisme enzymatique et mitochondrial.* Ph.D. Thesis université de Paris-Sud Orsay, France.

ROGNON (X.), ANDRIAMANGA (M.), MCANDREW (B.), GUYOMARD (R.), 1996 — Allozyme variation in natural and cultured populations in two Tilapia species: *Oreochromis niloticus* and *Tilapia zillii*. *Heredity*. 76: 640-650.

SHAKLEE (J.B.), ALLENDORF (F.W.), MORIZOT (D.C.), WHITT (G.S.), 1990 Gene nomenclature for protein coding loci in fish. *Transaction of the American Fisheries Society, 119*: 2-15.

SEYOUM (S.), KORNFIELD (I.), 1992a — Taxonomic notes on the *Oreochromis niloticus* subspecies-complex (Pisces:Cichlidae), with a description of a new subspecies. *Canadian Journal of Zoology*, 70: 2161-2165.

SEYOUM (S.), KORNFIELD (I.), 1992b — Identification of the subspecies of *Oreochromis niloticus* (Pisces:Cichlidae) using restriction endonuclease analysis of mitochondrial DNA. *Aquaculture*, 102: 29-42.

TREWAVAS (E.), 1983 — *Tilapiine fishes of the genera Sarotherodon, Oreochromis and* Danakilia. British Museum (Natural History), London.

Preliminary results on morphometric differentiation between natural populations of the Nile tilapia *Oreochromis niloticus* (Perciformes, Cichlidae)

Guy G. Teugels
Ichthyologist

Emmanuel Vreven
Ichthyologist

Introduction

The Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is one of the most important cultured freshwater fishes in the World. It naturally occurs in West Africa, the Nile and East Africa. TREWAVAS (1983) and TREWAVAS and TEUGELS (1991) listed seven subspecies: *O. n. niloticus* known from the Senegal, Gambia, Niger, Volta and Chad basins in West Africa, the Jebel Marra between Lake Chad and the Nile, the Nile from below the Albert Nile to the delta and the Yarkon River near Jaffa (Israel); *O. n. eduardianus* (Boulenger, 1912) known from the Lakes Edward and George basins, Lake Kivu, the Ruzizi River and Lakes Tanganyika and Albert; *O. n. baringoensis* Trewavas, 1983 only known from Lake Baringo (Kenya); *O. n. sugutae* Trewavas, 1983 only known from the Suguta River and its tributary the Kapedo

River and its warm alkaline springs; *O. n. vulcani* (Trewavas, 1933) known from Lake Turkana (Kenya) and its affluent streams and the crater lakes on its central island; *O. n. cancellatus* (Nichols, 1923), known from Lake Tsana and other lakes and rivers of Ethiopia, excluding the Blue Nile, Lake Turkana and certain hot springs in the Awash system; *O. n. filoa* Trewavas, 1982, only known from hot alkaline springs in the Awash National Park, Shoa, near Addis Ababa (Ethiopia).

SEYOUM and KORNFIELD (1992) described an additional subspecies, *Oreochromis niloticus tana*, only known from Lake Tana. The same authors reassigned *O. n. cancellatus* and *O. n. filoa* to *O. cancellatus* as *O.c. cancellatus* and *O. c. filoa* respectively. Their results, however, have been seriously questioned by AGNESE *et al.* (1997).

As part of a multidisciplinary programme on the characterization of species and strains used in aquaculture in Africa, an important collection of Nile tilapias was made originating from nearly allover its distribution range. In this paper, we study the morphometric variation between these different populations.

Material and methods

Two hundred and ninety two specimens of *Oreochromis niloticus* were studied morphometrically. Subspecific identifications follow TREWAVAS (1983). Representatives of all subspecies were examined except for *O. n. filoa.* Table 1 gives details on their origin, number of specimens examined and their size. All the material is deposited in the Musée royal de l'Afrique centrale, Tervuren, Belgium. Twenty five measurements and eigth meristic counts were taken on each specimen using dial calipers. Measurements and counts follow VREVEN *et al.* (in press). Only those specimens for which a complete data set was available, have been used for further analysis.

Origin	n	standard length (mm)
O. n. niloticus		
Dagana (Senegal)	18	77.4-252.6
Selingue (Mali)	24	95.0-138.5
Bamako (Mali)	17	73.5-219.4
Battor (Ghana)	7	180.0-252.4
Lake Chad (Chad)	20	140.6-279.3
Ndjamena (Chad)	17	97.7-156.9
Lake Manzalla (Egypt)	16	122.1-178.0
Cairo (Egypt)	17	136.9-246.7
O. n. eduardianus		
Lake Edward(Uganda)	28	152.9-223.9
O. n. baringoensis		
Lake Baringo (Kenya)	24	149.8-185.0
O. n. sugutae		
Kapedo, Suguta River (Kenya)	11	69.9-163.2
O. n. vulcani		
Lake Turkana (Kenya)	28	132.6-187.6
O. n. cancellatus		
Lake Awasa (Ethiopia)	15	99.6-162.1
Lake Langano (Ethiopia)	6	87.2-118.0
Lake Ziway (Ethiopia)	21	99.9-122.1
Lake Koka (Ethiopia)	13	105.9-159.2
Sodore, Debra Zet (Ethiopia)	10	95.0-138.5

Table 1
Population origin, number of specimens examined and minimum and maximum standard lengths for the different subspecies of *Oreochromis niloticus*.

Data obtained were submitted to factor analysis using a principal component analysis (PCA) (STATISTICA, statsoft, versions 3.1 and 5.0). Measurements were log-transformed before the PCA was run on the covariance matrix. An independent PCA was run on the correlation matrix from the untransformed count data.

Results

Populations of West Africa and the Nile (Egypt)

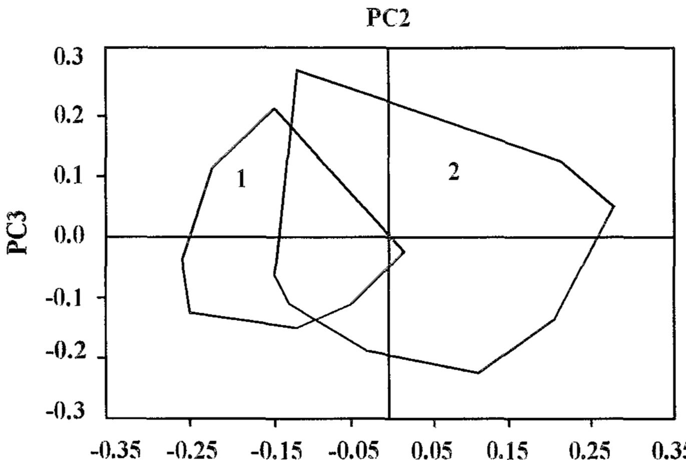


Figure 1
Plot of a principal component analysis using 25 log-tranformed metric variables on specimens examined of the natural populations of *Oreochromis niloticus niloticus sensu* TREWAVAS (1983) from Egypt (1) and from West Africa (2).

VREVEN *et al.* (in press) recently studied the morphometric and genetic variation in natural populations and cultured strains of *Oreochromis niloticus niloticus*, using part of the material examined in this study. We refer to that paper for details on morphometric variation in the natural populations of *Oreochromis niloticus niloticus sensu* TREWAVAS (1983). A summary is given in figure 1: the plot of a PCA on 25 log-transformed metric variables shows that the majority of West African specimens are located on the positive sector of the second component, while the specimens from Egypt are situated on the negative sector. The second component is merely defined by the caudal peduncle length (generally longer in specimens from Egypt) and the toothed

pharyngeal bone width (smaller in specimens from Egypt). A comparison of meristic counts between the different populations did not reveal differences between them.

Populations from West Africa, the Nile (Egypt) and Lake Edward

The results of a PCA on 25 log-transformed metric variables for populations of *Oreochromis niloticus* from West Africa, the Nile and Lake Edward are illustrated in figure 2. Interestingly, all specimens from the Nile and Lake Edward, except one, are located on the negative sector of the second component, while the majority of specimens from West Africa are located on the positive sector. The same variables as above define the second component. It should be noted that, following TREWAVAS (1983), the Nile specimens (Lake Manzalla and Cairo) are identified as *O. n. niloticus* while those from Lake Edward belong to *O. n. eduardianus*.

Populations from East Africa

The plot of a PCA on 25 log-transformed metric variables for the subspecies of *Oreochromis niloticus* from East Africa, is given in figure 3. The population from Lake Turkana (= *O. n. vulcani*) can easily be distinguished from the populations from Lake Baringo (= *O. n. baringoensis*), Suguta (= *O. n. sugutae*). A small overlap between the Lake Turkana (= *O. n. vulcani*) and the Lake Edward (= *O. n. eduardianus*) populations is noted.
The Lake Turkana population is entirely situated on the positive sector of the second component and the negative sector of the third component.
The second component is mainly defined by the anal spine and the dorsal spine lengths (generally longer in the Lake Turkana population) and in a lesser extent the lower jaw length. The third component is merely defined by the pelvic fin length and the anal fin length.

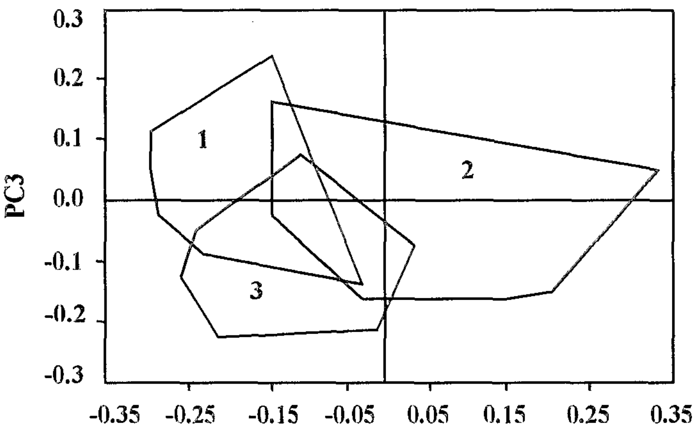


Figure 2
Plot of a principal component analysis using 25 log-transformed metric variables for populations of *Oreochromis niloticus* from Lake Edward (1), West Africa (2) and Egypt. (3)

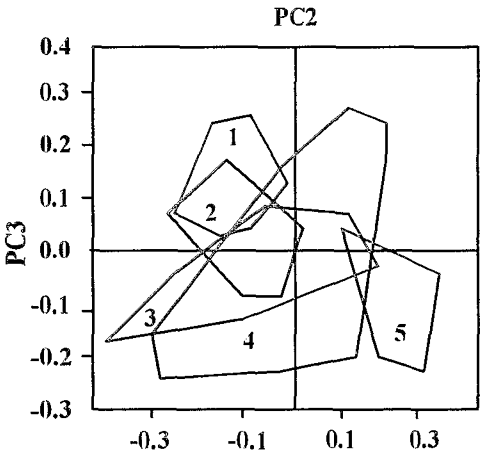
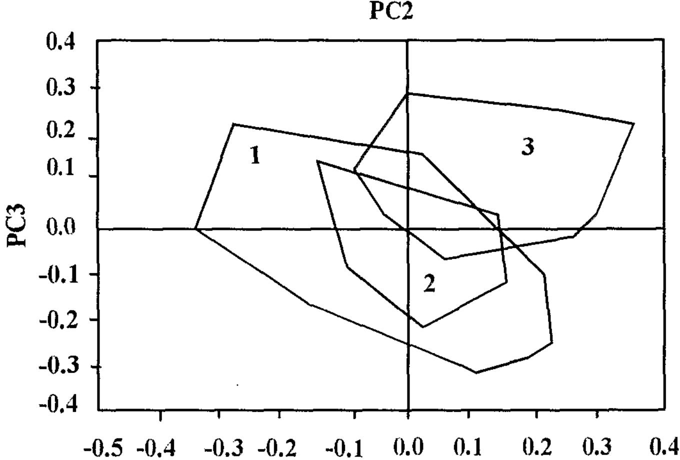


Figure 3
Plot of a PCA on 25 log-transformed metric variables for the subspecies of *Oreochromis niloticus* from East Africa:
(1) *O. n. barigoensis*, (2) *O. n. sugutae*, (3) *O. n. eduardianus*, (4) *O. n. cancellatus*, (5) *O. n. vulcani* .

Most important differences resulting from the analysis of meristic counts were the higher number of gill rakers in the Lake Edward (= *O. n. eduardianus*) and the Lake Turkana (= *O. n. vulcani*) populations.

All populations examined of Oreochromis niloticus



I Figure 4
Plot of a PCA on 25 log-transformed metric variables for all the populations examined of *Oreochromis niloticus*, indicating separately the specimens from East Africa (1), the Nile (Egypt) (2) and West Africa (3).

A PCA on 25 log-transformed metric variables for all the populations examined and identified following TREWAVAS (1983) is given in figure 4. Most important result from this analysis is once again the almost complete overlap between the Nile specimens and the East African populations.

The plot of a PCA on 5 meristic counts for all the populations examined and identified following TREWAVAS (1983) is given in figure 5. *Oreochromis niloticus niloticus*, *O. n. vulcani* and

O. n. eduardianus are mainly situated on the negative sector of the first component, while the other subspecies are mainly located on the positive sector. The first component in this analysis is merely defined by the number of gill rakers on the cerato- and hypobranchials of the first gill arch.

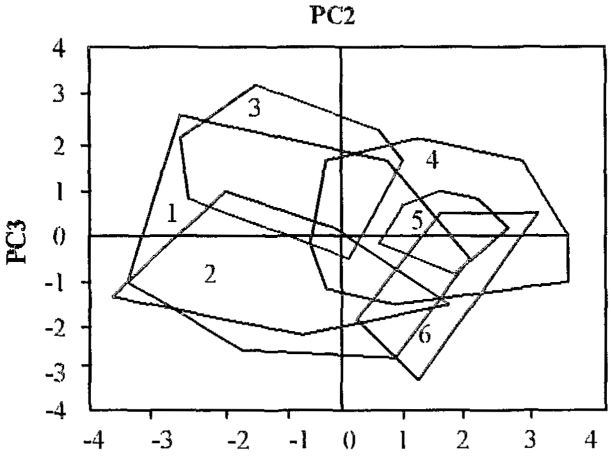


Figure 5
Plot of a PCA on 5 meristic counts for all the populations examined of *Oreochromis niloticus*, identified following TREWAVAS (1983), *O. n. niloticus* (1), *O. n. vulcani* (2), *O. n. eduardianus* (3), *O. n. cancellatus* (4), *O. n. baringoensis* (5), *O. n. sugutae* (6).

Discussion

The most important result of this preliminary study is undoubtedly the fact that morphometrically the populations from the Nile (Lake Manzalla and Cairo) seem closer to the East African populations than to the West African populations of *Oreochromis niloticus*. ROGNON *et al.* (1996) studying allozyme variation in West African and Nile populations of this species, reached the same conclusion

because their Nile population (also from Lake Manzalla) clusters exclusively with a population from Lake Turkana. Allozyme data based on tissue-electrophoresis of the same material as studied in this paper, confirmed this (VREVEN *et al.*, in press). TREWAVAS (1983) considered both, the West African and the Nile populations as belonging to *O. n. niloticus*. Therefore their arrangement in the same subspecies can be questioned.

From our results on the study of the East African populations, it is clear that the Lake Turkana specimens, identified as *Oreochromis niloticus vulcani* clearly differ from all the other subspecies. From the same results it is also clear that the *O. n. cancellatus* complex shows a high degree of morphometric polymorphism. Remarkably the different geographic populations are almost all distinctly marked in the *O. n. cancellatus* polygone (not illustrated), with the more northern populations (Sodore and Lake Koka) and the more southern populations (Lakes Ziway, Awasa and Langano) located close to each other. This pattern probably indicates a clinal variation which is an argument in favour of considering these populations as belonging to the same subspecies. From our morphometric analysis we did not find sufficient evidence to support the conclusion of SEYOUM and KORNFIELD (1992) that *O. n. cancellatus* is a separate species that contains two subspecies.

The results presented in this study indicate that the subspecific classification of *Oreochromis niloticus* as given by TREWAVAS (1983) can be questioned. The results however are still preliminary: not all subspecies have been examined (*e.g. O. n. filoa* and *O. n. tana*) and for some subspecies only few specimens have been examined. This is the subject of forthcoming research.

Acknowledgements
This work as part of the GENETICS program, was made possible thanks to funding from the European Union (contract ERBTS3*CT920079)

References

AGNESE (J.F.), ADEPO-GOURENE (B.), ABBAN (E.K.), FERMON (Y.), 1997 — Genetic differentiation among natural populations of the Nile tilapia *Oreochromis niloticus* (Teleostei, Cichlidae). *Heredity*, 79: 88-96

ROGNON (X.), ANDRIAMANGA (M.), MCANDREW, (B.) GUYOMARD (R.), 1996 — Allozyme variation in natural and cultured populations in two Tilapia species: *Oreochromis niloticus* and *Tilapia zillii*. *Heredity*, 76: 640-650.

SEYOUM (S.), KORNFIELD (I.), 1992 — Taxonomic notes on the *Oreochromis niloticus* subspecies-complex (Pisces:Cichlidae), with a description of a new subspecies. *Can. J.. Zool.*, 70: 2161-2165.

TREWAVAS (E.), 1983 — *Tilapiine fishes of the genera* Sarotherodon, Oreochromis *and* Danakilia. British Museum (Natural History), London.

TREWAVAS (E.), TEUGELS (G.G.), 1991 — Oreochromis. pp. 307-346. In DAGET (J.), GOSSE (J.-P.), TEUGELS (G.G.), THYS VAN DEN AUDENAERDE (D.F.E.), eds.: *Check List of the Freshwater fishes of Africa. Cloffa 4.* ISNB (Brussels), Mrac (Tervuren) and Orstom (Paris).

VREVEN (E.), ADEPO-GOURENE (B.), AGNESE (J.-F.), TEUGELS (G.G.), in press — Morphometric and genetic variation in natural populations and cultured strains of the Nile tilapia *Oreochromis niloticus* (Teleostei: Cichlidae). *Belg. J. Zool.*.

Morphometric and allozyme variation in natural populations and cultured strains of the Nile tilapia *Oreochromis niloticus niloticus* (Teleostei, Cichlidae)

Emmanuel J. Vreven
Ichthyologist

Béatrice Adépo-Gourène
Geneticist

Jean-François Agnèse
Geneticist

Guy G. Teugels
Ichthyologist

The Nile tilapia, *Oreochromis niloticus* (LINNAEUS, 1758) endemic to Africa has been introduced in many parts of the World for aquaculture purposes. In particular the subspecies *O. niloticus niloticus* is at present one of the most cultured freshwater fishes.

Several authors published on the meristic and morphometric characters of this species (*e.g.* DAGET, 1954 for local populations; TREWAVAS, 1983 for natural populations from the major part of its distribution range; GOURENE and TEUGELS, 1993 for cultured strains). Several others have published on the allozyme variation (*e.g.* BASIAO and TANIGUCHI, 1983; MCANDREW and MAJUMDAR,

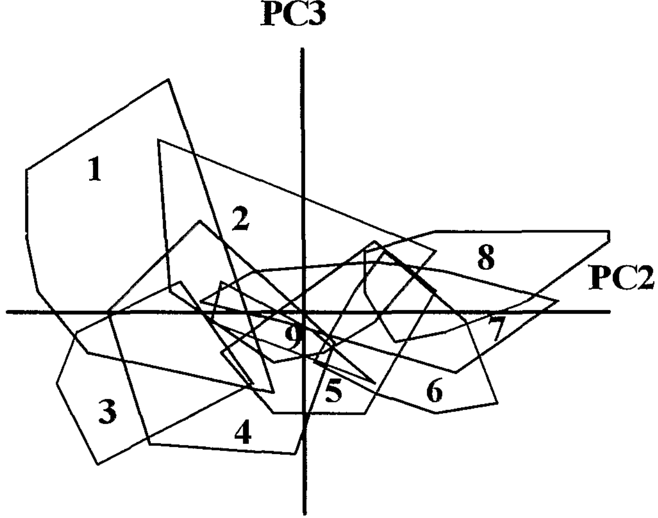
1983; SEYOUM and KORNFIELD, 1992 for cultured stocks; ROGNON *et al.*, 1996 for West African natural and cultured populations; AGNESE *et al.*, 1997 for natural populations from allover the distribution range).

Origin	N (morphometry)	Standard Length (mm)	N (allozyme study)
Natural populations			
Dagana, Senegal	18	77.4 - 252.6	63
Selingue, Mali	24	95.0 - 138.5	58
Bamako, Mali	17	73.5 - 219.4	22
Battor, Ghana	7	180.0 - 252.4	7
Lake Chad, Chad	20	140.6 - 279.3	22
N'Djamena, Chari , Chad	17	97.9 - 156.9	30
Cairo, Nile, Egypt	17	136,9 - 246,7	18
Lake Manzalla, Egypt	16	122.1 - 178.0	30
Lake Edward, Uganda	28	152.9 - 223.9	30
Cultured strains			
Bouake strain	29	95.0 - 138.5	55
Volta strain	32	101.0 - 136.8	50
Quarun strain	20	154.7 - 225.5	20

Table 1
List of natural populations and cultured strains examined of *Oreochromis niloticus*.

As part of a multidisciplinary project on the characterisation of species and populations used in aquaculture we examined the morphology and allozyme variation of nine natural populations and three cultured strains of *Oreochromis niloticus*. Origin and sample size of the populations and strains are listed in Table I. For each specimen twenty-five measurements and eight counts were taken. The morphometric results obtained were log-transformed and

submitted to principal component analysis using the STATISTICA package. For allozyme studies, standard horizontal gel electrophoresis was carried out to investigate the products of 25 loci. The allozymic data were analysed using the PHYLIP sofware package.



I Figure 1
Plot of a principal component analysis on log-transformed data of 25 metric variables for 142 specimens of *Oreochromis niloticus* originating from 9 natural populations from West Africa, the Nile and Lake Edward. The numbers given in the figure correspond to the following natural populations: Lake edward (1), Bamako (Niger) (2), Lake Manzalla (3), Cairo (Nile) (4), N'Djamena (Chari) (5), Lake Chad (6), Dagana (Senegal) (7), Selingue (Niger) (8), and Battor (Volta) (9).

Results obtained by a principal component analysis on the morphological data of all natural populations from West Africa, Egypt and Lake Edward are given in figure 1. Two major groups are discerned : all specimens from Egypt (Cairo and Lake Manzalla) and Lake Edward, except one, are located on the negative sector of

the second component, while the majority of the specimens from West Africa are located on the positive sector of this component. The latter is mainly defined by the caudal peduncle length, the toothed pharyngeal bone length and width.

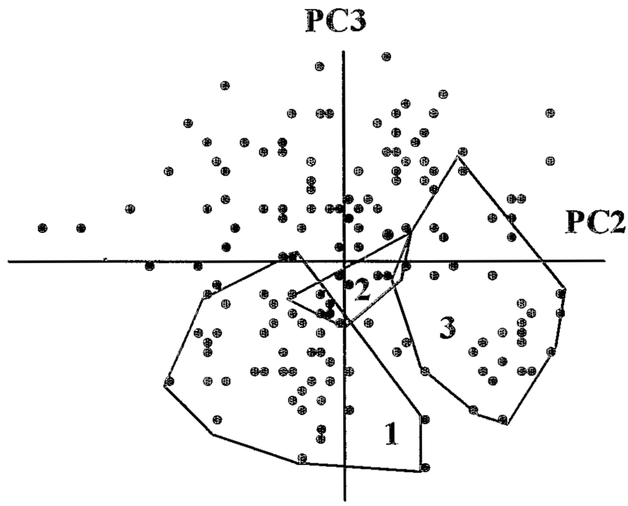


Figure 2
Plot of a principal component analysis on log-transformed data of 25 metric variables for 169 specimens of *Oreochromis niloticus*: (1) Lake Edward, (2) Battor (Volta) and (3) the cultured Bouake strain.

The results of the same analysis with addition of the Bouake strain are illustrated in figure 2. This cultured strain results from an interbreeding between specimens from the Volta basin and Lake Edward. This is however not confirmed by the results obtained as only a slight overlap is noted between the Bouake strain and the Battor (Volta) population; furthermore the Bouake strain is completely separated from the population from Lake Edward.

The results of further analysis with addition of the Volta strain are given in figure 3. Note that the Volta strain, descending from a

natural population of the Volta basin, only slightly overlaps with the Battor (= Volta) population. Interestingly, the Volta strain almost completely overlaps with the Bouake strain (not illustrated).

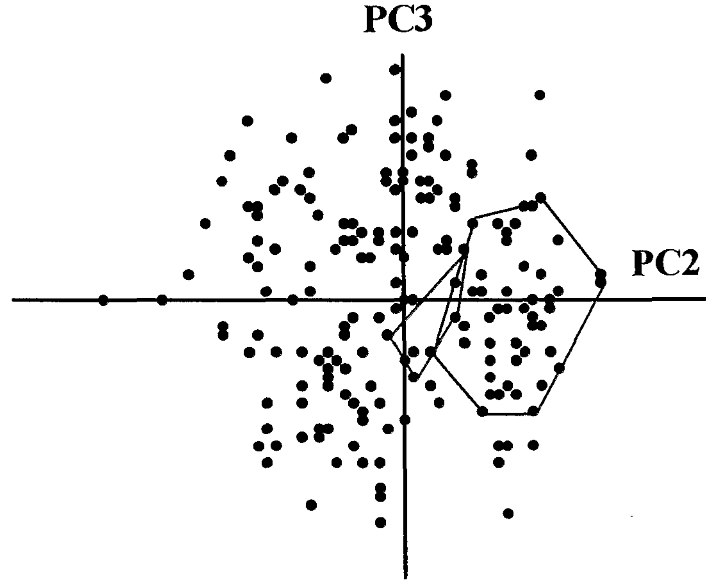


Figure 3
Plot of a principal component analysis on log-transformed data of 25 metric variables for 200 specimens of *Oreochromis niloticus* from the cultured Bouake and Volta strains and 9 natural populations from West Africa, the Nile and Lake Edward: Volta (Battor) (1), Volta strains (2).

The results of an analysis with addition of the Quarun strain are given in figure 4. Noteworthy here is that in this figure most of the specimens belonging to cultured strains are located on the negative sector of the third component, which is merely defined by the lower jaw length, body depth and pelvic fin length, while the majority of the natural populations are situated on the positive sector of this component.

In conclusion the morphometric analysis of the natural populations from West Africa did not enable us to indicate clearly marked differences between them. According to TREWAVAS (1983), natural populations from the Lower Nile system in Egypt belong to the same subspecies *O. niloticus niloticus* as those from West Africa. Morphologically, however, the majority of the specimens from both geographical region can be distinghuised from each other. Moreover the Nile specimens are morphologically closer to the Lake Edward specimens, which, following TREWAVAS (1983), belong to the subspecies *O. niloticus eduardianus*.

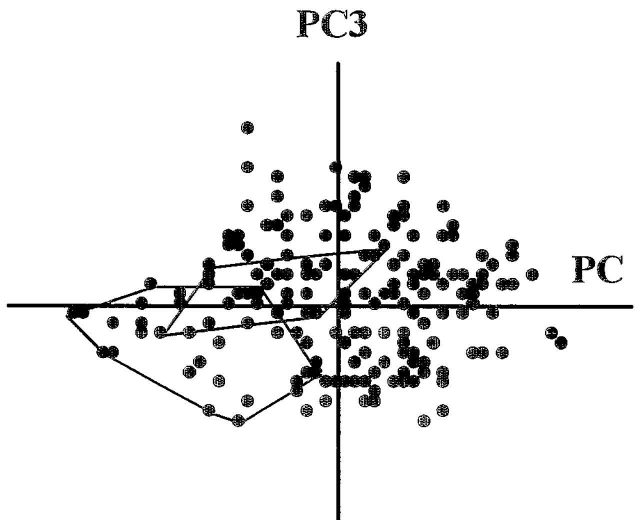


Figure 4
Plot of a principal component analysis on log-transformed data of 25 metric variables for 220 specimens from Bouake strain, Volta strain, Quarun strain and from 9 natural populations from West Africa, Lake Edward and the Nile; Quarun strain (1), Cairo and Lake Manzalla populations (2).

The morphometric results are confirmed by the allozyme study. Thirteen of the 25 loci were polymorphic and domestic strains did

not show lower H and P values than natural populations, indicating that they did not loose genetic polymorphism. The genetic relationships between the different samples studied is given in figure 5. The populations are clustered in two major groups: the West African natural populations (Dagana, Selingue, Bamako, N'Djamena, Chad and Volta) with one domestic strain (Volta) and the Nile drainage natural populations (Manzalla, Cairo and Lake Edward) with two domestic strains (Quarun and Bouake).

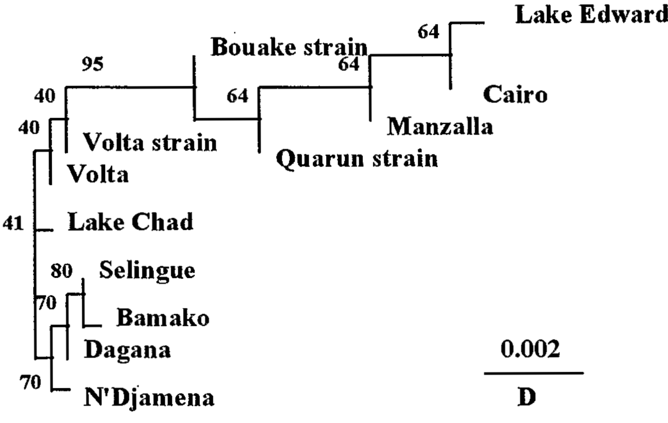


Figure 5
Consensus unrooted tree produced by PHYLIP for 9 natural populations and 3 cultured strains of *Oreochromis niloticus*. Number at each node indicates the percentage obtained using bootstrapping.

An overall comparison of all natural populations and all cultured strains examined, showed important morphological differences between both. It is thus obvious that the external morphology is considerably influenced by the environmental conditions in captivity. Factors such as lack of current in ponds, undoubtedly affect the external morphology this being expressed for example in the reduction of the body depth. In how far captivity conditions affect the growth rate and thus the aquaculture productivity is the subject of further investigation.

Acknowledgements
This work as part of the GENETICS program, was made possible thanks to funding from the European Union (contract ERBTS3*CT920079)

References

AGNESE (J.-F), ADEPO-GOURENE (B.), ABBAN (E.K.), FERMON (Y.), 1997 — Genetic differentiation among natural populations of the Nile tilapia *Oreochromis niloticus* (Teleostei, Cichlidae), *Heredity*, 79: 88-96.

BASIAO (Z.U.), TANIGUCHI (N.), 1983 — An investigation of enzyme and other protein polymorphism in Japanese stocks of tilapias *Oreochromis niloticus* and *Tilapia zillii*. *Aquaculture*, 38: 335-345.

DAGET (J.), 1954 — *Les poissons du Niger supérieur.* Mém. Ifan., 36: 1-391.

GOURENE (G.), TEUGELS (G.G.), 1993 — Différenciation morphologique de souches des tilapias *Oreochromis niloticus* et *O. aureus* (Teleostei; Cichlidae) utilisées en pisciculture. *Cybium*, 17: 343-355.

MCANDREW (B.J.), MAJUMDAR (K.C.), 1983 — Tilapia stock identification using electrophoretic markers. *Aquaculture*, 30: 249-261.

ROGNON (X.), ANDRIAMANGA (M.), MCANDREW (B.), GUYOMARD (R.), 1996 — Allozyme variation in natural and cultured populations in two Tilapia species : *Oreochromis niloticus* and *Tilapia zillii*. *Heredity*, 76: 640-650.

SEYOUM (S.), KORNFIELD (I.), 1992 — Taxonomic notes on the *Oreochromis niloticus* subspecies-complex (Pisces:Cichlidae), with a description of a new subspecies. *Can. J. Zool.*, 70: 2161-2165.

TREWAVAS (E.), 1983 — Tilapiine fishes of the genera *Sarotherodon, Oreochromis* and *Danakilia*, British Museum (Natural History), London.

Zoo-technical characterization of four strains of *Oreochromis niloticus*

Olga Assemien
Biologist

Germain Gourène
Ichthyologist

Introduction

Oreochromis niloticus is the species the most widely used in aquaculture in continental waters in Africa, because of its rapid growth, its easy and continuous reproduction, and its robustness. Many studies in the disçiplines of reproductive physiology, genetics, and culture systems have been carried out in order to characterize the different sub-species or strains to distribute. This study, presents the results of the zoo-technical characterization of four strains of *Oreochromis niloticus*.

Materials and methods

In this trial, four strains of *Oreochromis niloticus* were compared ; a domestic strain, the Bouaké strain (BKE), and three natural

strains : Ghana (GHA), Niger (NIG), and Senegal (SEN). These last three strains arrived at the Idessa (Institut des savannes) pisciculture station (Bouaké) in June 1994, May 1993, and December 1993 respectively. The samples were initially collected from Lake Volta (GHA), and the Senegal (SEN) and Niger (NIG) rivers. The domestic strain is a result of the crossing on the Idessa station of a strain from theVolta basin (Burkina Faso, arrived in 1957) and a strain from the Nile basin (Uganda, arrived in 1968). The whole of the biological material was made up of juveniles which were grown-out then placed in reproduction in cement tanks for growth comparisons.

Eleven ponds of 50 m^2 each were used, three ponds for each strain with the exception of the GHA strain, where individuals were placed into two ponds due to a lack of available juveniles. The stocking density used was 2.2 fish per m^2 (110 fish per pond). The tests were carried out over a period of five months (150 days). Months 1, 2, 3, 4, and 5 had 28, 30, 29, 30, and 32 days respectively. The fish were fed with Faci (Fabrique d'aliment de Côte d'Ivoire) granulated feed « Tilapia 2 GE »(containing 30% protein of which 10% is of animal origin). The feed ration was identical for all ponds. The ration was based on the group having the highest mean weight. The fish were sampled each month in order to adjust the ration. All fish were individually weighed at stocking and draining.

To characterize the zoo-technical performances of each strain, the evolution of mean weights, daily individual growth rate and the feed conversion ratios were first examined with an Anova and then the Newman-Keuls or Student's t test with a 5% tolereance.

Results

Figure 1 shows the evolution of mean weights of the strains at the different samplings. The general trend shows an increase in mean

weights of all strains during the entire grow-out period. The Anova of mean weights of the strains shows significant differences at every sampling. At stocking, the BKE (71.3 g +1.3), GHA (69.8g+0.2) and SEN (70.6g+1.3) strains had statistically identical mean weights, greater than that of the NIG strain (58.9g+0.1).

At the first sampling, the three srains BKE (143.5g+6.1), GHA (132.8g+3.0) and SEN (147.5g+6.2) had statistically similar mean weights but the GHA strain was slightly lower than the other two. The NIG strain (125.3g+4.7) had a mean weight clearly lower than the other two.

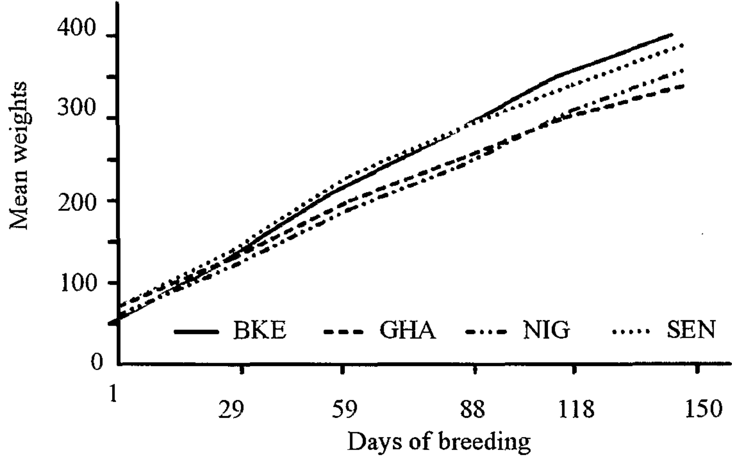


Figure 1
Evolution of mean weights at the different samplings.

We therefore have two groups, one made up of the BKE, GHA and SEN strains, and the other of the NIG strain.

At the second, third and fourth samplings we see that among the three strains that had identical initial mean weights, the GHA strain

now has mean weights inferior to those of the BKE and SEN strains.

Also, the NIG strain which had the lowest mean weight, now has the same mean weight statistically as that of the GHA strain.

It thus appeared two groups, the BKE and SEN strains in one hand the GHA and NIG strains in the other. The first two strains cited have higher mean weights.

At draining, we found three groups. The first is made up of the BKE (426.8g+22.8) and SEN (413.0g+19.3) strains. The second has the NIG (364.0g+9.4) strain. The last is the GHA (338.8g+3.6) strain. The strains in the first group have mean weights greater that that of the strain in the second group. The second group has mean weights greater than that of the third group.

Throughout the the grow-out period the BKE (2.4g/d+0.2) and SEN (2.3g/d+0.1) strains had better daily individual growth rates than the GHA strain (1.8g/d+0.0).

Concerning the NIG strain (2.0g/d+0.1), it holds an intermediate position between the two groups, not significantly different from the BKE and SEN strains on the one hand or the GHA strain on the other.

During the 150 days of grow-out, the feed conversion ratio of the GHA strain (2.4+0.1) is greater than that of the BKE (1.8+0.1) and SEN (1.9+0.2) strains. The ratio for the NIG strain (2.0+0.0) is not different from that of the GHA strain or the BKE and SEN strains.

Conclusion

From a zoo-technical point of view, based on the evolution of mean weights, daily individual growth rates and feed conversion ratios, the strains showing the best growth performances are the BKE and SEN strains. In Côte d'Ivoire, given the fact that the SEN strain which is imported, has no real advantages over the BKE strain, this

last remains the best for *Oreochromis niloticus* culture. In Senegal, the strain originating in this country should be distributed because it shows good performances. The GHA strain is the least performant of the four tested. It should be noted that the BKE strain can already be found in Côte d'Ivoire in the Bia and the Tanoé, two water systems shared by these countries. As for the NIG strain, it should be distributed in its country of origin, because it shows good growth potential.

Acknowledgements

This work as part of the GENETICS program, was made possible thanks to funding from the European Union (contract ERBTS3*CT920079)

Morphological and genetic differentiation of West African populations of *Sarotherodon melanotheron* Ruppel, 1852 (Teleostei, Cichlidae)

Béatrice Adépo-Gourène
Geneticist

Jean François Agnèse
Geneticist

Laurent Pouyaud
Geneticist

Guy G. Teugels
Ichthyologist

Marc M. Hansen
Ichthyologist

Introduction

Sarotherodon melanotheron is a tilapia species which lives in lagoons and estuaries from Senegal to Angola. It is also found in the sea, notably off the coast of Dakar or in Guinea. TREWAVAS in 1983 described five subspecies based on morphological characteristics whose distributions follow:

- *S. m. paludinosus*, in some freshwater regions of Dakar
- *S. m. heudelotii*, from Senegal to Guinea
- *S. m. leonensis*, from Sierra Leone to Liberia

- *S. m. melanotheron*, from Côte d'Ivoire to Cameroon
- *S. m. nigripinis*, from the Rio Muni to Zaïre.

This species, because of its good salinity tolerance interests aquaculturists. However, the low (0.45 g/d) daily growth rates observed until now have discouraged its use.

In this context, the research on genetically differentiated populations would be the first step in the quest for populations having zoo-technical aptitudes of interest for aquaculture. In effect, we can hope that an eventual genetic differentiation would be accompanied by physiological type differences and display the latter to their best.

Material and methods

This work was carried out under the auspices of the Genetics progam, in the ichthyology laboratories in Tervuren (Belgium), the genetics lab of the Centre de Recherches Océanologiques in Abidjan (Côte d'Ivoire) and by the Genome and Populations laboratory of Montpellier (France). Twenty-nine samples were analyzed consisting of 911 specimens coming from different West African hydrographic basins (fig.1): In Sénégal, Saint Louis (2 samples), Lake Redba, Hann, Dakar, Somone, Kaolack, Foundiougne, Missirah; in Gambia, Banjul; in Guinea, Diouloulou, Kandiaffara, Koba, Forrecariah; In Ivory Coast, Grand Bérébi, Grand Lahou (2 samples), Tiegba, Adiopodoumé (3 samples), Lake Bakré, Biétry Lagoon (2 samples), Anga (2 samples), Lake Ayamé; in Benin, Cotonou; in Congo, Bas Kouilou.

In the morphological study, 22 measurements were taken on each specimen. These were: (1) total length, (2) standard length, (3) head length, (4) snout length, (5) eye diameter, (6) inter-ocular distance, (7) preorbital bone length, (8) width of the toothed zone of the pharyngial bone, (9) length of the pharyngial bone, (10) body height, (11) caudal peduncle height, (12) cadudal peduncle length, (13) predorsal distance, (14) prepectoral distance, (15) preventral

distance, (16) preanal distance, (17) dorsal fin length, (18) greatest dorsal spine length, (19) pectoral fin length, (20) ventral fin length, (21) anal fin length, (22) third anal spine length (upper, lower and on the caudal) and number of scales around the caudal peduncle.

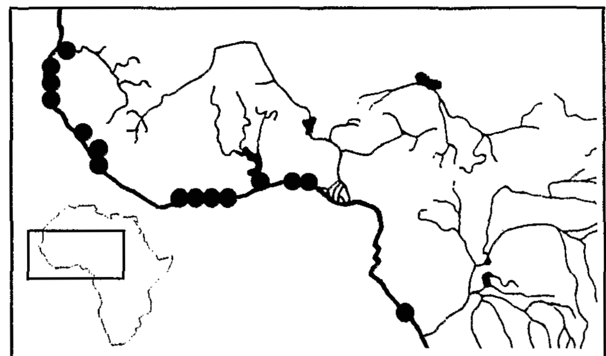


Fig. 1
Collecting sites of *S. melanotheron* samples.

The meristic characteristics analyzed for each specimen follow: number of branchiospines on the lower part of the first branchial arch, number of spines in the dorsal fin, number of branched rays in the dorsal fin, number of spines in the anal fin, number of branched rays in the anal fin, number of scales in the lateral line.

Statistical analyses of the data were carried out using the CSS: Statistica program (Statsoft, version 3.1).

Electrophoretic enzymatic protein analysis and the microsatellite study are the two methods used in the populations' genetics study. Concerning the enzymatic protein electrophoresis, 27 loci were analyzed. The genetic variability was evaluated with the help of two indices: i) the polymorphism rate P which corresponds to the number of polymorphic loci compared to the total number of loci

studied, ii) mean heterozygosity (H) calculated using NEI'S formula (1978).

The genetic divergence among the populations was calculated using the "Neighbor joining UPGMA method" (SAITOU and NEI 1987) with the Neighbor program by J. Felsenstein (Department of Genetics, University of Washington, Seattle, Washington 98195).

The allele frequency table was transformed into an allele present/absent matrix (1/0). This matrix was treated with the parsimony algorithm (ECK and DAYOFF, 1966 ; KLUG and FARRIS, 1969) from J. Felsenstein's Mix program.

The microsatellite study consisted of the analysis of the following 4 loci : 79 M, 79 H, 73 C and locus 28. Mean genetic diversity was estimated using the mean theoretical heterozygosity rate (H). Genetic differentiation among samples was evaluated with WEIR and COCKERHAM'S (1984) unbiased estimator. Reynolds distances, determined using estimators, allowed the construction of a phylogenetic network using the Neighbor joining method of the Phylip program of J. FELSENSTEIN (1989).

Results and discussions

The various types of principal component analyses carried out on the morphometric data (*i. e.* analyses of metric data alone or in association with merisitic data) gave almost identical figures. In this figure (fig. 2), all the populations overlap either partially (the case of Senegal and Guinea) or completely as is the case of the Gambian population being overlapped by those of Côte d'Ivoire, whereas the Congo population is the more isolated.

The Congo population's isolation is due to the fact that the latter clearly distinguishes itself by the combination of the following characteristics: head length, snout length, length/width relationship of the lower pharyngial bone, dorsal fin length, ventral fin length, anal fin length, number of branchiospines on the lower part of the

first branchial arch and number of branched rays in the dorsal fin. This population corresponds to the description of the *S. m. nigripinnis* population given by TREWAVAS (1983). The Côte d'Ivoire populations belong to a single subspecies which according to TREWAVAS, 1983 is the *S. m. melanotheron* subspecies. In the Guinea populations, TREWAVAS' 1983 criteria show that the Bofon population belongs to the subspecies *S. m. leonensis*. The second population is that which is overlapped by those of Senegal and Gambia. According to TREWAVAS' 1983 definition, these Guinea, Senegal and Gambia populations belong *to S. m. heudelotii.*

Finally, the partial overlapping observed between all the populations shows the necessity of refining the notion of subspecies as proposed by TREWAVAS (1983).

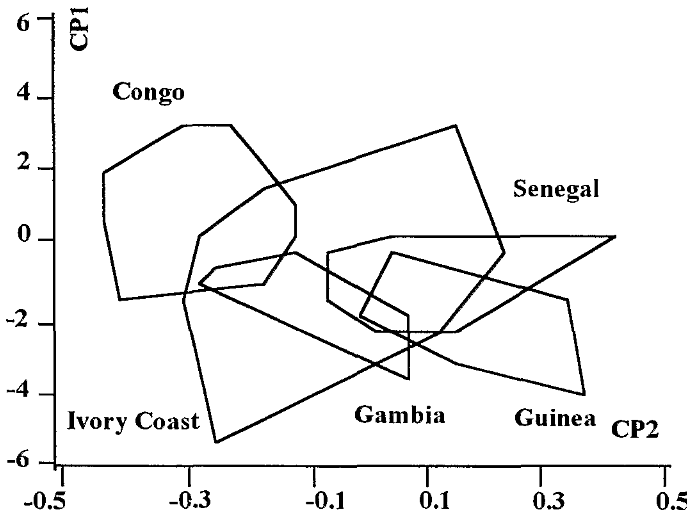


Fig. 2
result of principal component analyses carried out on the morphometric data (*i. e.* analyses of metric data alone or in association with merisitic data).

Seventeen of the 27 loci analyzed by enzymatic protein electrophoresis were polymorphic. Some were expressed by 4 or 5 alleles.

If we observe the allele frequency distribution at the Aat-2, Acp-1 and Gpi-2 loci, in all of the samples analyzed, we notice that the genetic differentiation of *S. melanotheron* presents as a clinal geographic variation (Fig. 3). The three clines observed are all superimposed. The slopes, more or less abrupt, are located in the region between the Guinea-Sierra Leone border (point 13) and the Liberia-Côte d'Ivoire border (point 14). This region represents the transition between the two relatively differentiated forms.

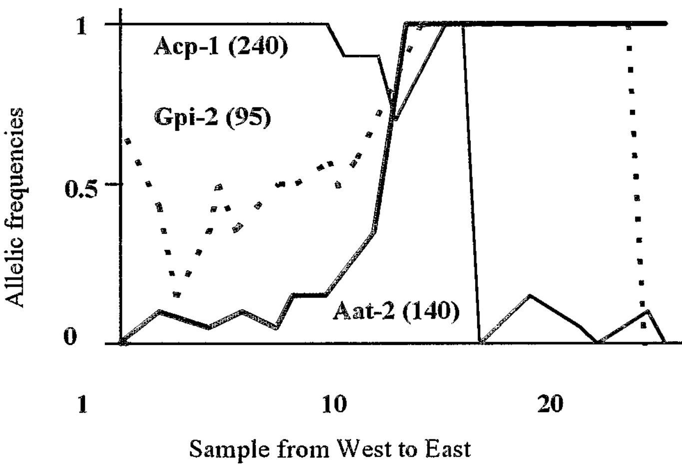


Fig. 3
Clinal geographic variation of the allele frequencies distribution at the Aat-2, Acp-1 and Gpi-2 loci, in all of the samples analyzed.

The mean polymorphism rate is 13.33 %. This rate is lower than that found in *Tilapia guineensis* (16.9 %); but it is higher than that found in *T. zillii*: 3.45 % .

The mean heterozygosity rate is 4.4%. This rate is comparable to those found in *Tilapia guineensis* (6.4%) and *T. zillii* (3.4%). This rate is also close to that estimated by McAndrew and Majumdar (1983) in *S. gallileus* (4.3%) and by Rognon (1993) in *Tilapia guineensis*.

In table 1 samples were regrouped by region. A relationship exists between the values of these parameters and the geographic location of the populations. In effect, it is in the Western zones of West Africa, that is, Senegal and Guinea, that the genetic variability is the greatest. Contrarily, in the more Eastern regions like Côte d'Ivoire, Benin and Congo, the genetic variability is half as great.

Geographic area	Senegal	Guinea	Ivory Coast	Benin-Congo
Polymorphism (%)	17.15	17.26	9.68	9.25
Heterozygosity	0.06±0.02	0.057±0.02	0.032±0.02	0.026±0.01

Table 1
Geographic variations of polymorphism and heterozygosity in *S. melanotheron* populations.

The geographic distribution of allele frequencies, polymorphism and heterozygosity rates show the presence of two relatively differentiated groups.

From the genetic distance matrix (Nei, 1978), a dendrogram was made using the "Neighbor joining" method. The branch length is proportional to the genetic distance separating the populations. We find the two previously defined groups: the Senegal and Guinea populations on one hand, the Côte d'Ivoire, Benin and Congo populations on the other. A more detailed analysis shows that the Lower Kouilou population from Congo is very differentiated from the other populations from Côte d'Ivoire or Benin. In fact, it forms an independent subgroup.

Finally, a dendrogram (Fig. 4) that was obtained with the parsimony technique using an alleles present/absent matrix allowed observation of the same previously mentioned groupings of the populations.

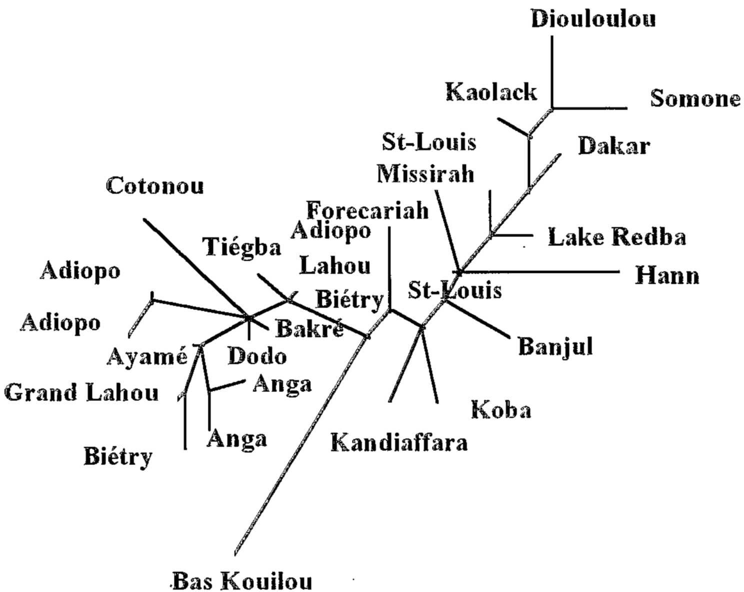


Fig. 4
Dendrogram obtained with the parsimony technique using an alleles presence/absence matrix.

If the different subspecies studied are shown on the figures, we see an affirmation of the groupings based on the morphological systematic and those revealed through enzymatic protein electrophoresis.

- The populations from Senegal and Guinea belong to the subspecies *S. m. heudeulotii*,
- The populations from Côte d'Ivoire and Benin belong to the subspecies *S. m. melanotheron*,

- The population from the Lower Kouilou in Congo belong to the subspecies *S. m. nigripinnis*.

Concerning the microsatellites, two (79M, 79H) of the four loci analyzed were monomorphic in all samples. The loci 28 and 73C had 6 and 26 alleles respectively. The mean theoretical Heterozygosity (H) varies from 32 to 78%. All samples presented relatively elevated value. Only the Cotonou sample where the specimens are homozygous for all loci studied showed a null mean heterozygosity value. The genetic differences are few, of a strictly qualitative order, and concern few of the alleles of which only allele 126 of locus 28 distinguishes the two groups. The first group is made up of samples from Western Côte d'Ivoire. The second includes not only samples from Côte d'Ivoire, but also those from the Eastern regions of this country. These groups are identical to those revealed by enzymatic electrophoresis. These two groups are also found in the phylogenetic network built using genetic distances. However, the microsatellite markers analyzed in this study did not allow identification of the different subspecies described by TREWAVAS (1983) for this region.

Conclusion

The morphological and genetic study of *S. melanotheron* populations has clearly shown that these latter can be placed into three groups, each one corresponding to a subspecies described by TREWAVAS (1983). However, our results show the need for the refinement of the classification proposed by TREWAVAS (1983). These results show that the genetic differentiation between the populations is clinal. It is therefore difficult to apply the concept of subspecies to them. The great genetic diversity observed between the samples may lead to different zoo-technical behaviors. Also, this study may open up new avenues of research into the zoo-technical characteristics of *Sarotherodon melanoteron* populations in the hopes of selecting more performing aquacultural strains.

Acknowledgements

The authors would like to thank Mrs. A. Diallo and A. Pariselle for their participation in the capture of wild fish from Senegal and Congo. This work as part of the GENETICS program, was made possible thanks to funding from the European Union (contract ERBTS3*CT920079) and Orstom (l'Institut français de recherche scientifique pour le développement en coopération).

References

ECK (R.V.), DAYOFF (M.O.), 1966 — *Atlas of protein sequence and structure 1966*. National Biomedical Research Foundation, Silver Spring, Maryland.

FELSENSTEIN (J.), 1989 — *PHYLIP (Phylogeniy Inference Package), version 3.5.* Seattle,University of Washington, Washington USA .

KLUGE (A.G.), FARRIS (J.S.), 1969 — Quantitative phyletics and the evolution of anurans. *Syst. Zool.*, 18: 1-32.

MCANDREW (B.J.), MAJUMDAR (K.C.), 1983 — Tilapia stock identification using electrophoretic markers. *Aquaculture*, 30: 249-261.

NEI (M.), 1978 — Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89: 583-590.

ROGNON (X.), 1993 — *Diversité génétique et relations phylogénétiques chez les tilapias (Pisces : Cichlidae). Comparaison des données du polymorphisme enzymatique et mitochondrial.* Thèse de doctorat de l'université Paris-XI, Orsay. 175 p.

TREWAVAS (E.), 1983 — *Tilapine fishes of the genera* Sarotherodon, Oreochromis *and* Danakilia. British Museum. Natural History, London 583 p.

WEIR (B.), COCKERHAM (C.C.), 1994 — Estimating F-statistics for the analysis of population structure. *Evolution*, 41 (2), 1358-1370.

Comparison of brackish water growth performances of *Sarotherodon melanotheron* (Cichlidae) from three West African populations

Sylvain Gilles
Aquaculture researcher

Jean-Baptiste Amon-Kothias
Hydrobiologist

Jean-François Agnèse
Geneticist

Introduction

Tilapia are common in tropical freshwater aquaculture, not only in Africa where they originated, but also in Asia and South America (PULLIN, 1988). Red tilapia, which are hybrids between *Oreochromis mossambicus* or *O. hornorum*, and *O. niloticus* or *O. aureus* are sometimes raised in sea water (WATANABE *et al.*, 1989). Culture of tilapia in lagoon and estuarine environments remains poorly developed because few of the species have both rapid growth and the ability to withstand marked variations in salinity (PAYNE, 1983). *Sarotherodon melanotheron* is a paternal mouthbrooding tilapia, which inhabits salt, brakish and freshwaters in West Africa (PAULY, 1976; PRUNET and BORANCIN, 1989;

TEUGELS and THYS VAN DEN AUDENAERDE, 1992). The growth rates of populations that inhabit lagoons in Côte d'Ivoire (LEGENDRE, 1986; LEGENDRE and ECOUTIN, 1989) are low (0.4 to 0.5 g/day for 100g fish). Therefore this species have never been inventoried as a good candidate for aquaculture in brackish or salt waters (STICKNEY, 1986). TREWAVAS (1983) showed that populations of *S. melanotheron* are morphologically differentiated and described five subspecies: *S. m. heudelotii* (DUMERIL, 1859), from Senegal to Guinea; *S. m. paludinosus* (TREWAVAS, 1983), in certain freshwaters near Dakar, Senegal; *S. m. leonensis*, (THYS VAN DEN AUDENAERDE, 1971), from Sierra Leone to Liberia; *S. m. melanotheron* (RUPPEL, 1853), from Côte d'Ivoire to Cameroon; *S. m. nigripinnis* (GUICHENOT and DUMERIL, 1859), from Equatorial Guinea to Angola. Recent genetic studies made on populations of *S. melanotheron* (POUYAUD, 1994; POUYAUD and AGNESE, 1996,) show that this species is composed of genetically well differentiated populations, and three genetic groups can be defined: (1) populations from Cameroon Senegal and Guinea, (2) populations from Sierra Leone to and (3) populations from Gabon and Congo. This level of differentiation may also imply that populations have physiological and behavioural differences. Consequently, comparisons of growth performance were made between three genetically and morphologically differentiated populations, from Senegal (*S. m. heudelotii*), Côte d'Ivoire (*S. m. melanotheron*), and Congo (*S. m. nigripinnis*), with the aim of assessing aquaculture potential.

Material and Methods

Test Fish

Fish from three populations of *S. melanotheron* were tested under intensive culture conditions in brackish water. The fish which were hatched in captivity were the offspring of wild parents. *S. m. heudelotii* (S) were collected from the Niayes of Thiaroye in the suburbs of Dakar, Senegal during April 1993 (32 individuals,

mean weight 63g, 16 males and 16 females). Broodstock *S. m. melanotheron* (I) were obtained from Layo experimental station in Ebrié Lagoon (Côte d'Ivoire) in October 1993 (40 individuals, mean weight 70g, 20 males and 20 females) and broodstock *S. m. nigripinnis* (C) (16 individuals, mean weight 53g, 8 males and 8 females) were collected at Bas Kouilou (lower Kouilou drainage in Congo) during May 1993.

Imported broodstocks were held in quarantine for six months and were then transferred to Layo aquaculture station. Fish were held under conditions which prevented the release of exotic fish into the natural environment.

First experiment

The wild parental fish were kept in tanks (1m*1m*1m) for 21 days, and on day 22, all brooders were removed and fry sorted.

Triplicate groups of 100 fry (between one and three weeks old) from each population were stocked into concrete tanks (33 fish/m^3).

Each group was labelled C1, C2 and C3; originating from the Congo; S1, S2 and S3 for population from Senegal; and I1, I2 and I3 for population from Côte d'Ivoire.

The fry had mean initial weights varying from 0.22 to 3.79g (Table 1). The growth trial was conducted in 4m^3 (2m*2m*1m) concrete tanks, and water was renewed (3m^3/h) with brackish water pumped from the lagoon.

The 176-days experimental period (4 January to 30 June 1994) coincided with the dry and then the rainy seasons so the physical and chemical characteristics of the water varied with time: salinities from 6 (February) to 0 (June), temperatures from 31.5°C (April) to 29°C (June).

Dissolved oxygen, maintained by mechanical agitation, varied from 4.4 mg/l at 5:00 PM to 4.5mg/l at 8:00 AM. Turbidity also varied, with Secchi disc values ranging from 70 cm (February) to 10 cm (June).

measures	C1	C2	C3	S1	S2	S3	I1	I2	I3
IMW (g)	0.35	0.36	0.32	0.22	2.72	3.79	0.55	0.77	0.62
SE	0.11	0.11	0.11	0.09	0.94	0.39	0.24	0.39	0.29
FMW (g)	44.3	31.9	29.1	74.5	106.7	116.1	40.8	37.9	40.8
SE	10.1	6.9	4.7	8.9	9.6	12.8	7.3	4.5	7.8
SR %	22	60	70	55	77	72	84	91	87
SLOPE * 10^2	1.41	1.26	1.10	1.81	1.85	1.84	1.35	1.25	1.26
SE * 10^2	0.07	0.04	0.03	0.04	0.01	0.02	0.04	0.03	0.05
INT	1.39	1.25	1.25	1.31	1.74	1.94	1.37	1.39	1.47
R^2	97.7	98.9	99.3	99.6	99.9	99.8	97.7	98.9	99.3

Table 1
Growth performances of all groups in the first experiment. IMW and SE: initial mean weights and standard errors; FMW and SE: final mean weights and standard errors; SR%: survival rates percentages; SLOPE *10^2 and SE *10^2: slopes of the regression lines of the growth curves and standard errors multiplied by 10^2; INT: intercepts of the regression lines; R^2: the R-squared.

The fish were fed with crumbles (0.5 to 1.5mm; containing 30% protein, 6% lipid, 7% cellulose, Vit.A 8000 I.U./kg, Vit.D3 2000 I.U./kg, Vit.E 100mg/kg, Vit.C 130mg/kg), twice every day. Every 15 days, the total weight of each tank of fish was monitored to adjust the daily feeding rate wich corresponded to 5% of the total biomass. Fish were individually weighed at the beginning and at the end of the experiment. During the experiment the total weight of each group, the number of individuals, the presence of mating individuals (individuals with sexual coloration) and the presence of mouthbrooding males were noted.

Second experiment

In the second experiment two populations originating from Senegal and Côte d'Ivoire were compared. Parental fish were held in tanks

(1m*1m*1m) for 56 days, and on day 57, all adults were removed and fry sorted. Three replicates of 50 fish from each population were placed in 1.6m^3 cages (31 fish/m^3), and labeled Sx, Sy, Sz and Ix, Iy, Iz. for Senegal and Côte d'Ivoire populations respectively.

Mean weights of the fish in all six replicates were similar (Table 4), weights (around 10g) corresponding to 6-week old fish in the Senegalese population and 8-week old fish in the Côte d'Ivoire population.

The 1.6m^3 (1.5m*1.5m*0.7m) cages, made of 6mm (side) mesh net, were held in place with stakes and rested on the bottom of a 1ha pond pump-fed with brackish or sea water. The 168-day experimental period (3 November 1994 to 20 April 1995) coincided with the dry season. Salinity varied over time: from 18 (November) to 35 (April). Water temperatures ranged from 29 to 31°C. Dissolved oxygen varied little during the experiment, averaging 6.0 mg/l at 8:00 AM and 12.5mg/l at 5:00 PM. The water was light green in colour and there was little suspended mineral matter.

The feeding and sampling protocols were as in the first experiment.

Data analysis

The survival rate was noted for each experiment. The different values observed were tested using a Chi^2 test (SNEDECOR and COCHRAN, 1957)

In order to compare growth rates, cube roots of weigths were taken, and a linear model applied: $Y=aX^{1/3}+b$, where Y is time in days and X the mean weight in g.

Growth rate equations were calculated from day 14 onwards to account adaptation of the fish to their new rearing environment.

Regression equations were compared for differences of slopes using ANCOVA F-test on mean squares (SNEDECOR and COCHRAN, 1957).

Results

First experiment

The results are summarized in Table 1.

Survival rates were not statistically different (Chi^2 Test, 5%) except for C1 replicate which showed the lowest survival rate (22%). Fish of the Côte d'Ivoire population showed the best survival (mean of 87%) whereas cumul was lowest in the Congo fish (mean of 51%), this value was caused by the low survival noted for the C1 replicate. Most mortalities occurred early in the experiment at a time when the fish were smallest and the salinity of the water highest.

The slopes and intercept of the growth rate equations for each replicate are shown in Table 1.

Cube roots transformation of the data enabled growth rates (slopes of the equations) to be compared. Table 2 summarizes the results of the statistical comparison (F test on mean squares) of the different slopes observed. Despite there being differences in initial weights in the three S groups, the corresponding slopes were not significantly different ($p>0.05$). During the experiment the growth of these groups followed the same model. This observation enables us to compare the slopes of the three S population groups with those of the C and I populations. In I population the slopes of the three groups were not significantly different ($p>0.05$). In C population, C3 group show a significantly lower slope than C1 group ($p>0.01$). Slopes observed in population S were statistically higher ($p<0.001$) than those observed in populations C and I while these two populations have not statistically different slopes except C3 group which had a statistically significant lowest slope with I1 group ($p>0.001$). In all groups there was no difference in weight between males and females.

Replicates	S3	S2	S1	I3	I2	I1	C3	C2
C1	***	***	***	~	~	~	*	~
C2	***	***	***	~	~	~	~	
C3	***	***	***	~	~	**		
I1	***	***	***	~	~			
I2	***	***	***	~				
I3	***	***	***					
S1	~	~						
S2	~							

Table 2
Statistical comparison (F test on mean squares) of the different slopes of the equations of the growth rates curves observed in the first experiment, with ~: P>0.05, *: 0.05>P>0.01, **: 0.01>P>0.001 and ***: P<0.001.

	First experiment			Second experiment	
Sexual characters	C	S	I	S	I
Appearence of first sexual colorations					
mean age of fishes (day)	70	91	70	154	154
Mean Weight (g)	8	26	7	93	47
Appearence of first spawn					
mean age of fishes (day)	98	133	98	168	168
Mean Weigh (g)t	9	56	13	107	52

Table 3
Mean age (days after hatching) and mean weigth of fish at the appearances of the first sexual colorations and the first spawns in both experiments.

Sexual dimorphism in body coloration of mature individuals of the three populations differed in both expression and time:
- mature males of Côte d'Ivoire Population permanently have metallic yellow opercula, whereas females have transparent opercula which show the red colour of the gills;

- when fish of the S population are spawning both sex have a black coloration on the edge of the caudal fin and at the throat;
- mature fish of both sex in the Congo population permanently display numerous black spots concentrated under the throat.

Table 3 summarize the observations. The appearence of sexual coloration and first spawn were simultaneous in both C and I population while it occured later in S population.

Second experiment.

The results are summarized in Table 4. The differences observed in survival rates (from 78 to 90%) was not significative (Chi² Test, 5%) despite the fact that I groups showed the highest values. In I population there was no initial mortality, but a slight morbidity appeared after the 50th day and continued through the end of the experiment. In the S population mortalities were noted during the first 30 days and disappeared thereafter.

Measures	Sx	Sy	Sz	Ix	Iy	Iz
IMW (g)	10.1	7.9	8.8	8.9	10.2	9.3
SE	1.2	0.8	0.9	1.2	1.4	1.4
FMW (g)	152.2	146.8	156.2	76.2	83.4	83.1
SE	24.3	19.1	20.3	19.0	23.3	22.4
SR %	82	78	80	86	90	88
SLOPE*10^2	1.73	1.75	1.80	1.36	1.39	1.43
SE*10^2	0.04	0.08	0.05	0.06	0.04	0.04
INT	2.78	2.74	2.71	2.28	2.32	2.27
R^2	99.4	98.1	99.2	98.3	99.2	99.1

Table 4
Growth performances of all replicates in the second experiment.
IMW and SE: initial mean weights and standard errors; FMW and SE: final mean weights and standard errors; SR%: survival rate percentages; SLOPE *10^2 and SE *10^2: slopes of the regression lines of the growth curves and standard errors multiplied by 10^2;
INT: intercepts of the regression lines; R^2: the R-squared.

The equations of the growth rate curves are shown in Table 4 (slope, intercept and R^2). Table 5 summarize results of the statistical comparison (F test on mean squares) of the different slopes observed. No significant differences were found between the intrapopulation slopes ($p>0.05$) whereas the interpopulation slopes were significantly different ($p<0.05$). In all groups, there was no difference in weight between males and females.

The appearance of secondary sexual characteristics and spawns (Table 3) occurred simultaneously for both populations when fish were 154 and 168 days old respectively.

Replicates	Sz	Sy	Sx	Iz	Iy
Ix	***	**	***	~	~
Iy	***	***	***	~	
Iz	***	**	***		
Sx	~	~			
Sy	~				

Table 5
Statistical comparison (F test on mean squares) of the different slopes of the equations of the growth rates curves observed in the second experiment, with ~ : P>0.05, *: 0.05>P>0.01, **: 0.01>P>0.001 and *** P<0.001.

Discussion

In both experiments, though survival rates were not statistically different (except for C1), the I groups always had the lowest mortalities. It can also be noted that this survival was characterized by a high initial mortality for population S and C. This could be due to the non-indigenous status of these two populations, rendering them more sensitive to the stress of manipulations. In these

conditions, the densities of I groups were always higher than those of S. groups. This could affected their growth rates. LEGENDRE *et al.* (1989) showed that rearing densities varying from 20 to 150 fishes/m^3 do not affect growth rates of *S. melanotheron* from Côte d'Ivoire. Our results also showed that the S1 group in the first experiment which had a low survival rate did not have a higher growth rate value. For these raisons, it is likely that survival rates and the final densities observed in both experiments (25 fish/m^3 for S, and 30 fish/m^3 for I) did not affect the growth performances of each S and I group. In C population, C1 group had the higher growth rate and the lowest survival rate. Final density in C1 was 7 fishes/m^3. This density is far from those used in LEGENDRE *et al.* (1989) and the others observed in this study. Then we can conclued that population S had significantly higher growth rates than population I and C.

Comparison of the growth rates within each population for both experiments showed no statistically significant differences ($p>0.05$).Therefore fish from this species had the same growth rate at salinity varying from 0 to 35g/l. On the contrary, reproductive behaviors were different in both experiments. The first sexual coloration and first spawn occurred earlier in the first experiment (Table 3). In this experiment fish from S population reproduced later than those of two other populations. No differences were observed between S and I populations in the second experiment. LEGENDRE and ECOUTIN (1996) have shown that the time of the first appearence of sexual coloration and fecundity were different in wild, intensive and extensive captive conditions. Our results showed that salinity could also affect the reproductive behavior of this species. From these results it clearly apears that population from Senegal is a potential candidate for aquaculture, especially in waters such as lagoons or estuaries where the salinity varies. More studies are necessary to know if this growth capacities are consistent throughout the subspecies *Sarotherodon melanotheron heudelotii* or specific only to those populations living in the marshy areas of Dakar. It would therefore be useful to sample this subspecies throughout its distribution, which extends from Senegal to Guinea, and then to compare the zootechnical performances of the different populations.

Acknowledgements

The authors would like to thank Mrs. A. Diallo, A. Pariselle and L. Pouyaud for their participation in the capture of wild fish from Senegal and Congo, also R. Dugué and F. Duponchelle for their help in statistical analysis. This work as part of the GENETICS program, was made possible thanks to funding from the European Union(contract ERBTS3*CT920079) and Orstom (l'Institut français de recherche scientifique pour le développement en coopération).

References

LEGENDRE (M.), 1986 — Influence de la densité, de l'élevage monosexe et de l'alimentation sur la croissance de *Tilapia guineensis* et de *Sarotherodon melanotheron* élevés en Lagune Ebrié, (Côte d'Ivoire). *Rev. Hydrobiol. Trop.*, 19: 19-29.

LEGENDRE (M.), ECOUTIN (J.M.), 1989 — Suitability of brackish water tilapia species from Ivory Coast for lagoon aquaculture. I - Reproduction. *Aquat. Living Resour.*, 2: 71-79.

LEGENDRE (M.), HEM (S.), CISSE (A.), 1989 — Suitability of brackish water tilapia species from Ivory Coast for lagoon aquaculture. II - Growth and rearing methods. *Aquat. Living Resour.*, 2: 81-89.

LEGENDRE (M.), ECOUTIN (J.M.), 1996 — Aspects of the reproductive strategy of *Sarotherodon melanotheron*: Comparison between a natural population (Ebrie Lagoon, Côte d'Ivoire) and different cultured populations. Translated from French by (C.) LHOMME-BINUDIN. In PULLIN (R.S.V.), Legendre (M.), LAZARD (J.L.), AMON-KOTHIAS (J.B.), PAULY (D.), ed.: *TheThird International Symposium on Tilapia in Aquaculture.* Iclarm. Proc. 41: 326-338

PAULY (D.), 1976 — The biology, fishery and potential for aquaculture of *Tilapia melanotheron* in small West African lagoons. *Aquaculture*, 7: 33-49.

PAYNE (A.I.), 1983 — *Estuarine and salt tolerant tilapias.* In Proceedings, International Symposium on Tilapias in Aquaculture. Tel Aviv University, Tel Aviv, p 534-543

POUYAUD (L.), 1994 — *Génétique des populations de tilapias d'intérêt aquacole en Afrique de l'Ouest. Relations phylogénétiques et structurations populationnelles.* Thèse de l'université de Montpellier-II, France. 182 p.

POUYAUD (L.), AGNESE (J.F.), 1996 — Genetic differenciation in several stocks of *Sarotherodon melanotheron* and *Tilapia guineensis* from Côte d'Ivoire, Senegal and Gambia. Translated from the French by (C.) LHOMME-BINUDIN. In PULLIN (R.S.V.), Legendre (M.), LAZARD (J.L.), AMON-KOTHIAS (J.B.), PAULY (D.), eds.: *The Third Int. Symp. on Tilapia in Aquaculture.* ICLARM Conf. Proc. 41: 368-376

PRUNET (P.), BORANCIN (M.), 1989 —
Physiology of salinity tolerance
in Tilapia: An update of basic
and applied aspects.
Aquat. Living Resour., 2: 91-97.

PULLIN (R.S.V.), 1988 —
Ressources génétiques en tilapias pour l'aquaculture. Atelier
sur les ressources génétiques
en tilapias pour l'aquaculture 23-24
mars 1987, Bangkok, Thaïlande.
ICLARM Conference proceeding.
(R.S.V.) PULLIN editor, 129 p.

SNEDECOR (G.W.), COCHRAN (W.G),
1957 —
Statistical Methods. The Iowa State
University Press, Ames, Iowa, USA,
49 p.

STICKNEY (R.R.), 1986 —
Tilapia Tolerance of Saline Waters:
A Review. *Progressive Fish-Culturist*,
48: 161-167.

TEUGELS (G.G.), THYS VAN DEN
AUDENAERDE (D.F.E.), 1992 —
Cichlidae. *In: Faune des poissons d'eau douces et saumâtres de l'Afrique de l'Ouest.* LEVEQUE (C.), PAUGY (D.),
TEUGELS (G.G.), eds.
Collection Faune Tropicale 28. Orstom
éditions/Mrac, 714-779.

TREWAVAS (E.), 1983 —
Tilapiine fishes of the genera Sarotherodon, Oreochromis
and Danakilia. British Museum
(Natural History),
London, UK, 583 p.

WATANABE (W.O.), ERNST (D.H.),
OLLA (B.L), WICKLUND (R.I.), 1989 —
*Aquaculture of red Tilapia (*Oreochromis sp*) in marine environments: state of the art.*
In: Advances in Tropical Aquaculture.
Tahiti, Feb. 20 - March 4, 1989.
Aquacop. Ifremer.
Actes de Colloque 9: 487-498.

Genetic diversity analysis of *Oreochromis shiranus* species in reservoirs in Malawi

Aggrey J. D. Ambali
Geneticist

Roger W. Doyle
Geneticist

Introduction

It is estimated that there are more than 800 reservoirs in Malawi of size range of 0.4 - 7.7 ha, spread throughout the country (VINCKE, 1990). Most of them have capacity of less than 50,000 m^3 with catchment of less than 1-2 km^2. Artificial stocking of the reservoirs was carried out in early 1960s with *Oreochromis shiranus chilwae* and *Tilapia rendalli* especially those in the southern region. The reservoirs in general are rarely harvested and have not been managed for commercial fish production.

According to mode of ownership, reservoirs are classified into three categories namely; government owned; estate owned and communally owned. Government owned reservoirs are those that belong to government institutions like schools, the Forestry Departement and agricultural development projects, among others. These are located on public land. Estate owned reservoirs are located on leasehold land belonging to tea, tobacco and sugar estates. Communal reservoirs are located on customary land controlled by chiefs or heads of ethnic groups. Ownership of

communal reservoirs is unclear because they belong to every member of the ethnic group, although major activities carried out on the reservoirs by individuals have to be approved by the chief.

The government reservoirs were constructed between 1950 and later in the 1960s. They were mainly constructed for irrigation purposes, for instance those that belong to Forestry Department and Ministry of Agriculture were for supplying drinking water for livestock and irrigating tobacco seedlings. As a realized benefit, water from reservoirs is also used for domestic activities. Stocking of fish in the reservoirs was carried out during the colonial period and was done only once in almost all the reservoirs in the country.

Estate reservoirs were stocked by estate management (KANDOOLE and AMBALI, 1992). Limited investment is made in fishery management because most activities are concentrated on crop production from where estates derive almost all their gross margins.

Communal reservoirs were stocked by Fisheries Department during the colonial period but limited number of local community members recall when stocking took place. Some of them even believe that stocking was through streams that drain into the reservoirs. Fishing by village members is allowed although people still believe that the reservoirs belong to government.

The objective of the study was to determine genetic diversity of reservoir populations of *O. shiranus sp*; and determine the extent of genetic differentiation occurring in the reservoirs.

Materials and methods

Source of samples

Oreochromis shiranus sp samples were collected from seven reservoirs, namely Chilala (CHL), Bvumbwe (BVU), Mikolongwe (MKW), Mpemba (MPA), Mvonia (MVO), Bishop (BSR) and Bunda (BCR). Of these, five belong to government and private institutions and two are communal (Table 1).

Reservoir	Code	District/City	Type of ownership	n
Chilala	CHL	Blantyre	Government	40
Bvumbwe	BVU	Blantyre	Communal	42
Mikolongwe	MKW	Blantyre	Government	40
Mpemba	MPA	Blantyre	Government	32
Mvonia	MVO	Blantyre	Communal	40
Bunda	BCR	Lilongwe	Institutional	55
Bishop's	BSR	Mzuzu	Institutional	55

Table 1
Summary of the reservoirs studied and number of individuals analysed.

Species Composition

Sampling was carried out using a seine net cast across several areas of each reservoir. The net was pulled to the edge where fish caught were identified to the species they belonged. Random samples of *O. shiranus* were collected from catches and individual standard length was measured before blood or muscle were collected for DNA analysis.

DNA analysis

Blood samples were collected from BCR, BSR and MVO populations while muscle tissues were collected from BVU, CHL, MBA and MPA populations. The procedure outlined in BROOKER *et al.* (1994) was used to extract DNA from blood and a modification of the extraction procedure of KAMONRAT *et al.* (in prep) was used to extract DNA from muscle. Tissue of 3 mm^2 size was placed in a microtube and 1.0 mL high TE was added. The mixture was vortexed for 30 s and left to stand for 10 min before the supernantant was decanted. Two hundred and fifty µl MGPL lysis buffer and 2.5 µl Proteinase K were added to digest tissue in a 45°C waterbath for at least 3 h until the tissue had been completely

digested. The mixture was vortexed and spun in Eppendorf microcentrifuge for 5 min. The aqueous phase was transferred to new microtube where 500 µl TE was added before mixing by vortexing. Thirty five µl of 3 M NaCl and 750 µl of cold isopropanol were added to the mixture and vortexed until the solutions had completely mixed. The mixture was incubated at -80°C to precipitate DNA and then spun in the Eppendorf microcentrifuge for 10 min. The liquid was decanted and DNA pellete was washed with 500 µl of 70% cold ethanol by vortexing the mixture and then spinning for 5 min. Ethanol was decanted and the tube was spun again to remove residual ethanol. The pellete was air dried for 10-15 min before resuspending DNA in 100 µl TE.

Five polymorphic microsatellite loci were analyzed using primers developed by AMBALI (1996). Amplification was carried out in a Biocycler PCR machine where samples were at first cycled 7 times through the series: denaturation at 94°C for 1 min, annealing at primer specific temperature for 30 s and extension at 72°C for 1 s. These were followed by 28 cycles through the series: denaturation at 88°C for 30 s, annealing at primer specific temperature for 30 s and extension at 72°C for 1 s. PCR products were electrophoresed on a 8% denaturing acrylamide gel and sized using a M13 DNA ladder.

Data analysis

Mean and range for each population were calculated for the standard length measurements. These were compared to wild populations from lakes Chilwa, Chiuta, and Malombe. The exact Hardy-Weinberg test in GENEPOP version 1.2 (RAYMOND and ROUSSET, 1995) was used to compute observed and expected heterozygosity values, and to test for conformity to Hardy-Weinberg equilibrium (HWE) using the exact test by GUO and THOMPSON (1992).

The sequential Bonferroni correction was used to adjust significant level (LESSIOS, 1992). In the procedure, the tests were ordered from

the highest to the lowest according to their probability values. The highest probability, p_m was compared to the significant level α. If p_m was greater than α the comparison continued with the subsequent probabilities, each compared to $\alpha'_{i+1} = \alpha/(1+i)$ where i is the number of tests already performed.

BIOSYS-1 computer program (SWOFFORD and SELANDER, 1989) was used to compute a number of measures of genetic variation within and between sample populations. The following variables were computed to determine allelic diversity: number of alleles per locus, actual number of alleles per locus for each reservoir, mean number of alleles per locus per reservoir, effective number of alleles per locus per reservoir (CROW and KIMURA, 1970).

The DIPLOIDL program in GENEPOP was used to compute Wright's F-statistics (WRIGHT, 1978) according to WEIR and COCKERHAM (1984). The among-population component of genetic variance F_{ST} was computed to measure the proportion of total variation that could be ascribed to differences between population allele frequencies. F_{IS} values were also calculated to determine heterozygote deficiency and excess within populations.

The GENDIST program in PHYLIP version 3.4 (FELSENSTEIN, 1990) was used to compute CAVALLI-SFORZA and EDWARDS (1967) chord distance between species and subspecies. Mantel's test was carried to determine the correlation between geographic distance and CAVALLI-SFORZA and EDWARDS (1967) chord distance. The test was based on the null hypothesis that there was no correlation between genetic distance and the geographic distance between locations where the population samples were collected.

The MXCOMP program of NTSYS-pc was used to compute a product-moment correlation coefficient (*i.e.* normalized Mantel's statistic Z) for each pair of distance matrices (ROHLF, 1992). To determine if the correlations were significant, actual coefficients were compared to values produced by randomly permuting each matrix pair 1000 times.

Results

Fish species found in the reservoirs

Data on species caught in the reservoirs are provided in Table 2. The predominant species were *O. shiranus sp*, *T. rendalli*, *Barbus sp*, *Clarias gariepinus*, *Serranochromis robustus* and other species in the haplochromid family like *Pseudocrenilabrus philander* and *Astatotilapia calypterus*. *O. shiranus sp*, *T. rendalli* and *S. robustus* were artificially stocked while the other species were stocked through natural streams. With the exception of the MVO reservoir, *O. shiranus sp* was the most predominant species in the catches in all the reservoirs. *T. rendalli* was observed in five of the reservoirs but not in MPA and MVO. *C. gariepinus* was found in CHL, MKW and MVO reservoirs; the species was mostly abundant in MKW reservoir. *S. robustus* was only found in BVU reservoir while *Barbus sp* was found in CHL, MKW and MPA reservoirs.

Reservoir	O. sh.	T. rend	C. gar.	S. rob	Barbus
Chilala	+	+	+	-	+
Bvumbwe	+	+	-	+	-
Mikolongwe	+	+	+	-	+
Mpemba	+	-	-	-	+
Mvonia	+	-	+	-	-
Bishop's	+	+	-	-	-
Bunda	+	+	-	-	-

Table 2
Species composition of the catch in various reservoirs at the time of sampling. O. sh. (*O. shiranus sp*), T. rend (*T. rendalli*), C. gar (*C. gariepinus*), S. rob (*S. robustus*), Barbus (*Barbus sp*): (+) species present, (-) species not observed.

Mean and range standard length (SL) of *O. shiranus sp* found in the reservoirs and lakes are presented in Table 3.

Although differences in mean SL of the various populations did not necessarily imply that reservoir populations grew faster than those in lakes, they generally shed some light on individual size distribution found in the two types of waterbodies. *O. shiranus* populations in the reservoirs had higher mean SL than those in the lakes. The range suggests that reservoir populations were shifted towards large size distribution compared to lake populations. In the reservoirs where there were large predators like *C. gariepinus* and *S. robustus*, *O. shiranus* caught were of large size, implying that predators reduced the population of small tilapia individuals. *O. shiranus* samples analyzed for MVO reservoir were collected in March 1995 when the species was most abundant in the catch. Ten months later, in December 1995, when the water volume had declined due to drought, the species was less than 1% of the total catch, and the predominant species became *C. gariepinus*.

Reservoir/Lake	Mean SL (cm)	Range (cm)
Chilala	19.9	14.5 - 26.0
Bvumbwe	20.5	15.0 - 25.5
Mikolongwe	13.9	10.5 - 17.0
Mpemba	17.7	10.0 - 17.0
Mvonia	13.6	13.8 - 24.7
Bishop	-	-
Bunda	14.1	11.3 - 20.1
Lake Chilwa	11.4	9.9 - 14.5
Lake Chiuta	8.1	6.6 - 13.5
Lake Malombe	9.3	7.7 - 10.7

Table 3
Mean and range standard length (SL) of *O. shiranus sp* in the reservoirs and lakes.

Conformity to Hardy-Weinberg Equilibrium

Tests for conformity to Hardy-Weinberg Equilibrium (HWE) are presented in Table 4. According to the exact test, there were more locus-population combinations (77.1%) that showed no significant departure from the HWE equilibrium than those that showed significant departure from HWE (22.9%).

Populations	Os-7	Os-25	Os-7R	Os-64	Os-75
CHL	0.240	0.094	0.034	0.737	0.242
BVU	0.007	0.228	0.001	0.737	<0.001
MKW	0.910	<0.001	0.016	0.762	0.009
MPA	0.399	1.000	0.527	1.000	0.067
MVO	0.096	0.141	<0. 00 1	0.515	0.357
BCR	0.162	0.006	0.012	0.004	<0.001
BSR	0.471	0.893	0.197	0.001	0.021

Table 4
Level of significance of departure from HWE using the exact test.

Genetic diversity

Summary of observed and expected heterozygosity values is presented in Table 5. All loci were polymorphic in all the reservoir populations. Mean observed heterozygosity ranged from 0.537 ± 0.076 to 0.713 ± 0.034. The highest heterozygosity values were observed in BSR and BCR populations and in their decreasing order MKW, BVU, CHL, MPA and MVO, although their 95% confidence intervals (mean ± 2SE) suggest that heterozygosity values between populations were not significantly different.

Pop		Os-7	Os-25	Os-7R	Os-64	Os-75	Mean ± SE
CHL	Observed	0.676	0.667	0.724	0.650	0.450	0.633 ± 0.047
	Expected	0.789	0.708	0.838	0.707	0.421	0.693 ± 0.072
BVU	Observed	0.632	0.462	0.769	0.487	0.925	0.655 ± 0.087
	Expected	0.555	0.585	0.563	0.459	0.596	0.552 ± 0.024
MKW	Observed	0.571	0.306	0.974	0.667	0.789	0.661 ± 0.112
	Expected	0.594	0.585	0.829	0.644	0.819	0.694 ± 0.054
MPA	Observed	0.542	0.390	0.854	0.467	0.780	0.607 ± 0.090
	Expected	0.494	0.342	0.836	0.437	0.811	0.584 ± 0.101
MVO	Observed	0.353	0.575	0.795	0.425	0.538	0.537 ± 0.076
	Expected	0.500	0.642	0.767	0.481	0.711	0.620 ± 0.057
BCR	Observed	0.571	0.769	0.750	0.540	0.766	0.679 ± 0.051
	Expected	0.553	0.855	0.914	0.657	0.891	0.774 ± 0.071
BSR	Observed	0.783	0.760	0.750	0.600	0.674	0.713 ± 0.034
	Expected	0.827	0.792	0.861	0.683	0.871	0.807 ± 0.034

Table 5
Observed and expected heterozygosity at five microsatellite loci.

Measures of allelic variability are presented in Table 6. The average number of alleles per population ranged from 3.2 ± 0.37 to 10.2 ± 2.15. The effective number of alleles was high in more recently stocked reservoirs (BSR and BCR) than those which were stocked between 1950 and 1960 (*i.e.* reservoirs in Blantyre district) and where carnivorous species were observed.

Population structure

Levels of intra- and interpopulation variation are presented in Table 7. Inbreeding coefficient (F_{IS}) ranged from 0.040 to 0.143, with mean of 0.090; implying that there was heterozygosity deficiency at all loci in the populations. The F_{ST} values ranged from 0.147 to 0.370, with mean of 0.248.

Populations	Os-7	Os-25	Os-7R	Os-64	Os-75	Total	A
CHL	7 (4.5)	8 (3.31)	10 (5.66)	7 (3.30)	5 (1.71)	37 (18.5)	7.4±0.81 (3.7±0.66)
BVU	4 (2.21)	3 (2.36)	3 (2.25)	2 (1.82)	4 (2.43)	16 (11.1)	3.2±0.37 (2.2±0.10)
MKW	4 (2.40)	7 (2.26)	8 (5.49)	5 (2.73)	11 (5.20)	35 (18.2)	7.2±1.28 (3.6±0.70)
MPA	4 (1.93)	5 (1.51)	11 (5.72)	3 (1.75)	9 (5.04)	32 (16.0)	6.4+-0.09 (3.2±0.90)
MVO	2 (1.97)	3 (2.73)	7 (4.11)	2 (1.90)	4 (3.16)	18 (13.9)	3.6±0.93 (2.8±0.41)
BCR	8 (2.21)	10 (6.50)	14 (10.53)	6 (2.86)	14 (8.44)	52 (30.6)	10.2+9.15 (6.1±1.59)
BSR	8 (5.50)	10 (4.62)	13 (6.79)	4 (3.09)	11 (7.19)	46 (27.2)	9.6±1.63 (5.4±0.74)

Table 6
Measures of allelic variability at five loci in seven reservoir populations of *O. shiranus sp* (number of alleles per locus per population, total number of alleles per population, effective number of alleles per locus (in parentheses) mean ±SE number of alleles (A) and mean ± effective number of alleles (in parentheses).

Locus	F_{IS}	F_{ST}
Os-7	0.076	0.268
Os-25	0.078	0.305
Os-7R	0.040	0.147
Os-64	0.143	0.370
Os-75	0.130	0.175
All loci	0.090	0.248

Table 7
Levels of intra- and interpopulation variation at five loci in seven reservoir populations of *O. shiranus sp.*

Correlation between genetic distance and geographic distance

Matrices of CAVALLI-SFORZA and EDWARDS (1967) chord distance are presented in Table 8. Mantel's correlation coefficient between genetic distance and geographic distance (data not shown) was 0.265 suggesting that there was poor correlation between population genetic distance and geographic distance between reservoirs.

Populations	CHL	BVU	MKW	MPA	MVO	BCR
CHL						
BVU	0.145					
MKW	0.155	0.180				
MPA	0.161	0.164	0.024			
MVO	0.197	0.204	0.120	0.129		
BCR	0.148	0.135	0.122	0.114	0.157	
BSR	0.101	0.156	0.153	0.130	0.156	0.090

Table 8
CAVALLI-SFORZA and EDWARDS (1967)
genetic chord distance between reservoir populations.

Discussion

Species composition

The most dominant species in the reservoirs was *Oreochromis shiranus sp* which was stocked between 1955 and 1960 but there were no commercial fish harvesting operations in the reservoirs. It is speculated that with proper management an additional 80-140 tonnes per annum could be produced from the reservoirs in

Malawi (VINCKE, 1990). The occurrence of carnivorous species like *S. robustus* and *C. gariepinus* had considerable effect on the size distribution of tilapias in the reservoirs. The major problem was that there was no specific predator/prey ratio used in the reservoirs which was detrimental to the tilapia populations as observed in the MVO population during periods of recession. MATHOTHO (1975) recommends a stocking of no more than 6% predators. The proportion of *C. gariepinus* and *S. robustus* was on average above this rate. Production of large tilapia by using predators to control recruitment has been practiced in ponds. OFORI (1988) observed that a predator/prey ratio combination of 1:80 produced the largest average individual size tilapia compared to lower predator/prey ratios of 1/250 and 0. The total biomass was however highest in the treatments where there were no predators. Determination of optimum predator/prey ratio and the size of predator to stock is still problematic in tilapia culture. Lack of management of the reservoirs in Malawi exacerbated the predation problem because there was no culling carried out to reduce the predator population which kept growing through natural recruitment. It was therefore observed that the reservoir fishery was gradually being dominated by *C. gariepinus*.

Genetic diversity

Genetic variability was high in those relatively more recently stocked and managed reservoirs (BCR and BSR) where there was considerably low abundance of carnivorous species. The carnivorous species preyed upon tilapia to the extent that the effective population size of the prey population was reduced. Although the actual numbers of tilapia that remain in the reservoirs is not known, there is evidence showing that their recruitment is continuously controlled by predators. Population size is the most important factor in maintaining a high level of genetic variation in a stock (MEFFE, 1986). Population decline results in genetic variation for future generations being preserved in a relatively small number of individuals. The effective population size (N_e), which is equal to the harmonic mean $1/N_e = 1/[t(N_1 + N_2 + ... + N_t)]$ where t is the number of generations (FRANKLIN, 1990), becomes reduced. The

low Ne, subjects populations to dispersive processes of allele frequencies like genetic drift, bottlenecking and inbreeding.

AMBALI (1996) determined effective number of alleles in lake, reservoir and farm populations. The effective number of alleles in the reservoir populations of Blantyre district which were stocked between 1955 and 1960 were similar to the farm populations that were stocked from the National Aquaculture Centre (NAC) in the early and mid 1980s. The fact that the genetic variability in the reservoirs was close to that of recently domesticated populations implies that the rate of decline of allelic diversity might have been lower in the reservoirs than in the farm populations. For instance, none of the loci were monomorphic in the reservoir populations while there were monomorphic loci in the farm populations.

Despite the predators, the history of stocking in the reservoirs also points to the fact that government and communal reservoir populations were founded on a narrow genetic base. The sizes of founder populations were generally low. ICLARM and GTZ (1991) quote stocking rates in government owned reservoirs ranging from 34 tilapias, 2 haplochromids and 19 *S. robustus* per reservoir to 173 tilapias, 20 haplochromids and 26 *S. robustus*. The tilapias were usually a mixture of *O. shiranus sp* and *T. rendalli*, although there was more shiranus than rendalli.

Genetic distance

Mantel's correlation coefficient between genetic distance and geographic distance was low and not significantly different from zero. Lack of strong correlation was due to the fact that unlike BCR and BSR populations which were founded on single populations of pure strains, the reservoir populations in Blantyre were composite. Despite this, their genetic variation was low. MATHOTHO (1975) indicate that reservoirs in the southern region of Malawi were stocked with mixtures of *O. sh. shiranus* and *O. sh. chilwae*, although the proportions of the two species are not indentified and the actual reservoirs are not indicated. Although Mvonia and Chilala reservoirs were only 3.2 km apart, the alleles observed at locus Os-7 in the two populations (MVO and CHL) were different.

In the MVO population, two alleles observed at the locus were not observed in the CHL population. Alleles in MVO were of larger size than those in CHL. Possibility of contamination with other species of tilapia could not be ruled out, as MATHOTHO (1975) indicates that *O. mossambicus* and *O. placidus* were found in some of the reservoirs in the southern region for instance Lujeri Estate in Mulanje district.

Conclusion

The BSR and BCR reservoirs have demonstrated that genetic diversity can be maintained in the reservoirs over a long period of time. The major factors affecting the biodiversity in the Malawian reservoirs are management, predator/prey ratios and genetic diversity of the founder population. The BCR is the most productive and best managed reservoir in the country (ICLARM and GTZ, 1991) and the BSR in the north is also well managed where feeding is done on regular basis, harvesting is done twice a year and only about 200 kg of large fish is removed *per* harvest. There were no carnivorous species observed during sampling in the two reservoirs. A different situation in the communal and government reservoirs is observed. There is no management being carried out and there is no control in the population of carnivorous species and the effective population sizes of the founder populations were low.

The present setup shows that BCR and BSR can be used for *in situ* conservation but a lot of improvements need to be made on the communal and government reservoirs for them to be used for conservation. There is need to control continuous recruitment of the carnivorous species by carrying out scheduled culling operations. The Fisheries Department of Malawi is currently carrying out stock assessment and limnological experiments in the reservoirs in order to develop management procedures for enhancing productivity. This should be complemented with restocking programs utilizing pure strains of tilapia. The major social issue that needs to be resolved in

reservoir management is that of tenure. Like many other African countries, communal or state-owned reservoirs have presented problems of ownership and fishing rights to the effect that interest in fisheries management in reservoirs has declined in the recent years (ICLARM and GTZ, 1991).

References

AMBALI (A.J.D.), 1996 — *The relationship between domestication and genetic diversity of* Oreochromis species *in Malawi:* Oreochromis shiranus shiranus *(Boulenger) and* Oreochromis shiranus chilwae *(Trewavas).* Ph.D. thesis, Dalhousie University, 204 p.

BROOKER (A.L.), COOK (D.), BENTZEN (P.), WRIGHT (J.M.), DOYLE (R.W.), 1994 — Organization of microsatellites differs between mammals and cold-water teleost fishes. *Can. J. Fish. Aquat. Sci.*, 51: 1959-1966.

CARVALLI-SFORZA (L.L.), EDWARDS (A.W.F.), 1967 — Phylogenetic analysis: models and estimation procedures. *Evol.*, 32: 550-570.

CROW (J. F.), KIMURA (M.), 1970 — *An Introduction to Population Genetics Theory.* Harper & Row, Publishers, 591 p.

FELSENSTEIN (J.), 1990 — *PHYLIP (Phylogeny Inference Package) Version 3.3.* University of Washington, Seatle.

FRANKLIN (I. R.), 1990 — Evolutionary change in small populations. *In* M.E. SOULE and B.A. WILCOX (eds): *Conservation Biology: An Evolutionary-Ecological Perspective.* Sinaeuer Associates, Sunderland, MA. p 135-139.

GUO (S. W.), THOMPSON (E. A.), 1992 — Perfoming the exact test for Hardy-Weinberg proportion for multiple alleles. *Biometrics*, 48: 361-372.

Iclarm, GTZ, 1991 — *The Context of Small-scale Integrated Agriculture-Aquaculture Systems in Africa: a Case Study of Malawi.* Iclarm studies and Reviews 18. Iclarm, Manila, Philippines, and GTZ, Germany, 302p.

KANDOOLE (B. F.), AMBALI (A. J. D.), 1992 — *Socio-economic study of fish farming in the CNRFF Project.* Consultancy report submitted to EC Delegation, Malawi, 90p.

LESSIOS (H.A.), 1992 — Testing electrophoretic data for agreement with Hardy-Weinberg expectations. *Mar. Biol.*, 112: 517-523.

MATHOTHO (A.J.), 1975 — *A simple guide to rearing fish in small dams in Malawi.* Fisheries Bulletin, Malawi Department of Fisheries, 8 p.

MEFFE (G.K.), 1986 — Conservation genetics

and the management of endangered fishes. *Fisheries,* 11(1): 14-23.

OFORI (J.K.), 1988 —
The effect of predation by Lates niloticus on overpopulation and stunting in mixed sex culture of tilapia species in ponds, p. 69-73. In PULLIN (R.S.V.), BHUKASWAN (T.), THONGTHAI (K.), MACLEAN (J.L.), (eds): *The Second International Symposium on Tilapia in Aquaculture.* Iclarm Conference Proceedings 15, 623 p.

ROHLF (F.J.), 1992 —
NTSYS-pc. Numerical taxonomy and multivariate analysis system. Exeter Software, Setauket, NY.

SWOFFORD (D.L.), SELANDER (R.B.), 1989 —
BIOSYS-1: a computer programme for the analysis of allelic variation in population genetics and biochemical systematics; release 1.2. Illinois Natural History Survey, 43 p.

RAYMOND (M.), ROUSSET (F.), in press —
GENEPOP version 1.2: a population genetics software for exact test and ecumenism. *J. Hered.*

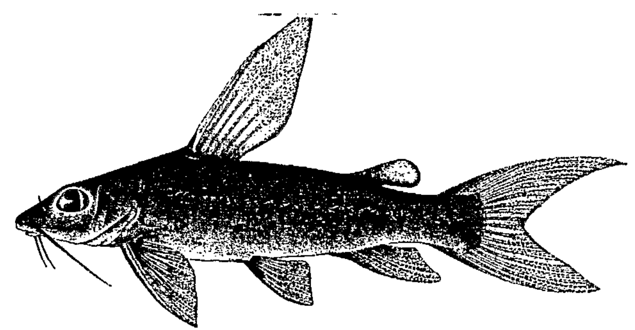
VINCKE (M.M.), 1990 —
Field verification of water bodies inventoried for aquaculture development inCentral and Northern Regions of Malawi. FAO. Rome, Italy, 46p.

WEIR (B. S.), COCKERHAM (C. C.), 1984 —
Estimating F-statistics for the analysis of population structure. *Evol.* 38: 1358-1370.

WRIGHT (S.), 1978 —
Chicago: University of Chicago Press. *Evolution and Genetics of populations. Vol 2. Theory of Gene Frequencies.* Chicago : University of Chicago press.

Catfishes

part 3



Biodiversity and aquaculture of African catfishes (Teleostei, Siluroidei): an overview

Guy G. Teugels
Ichthyologist

Germain Gourène
Ichthyologist

Introduction

Catfishes are a large group of predominantly freshwater fishes. TEUGELS (1996) listed 33 families, with 416 genera and 2584 species. The systematics of catfishes however, are far from being completely known. Especially in South America and in South-East Asia, several new species and even new genera are regularly being discovered. Therefore total taxa numbers continuously change. The most recent estimates include 32 families, 418 genera and 2612 species.

Catfishes belong to the Siluriformes, one of the four orders of the Ostariophysi (FINK and FINK, 1981) including also Gonorynchiformes (milkfishes, etc.), Cypriniformes (carps, suckers, etc.) and Characiformes (piranhas, etc). Ostariophysi are recognized in particular by a notable modification of the anterior four or five vertebrae (three in Gonorynchiformes) into the so-called Weberian apparatus, a connection of alternating ligaments and small bones between the gas bladder and the otic capsule

(CHARDON, 1968). It has an important role in sound perception and its development in Ostariophysi is undoubtedly related to their almost exclusive occurrence in freshwater, where often limited visibility reduces visual perception.

Within the Siluriformes, catfishes belong to the suborder Siluroidei. FINK and FINK (1981) and ARRATIA (1992) listed several synapomorphies to demonstrate the monophyly of this group most of them based on osteological features. An important variation exists in the external morphology of catfishes and it is therefore difficult to give a standard definition. General characters include the absence of scales (although in some South American families the body is covered with bony scutes), and the presence of up to four pairs of circumoral barbels, used for the detection of food. Dorsal and pectoral fins are often provided with a leading spine. An adipose fin is often present.

Catfishes occur in North, Central and South America, Africa, Eurasia, South-East Asia, Japan and Australia. TEUGELS (1996) reported on the proportional distribution of freshwater catfishes in the world: some 64% of all species known are confined to Central and South America about 19% occur in Africa and about 15% are found in Eurasia and South- East Asia. Some 2% of the species are present in North America.

African catfish families

Nearly one fifth of all known catfish species occur in Africa. Ten families are recognized. Two of them, Ariidae and Plotosidae, generally contain marine species and have a large distribution. Three others, Clariidae, Bagridae and Schilbeidae, are also present in southern Asia. All the others are endemic to Africa. At present 58 genera including 465 species are known.

Family Clariidae

This family occurs in Minor, South and South-East Asia and in Africa. Its highest diversity however is found in the latter continent. TEUGELS (1986a) reported 12 genera with 74 species in Africa, while only 2 genera with some 17 species are presently known from Asia (TEUGELS, 1996).

Clariidae are elongated catfishes with long dorsal and anal fins. A dorsal spine is missing but the pectorals are provided with a strong leading spine. An adipose fin is present in a few genera (*e.g. Heterobranchus*). They have four pairs of barbels. A remarkable character for this family is the presence of a suprabranchial organ, formed by folds of the second and the fourth branchial arches. With this organ, the fishes are able to practice aerial respiration, implicating that they can survive out of the water for a long time. They are also known for walking on land over distances of several hundred meters, breathing atmospheric air and using their pectoral spines as a support.

Within the Clariidae, the genus *Clarias* is the most speciose group, containing 32 species (TEUGELS, 1986b) in Africa and some 12 species in Asia (TEUGELS, 1996). This genus contains the most cultured African catfish (Figure 1), *Clarias gariepinus* (Burchell, 1822). Its culture started almost fifty years ago. Its large size (up to 1,5 m), its omnivorous feeding habits, its almost Panafrican distribution and its capacity to survive in poorly oxygenated water, favoured its selection for aquaculture purposes. For 1994, GARIBALDI (1996) reported a production of 3,978 metric tons in Africa and 1,057 mt in Europe. The species has also been introduced in South-East Asia, where it is a severe threat for the local fish fauna.

Clarias anguillaris (Linnaeus, 1758) is of minor importance in African fishculture. GARIBALDI (1996) reported a production of 1,185 mt in Africa for 1994. The species only occurs in West Africa and the Nile. Although other *Clarias* species have been studied for aquaculture purposes, their importance is at present limited.

Recently however the genus *Heterobranchus*, recognized by the presence of a large adipose fin and including four species (TEUGELS

et al., 1990), was introduced in aquaculture and shows most promising results. LEGENDRE *et al.* (1992) demonstrated that under identical conditions, *Heterobranchus longifilis* Valenciennes, 1840 has a growth rate which doubles that of *Clarias gariepinus*. At present, *H. longifilis* is cultured in Côte d'Ivoire (AGNESE *et al.*, 1995), Nigeria (NWADUKWE, 1993), Cameroon (NGUENGA *et al.*, 1996) and Zambia (HECHT *et al*, 1991). *Heterobranchus isopterus* Bleeker, 1863 is another species presently cultured in Côte d'Ivoire (DA COSTA *et al.*, 1996). Experiments with *H. bidorsalis* (Geoffroy Saint-Hilaire, 1809) have recently been conducted in Côte d'Ivoire (Gilles, pers. comm.) and in Nigeria (FAGBENRO *et al.*, 1993). Data on production of *Heterobranchus* species are not yet available.

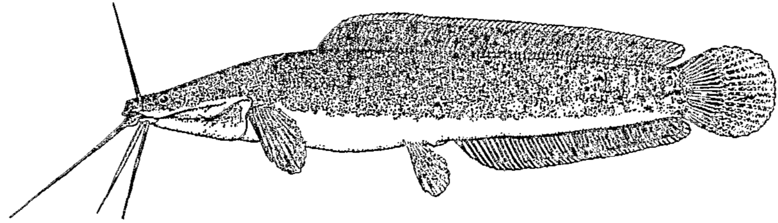


Figure 1
Clarias gariepinus; representative of the Clariidae.

Bathyclarias (sometimes referrred to as *Dinotopterus*) is another clariid genus which is actually being studied for aquaculture purposes in Malawi (Kaunda and Hecht, pers. comm.). Preliminary research by MSISKA *et al.* (1991) showed promising results. The genus merely differs from the large *Clarias* and *Heterobranchus* by the presence of a small adipose fin and by the lower jaw reaching beyond the upper jaw. The genus has a limited distribution: a species-flock (12 species) has been described from Lake Malawi, one species is known from Lake Tanganyika and another, originally described in *Heterobranchus*, is known from Lake Mweru and its

neighbouring rivers (TEUGELS, in prep.), in between Lakes Malawi and Tanganyika. Their taxonomy, growth, reproduction and feed requirements are presently being studied.

Finally some anguilliform clariid genera are presently being tested on their aquaculture potential. Although belonging to the same family, they substantially differ from other clariid genera. The body is extremely elongated and the head is strongly reduced. Several genera have been described but their taxonomy is problematic. In Belgium and the Netherlands experimental research is presently being done on *Gymnallabes typus* Günther, 1867 as a possible replacement for common eel-culture.

Family Bagridae

A detailed osteological and phylogenetical study led MO (1991) to split the Bagridae into three families: Bagridae, Claroteidae and Austroglanididae.

As a result of MO's revision the Bagridae are represented in Africa by only one genus, *Bagrus* containing 10 species. The genus is recognized by a moderately elongated body, compressed posteriorly, four pairs of barbels, strong dorsal and pectoral spines and a large adipose fin.

The genus has a large distribution including the Senegal, Niger, Volta, Chad and Nile basins, the East African Rift Lakes and the Zaire basin. Some species like *Bagrus docmak* (Forsskall, 1775) can attain a total length of over one meter. Although they are important food fishes, in those areas where they form an important part of the fish catches, they are presently not used in aquaculture.

Family Claroteidae

Following MO (1991) this family contains 13 genera, all endemic to Africa. TEUGELS (1996) mentioned 88 species. Two subfamilies are recognized, Claroteinae and Auchenoglanidinae. The former includes seven genera, the latter has five. The most important

difference between both sub-families is the presence of an accessory toothplate on the palate in the Claroteinae.

The external morphology varies considerably within the family. Generally, the body is moderately elongated. There are usually four pairs of barbels (three in *Auchenoglanis*). The dorsal and the pectoral fins have strong leading spines and an adipose fin is present.

Claroteidae have a large sub-saharian distribution. Within the Claroteinae *Clarotes* and in particular *Chrysichthys* are economically important. The latter has been introduced in aquaculture in the early 1980's (HEM, 1986; DIA and OTEME, 1986). *Chrysichthys nigrodigitatus* (Lacepéde, 1803) (Figure 2) is presently cultured in Côte d'Ivoire (OUATTARA *et al.*, 1993) and in Nigeria (EKANEM, 1993). For 1994, GARIBALDI (1996) mentioned a production of 1,503 mt in Africa.

Within the Auchenoglanidinae, especially *Auchenoglanis* is economically important in local catches. BARDACH *et al.* (1972), MICHA (1973) and PLANQUETTE (1976) published on the aquaculture potential of *Auchenoglanis occidentalis* (Valenciennes, 1840). The species, however, is at present not used in aquaculture.

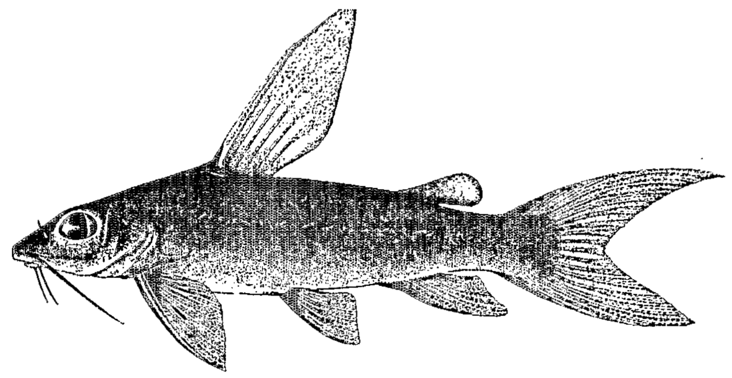


Figure 2
Chrysichthys nigrodigitatus, representative of the Claroteidae.

Family Austroglanididae

Originally included in the Bagridae, this family, containing one genus *Austroglanis* with three species is only known from the Orange and Olifantsriver in southern Africa.

Morphologically, the family is recognized by the presence of three pairs of barbels, strong dorsal and pectoral spines and a small, posteriorly placed adipose fin. The maximal size reported is 250 mm. They have no importance in aquaculture.

Family Malapteruridae

The electric catfish family Malapteruridae contains one genus *Malapterurus*. Three species are currently recognized as valid but the systematics of this group are poorly known and are presently being revised (Norris, in prep.).

Morphologically they are recognized by a somewhat cylindrical body, three pairs of barbels, the absence of a dorsal fin and the absence of spines in the pectoral fin. An adipose fin is present. They possess an electric organ of muscular origin which produces violent electric discharges up to 450 Volts.

Electric catfishes have a wide distribution in tropical Africa. Their maximal total length is 1,5 m. They have no aquaculture potential.

Family Mochokidae

Eleven genera, including 177 species are presently known for this family, which is endemic to Africa.

Their external morphology consists in a robust body, slightly compressed posteriorly. They have three pairs of barbels, of which the mandibular pair is branched in some genera (*e.g. Synodontis*). The dorsal and pectoral fins have strong spines. An adipose fin is present. Although the maximal length reported is 800 mm in a species of *Synodontis*, the mochokids are in general much smaller.

They have not been introduced in aquaculture but some species are popular aquarium fishes.

Family Amphiliidae

Amphiliids are small freshwater catfishes (maximal total length 195 mm but usually much smaller) endemic to tropical Africa. Nine genera including 60 species are known.

They have three pairs of barbels; in most genera dorsal and anal spines are lacking; an adipose fin is present. Many genera are rheophilic and display greatly enlarged pectoral and pelvic fins.

Amphiliidae are of no interest to aquaculture.

Family Schilbeidae

Schilbeidae are pelagic catfishes found in freshwaters of Africa and southern Asia. In Africa DE VOS (1995) recognized five genera with 34 species.

Morphologically schilbeid catfishes are recognized by a laterally compressed body and two to four pairs of barbels. A short dorsal fin may be present or absent. When present, it has a strong spine. An adipose fin is present or absent. The anal fin is very long and the pectoral fin has a strong spine.

Maximal total length reported is 590 mm in *Schilbe*. Some schilbeids are excellent and important food fish. No records on their introduction in aquaculture could be found.

Family Ariidae

Most ariids are marine catfishes with a worldwide distribution in tropical and subtropical regions. Four genera, *Anchorius* with two species, *Arius* with eight species, *Galeichthys* with two species and *Netuma* with one species, have been reported from Africa mostly

from coastal bays and estuaries. Some, however, are confined to freshwaters.

Morphologically they are recognized by a robust body, compressed posteriorly, three pairs of barbels, dorsal and pectoral fins with strong spines and the presence of an adipose fin.

Arius gigas Boulenger, 1911 is exclusively found in freshwater in the Niger and Volta basins. Its maximal length reported is 1.5 m. It has become however, extremely rare. They have not been used in aquaculture in Africa.

Family Plotosidae

Like the Ariidae, the Plotosidae are mostly marine catfishes found in the western Pacific and the Indian Ocean from the east coast of Africa to Australia. One genus, *Plotosus*, and three species have been reported from the east coast of Africa. They occasionally enter freshwater.

Plotosidae are recognized by an elongated body with a pointed tail. Four pairs of barbels are present. Two dorsal fins are present, the first with a strong leading spine, the latter very long and confluent with the caudal. Caudal and anal fins are also confluent. The pectorals have a strong spine. The maximal size reported is about 600 mm. They are not used in aquaculture.

References

AGNESE (J.-F.), OTEME (Z.J.), GILLES (S.), 1994 — Effects of domestication on genetic variability, fertility, survival and growth rate in a tropical siluriform; *Heterobranchus longifilis* Valenciennes 1840. *Aquaculture*, 131: 197-204.

ARRATIA (G.),1992 — Development and variation of the suspensorium of primitive catfishes (Teleoste: Ostariophysi) and their phylogenetic relationships *Bonn. Zool. Monogr.*, 32: 1-149.

BARDACH (J.E.), RYTHER (J.H.), MACLARNEY (W.O.), 1972 — *Aquaculture. The farming and husbandry of freshwater and marine organisms.* Wiley Interscience, New York: 868 p.

BLACHE (J.), MITON (F.), STAUCH (A.), ILTIS (A.), LOUBENS (G.), 1964 —
Les poissons du bassin du Tchad et du bassin adjacent du Mayo-Kebbi. Etude systématique et biologique. Mém. Orstom, 4: 1-485.

BOULENGER (G.A.), 1907 —
Zoology of Egypt. The Fishes of the Nile published for the Egyptian Government. 2 Vol., London, 578 p.

BOULENGER (G.A.), 1911 —
Catalogue of the freshwater fishes of Africa in the British Museum (Natural History). The Trustees, Vol. 2, 529 p.

CHARDON (M.), 1968 —
Anatomie comparée de l'appareil de Weber et des structures connexes chez les Siluriformes. *Ann. Mus. R. Afr. Centr.*, 169: 1-227.

DA COSTA (S.), GOURENE (G.), TEUGELS (G.G.), 1996 —
Embryologie, aspect extérieur du développement larvaire et maturité sexuelle du poisson-chat africain *Heterobranchus isopterus* (Siluroidei, Clariidae). *Belg. Journ. Zool.*, 126: 93-109.

DAGET (J.), 1957 —
Les eaux et les poissons de Haute Volta. Mém. Ifan, 50: 95-169.

DE VOS (L.), 1995 —
A systematic revision of the African Schilbeidae (Teleostei, Siluriformes). *Ann.Mus.R.Afr.Centr.*, 271: 1-450.

DIA (A.K.), OTEME (Z.J.), 1986 —
Etude de la qualité de l'aliment et de la densité sur le taux de survie des alevins de *Chrysichthys nigrodigitatus*. pp. 169-174. *In* Huisman (E.A.), ed.: *Aquaculture research in the Africa region.* Pudoc, Wageningen, 274 p.

EKANEM (S.B.),1993 —
Water temperature of typical ponds and its effect on *Chrysichthys nigrodigitatus* (Lacepede) culture. *J. Aquac. trop.*, 9: 141-149.

FAGBENRO (O.A.), ADEDIRE (C.O.), OWOSEENI (E.A.), AYOTUNDE (E.O.), 1993 —
Studies on the biology and aquaculture potential of feral catfish *Heterobranchus bidorsalis* (Geoffroy St. Hilaire 1809) (Clariidae). *Trop. Zool.*, 6: 67-79.

FINK (S.), FINK (W.L.), 1981 —
Interrelationships of the ostariophysan fishes (Teleostei). *Zool. J. Linn. Soc.*, 72: 297-358.

GARIBALDI (L.), 1996 —
List of animal species used in aquaculture. FAO Fish.Circ., 917: 1-37.

HECHT (T.), LUBLINKHOF (W.), KENMUIR (D.), 1991—
Induced spawning of the Vundu *Heterobranchus longifilis*, and embryo survival rates of pure and reciprocal clariid crosses. *S. Afr. J. Wildl. Res.*, 21: 123-125.

HEM (S.),1986 —
Premiers résultats sur la reproduction contrôlée de *Chrysichthys nigrodigitatus* en milieu d'élevage. pp.189-205. *In* Huisman (E.A.), ed.: *Aquaculture research in the Africa region.* Pudoc, Wageningen, 274 p.

LEGENDRE (M.), TEUGELS (G.G.), CAUTY (C.), JALABERT (B.), 1992 —
A comparative study on morphology, growth rate and reproduction of *Clarias gariepinus* (Burchell, 1822), *Heterobranchus longifilis* Valenciennes, 1840 and their reciprocal hybrids (Pisces, Clariidae). *J. Fish Biol.*, 40: 59-79.

MICHA (J.C.),1973 —
Etude des populations piscicoles de l'Ubangui et tentatives de sélection et d'adaptation de quelques espèces à l'étang de pisciculture. Not. Doc. Pêches Piscic., CTFT: 110p.

MO (T.), 1991 —
Anatomy, relationships and systematics of the Bagridae (Teleostei: Siluroidei)

with a hypothesis
of siluroid phylogeny. *Theses Zoologicae*, 17: 1-216.

MSISKA (O.V.), RASHIDI (B.B.A.), KAMVABINGU (Z.E.), 1991 —
Growth performance of a planktivorous clariid, *Bathyclarias loweae* in an experimental pond.
Aquabyte, 4: 5.

NGUENGA (D.), BREINE (J.J.), TEUGELS (G.G.), OLLEVIER (F.), 1996 —
Artificial propagation of the African catfish *Heterobranchus longifilis* (Siluroidei; Clariidae): description of a simple technique to avoid sacrifying male broodfish for the obtention of milt.
Aquaculture, 143: 215-217.

NWADUKWE (F.O.), 1993 —
Inducing oocyte maturation, ovulation and spawning in the African catfish, *Heterobranchus longifilis* Valenciennes (Pisces: Clariidae), using frog pituitary extract.
Aquac. Fish. Manag. 24: 625-630.

OUATTARA (M.), KOUAKOU (K.), GOURENE (G.), 1993 —
Une approche technologique peu onéreuse de production de larves et d'alevins de *Chrysichthys nigrodigitatus* (Pisces; Bagridae).
Agron. Afr., 5: 33-38.

PLANQUETTE (P.), 1976 —
Contribution à l'étude de poissons utilisables en aquaculture en eaux chaudes. Observations sur la croissance et la reproduction d'*Auchenoglanis occidentalis*.
Not. Doc. Pêches Piscic., CTFT, 12: 1-11.

TEUGELS (G.G.), 1986a —
Clariidae. pp 66-101. In Daget (J.), Gosse (J.P.), Thys van den Audenaerde (D.F.E.), eds.: *Check-List of the Freshwater Fishes of Africa.*Cloffa 2 ISNB, Bruxelles-Mrac, Tervuren-Orstom, Paris, 520 p.

TEUGELS (G.G.), 1986b —
A systematic revision of the African species of the genus *Clarias* (Pisces; Clariidae). *Ann. Mus. R. Afr. Centr.*, 247 : 1-199.

TEUGELS (G.G.), 1996 —
Taxonomy, phylogeny and biogeography of catfishes (Ostariophysi; Siluroidei) : an overview.
Aquat. Liv. Res.

TEUGELS (G.G.), DENAYER (B.), LEGENDRE (M.), 1990 —
A systematic revision of the African catfish genus *Heterobranchus* (Pisces, Clariidae). *Zool. J. Linn. Soc.*, 98 : 237-257.

Intra- and interspecific morphometric variation in *Clarias gariepinus* and *C. anguillaris* (Siluroidei, Clariidae)

Guy G. Teugels
Ichthyologist

The genus *Clarias* with some 32 valid species is the third most diversified catfish genus on the African continent. Within the genus, TEUGELS (1986) recognized six subgenera. One of them, *Clarias* (*Clarias*) includes two species: *Clarias gariepinus* (Burchell, 1822) and *C. anguillaris* (Linnaeus, 1758). Especially the former is of great economic importance as it is the most cultured catfish in Africa and the third most cultured catfish species in the World (GARIBALDI, 1996).

Clarias gariepinus has an almost Panafrican distribution and also naturally occurs in Minor Asia (SKELTON and TEUGELS, 1992). *Clarias anguillaris* has a more restricted distribution and is only known from the Nile and West Africa (TEUGELS, 1986).

Morphologically, both species are very similar and although sympatrical populations in some river basins may be distinguished on other characters (*e.g.* BENECH *et al.,* 1993), the only reliable feature to distinguish both is the number of gill rakers on the first branchial arch. In *Clarias gariepinus*, this number is very high (up to 110) while in *C. anguillaris* it is rather low (up to 50). In both species the gill raker number increases with the standard length (TEUGELS, 1982).

As part of a multidisciplinary project on the characterization of species and populations used in aquaculture, we examined the morphometry of eight populations of *Clarias gariepinus* and six populations of *C. anguillaris*. Origin and sample size of these populations are listed in Table I. Thirteen measurements were taken on each specimen using dial calipers. They follow AGNESE *et al.* (1997). For each specimen, the number of gill rakers on the complete first branchial arch was counted. Results obtained were log-transformed and submitted to principal component analysis (Statistica package) using the matrix of covariance and to cluster analyses.

Population	*Clarias gariepinus* n	*Clarias anguillaris* n
Dagana (Senegal)	10	25
Selingue (Mali)	2	25
Bamako (Mali)	-	9
Layo (Côte d'Ivoire)	-	15
Hadide (Chad)	3	1
Ndjamena (Chad)	13	4
Lake Manzalla (Egypt)	37	-
Cairo (Egypt)	9	-
Lake Victoria (Kenya)	23	-
Sand River Dam (Swaziland)	9	-

Table I
Origin and sample size of populations examined of *Clarias gariepinus* and *C. anguillaris*.

Results of a principal component analysis using 13 log-transformed metric variables did not reveal significant differences between the populations examined of *Clarias anguillaris* (Fig. 1). They all (except for the population from Côte d'Ivoire) originate from river systems in West Africa (Senegal, Niger, Chad) and climatological

and geological events during the Late Quaternary explain for the bigger part the similarities in their faunal composition.

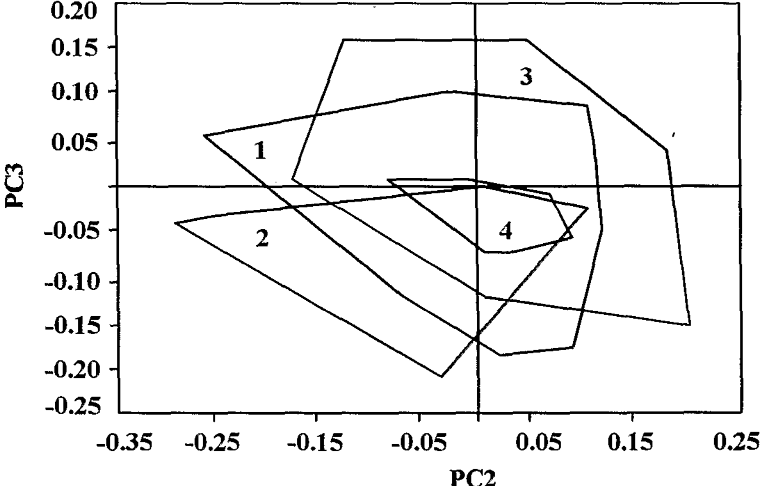


Figure 1
Plot of a principal component analysis on 13 log-transformed metric variables for all populations examined of *Clarias anguillaris*. Senegal (1); Côte d'Ivoire (2); Mali (Selingue + Bamako) (3); Chad (Hadide + Ndjamena) (4).

The same statement can be made for the West African populations (Senegal and Chad basins) and the Nile populations (Lake Manzalla and Cairo) of *Clarias gariepinus* which showed to be very similar and a principal component analysis of 13 log-transformed metric variables did not enable to separate them (Fig. 2). Historical contacts between the Nile and the West African river basins have been documented by ROBERTS (1975) and others.

An important morphometric separation between the Nile and the Lake Victoria specimens of *Clarias gariepinus* was observed (Fig. 2): the Nile specimens are located on the negative sector of the second component, while the Lake Victoria specimens are situated

on the positive sector of this component. The second component is merely defined by the width of the premaxillary toothplate, the width of the occipital process, the length of the occipital process and the dorsal fin length. GREENWOOD (1976) stated that although Lake Victoria is connected by river with the Nile, the Lake fauna is physically isolated by barriers that seemingly are impassable to fishes. The Lake Victoria and the Egypt populations of *Clarias gariepinus* however are both partly overlapped by the population from Chad, while that from the Senegal is intermediate between both. Possible hydrographic connections between the Chad and the Nile basins during the Pleistocene have been suggested by ROBERTS (1975) and are supported by the presence in Lake Victoria of taxa such as the cyprinid *Barbus apleurogramma* (see LEVEQUE, 1990).

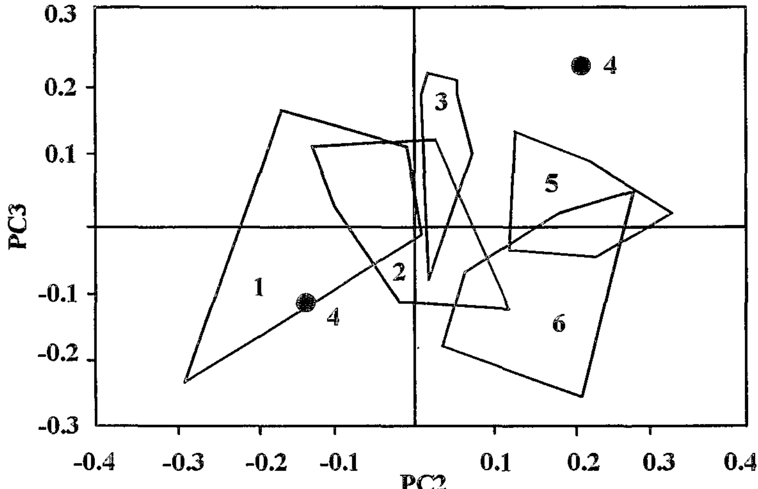


Figure 2
Plot of a principal component analysis on 13 log-transformed metric variables for all populations examined of *Clarias gariepinus*. Egypt (Cairo + Lake Manzalla) (1); Chad (Hadide + Ndjamena) (2); Senegal (3); Mali (Selingue) (4); Swaziland (5); Lake Victoria (6).

A comparison of the morphometric data of all populations examined of both species, showed an important overlap in particular between the *Clarias gariepinus* specimens from Lake Victoria and Swaziland and the *C. anguillaris* specimens (Fig. 3). When however the number of gill rakers is included in the analysis, the Lake Victoria and Swaziland populations clearly fall within the other *C. gariepinus* populations (Fig. 4), where they cannot be distinguished as a subgroup.

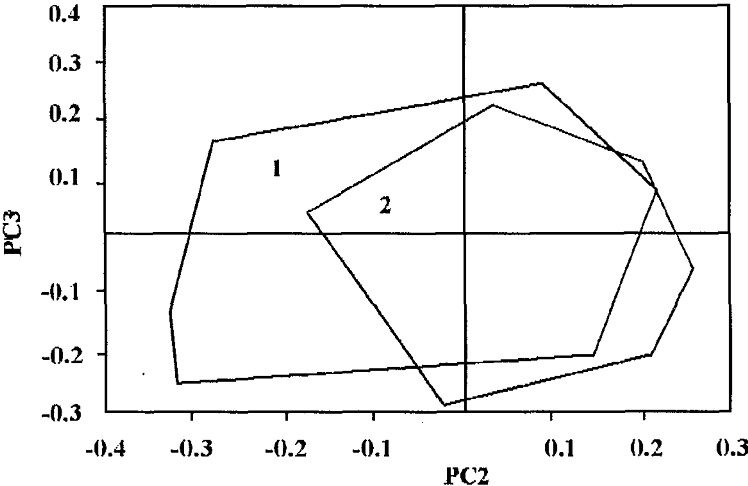


Figure 3
Plot of a principal component analysis on 13 log-transformed metric variables for all natural populations examined of *Clarias gariepinus* (1) and *C. anguillaris* (2).

In conclusion, populations from *Clarias anguillaris* showed limited morphometric variation. In populations from *Clarias gariepinus* however, those from West Africa and the Nile are morphometrically closer to each other than to those from Lake Victoria and southern Africa. Different colonization patterns, related to earlier

hydrographic connections are used to explain this intraspecific variation.

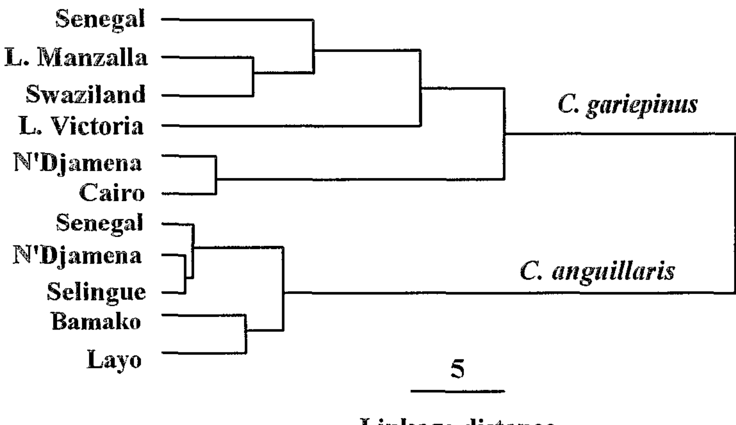


Figure 4
Cluster analysis (linkage rule: unweighed pair-group average; euclidean distance measure) using 13 log-transformed metric variables and the gill raker number for all populations examined of *Clarias anguillaris* and *C. gariepinus.*

Acknowledgements
This work as part of the GENETICS program, was made possible thanks to funding from the European Union (contract ERBTS3*CT920079)

References

AGNESE (J.F), TEUGELS (G.G.), GALBUSERA (P.), GUYOMARD (R.), VOLCKAERT (F.), 1997 — Morphometric and genetic characterization of sympatric populations of *Clarias gariepinus* and *C. anguillaris* (Siluroidei; Clariidae) from Senegal. *J. Fish Biol.*, 50, 1143-1157

BENECH (V.), TEUGELS (G.G.), GOURENE (G.), 1993 — Critère pratique pour distinguer deux poissons-chats africains, *Clarias*

anguillaris et *C. gariepinus* (Siluriformes; Clariidae). *Cybium.*, 17 (1): 83-85.

GARIBALDI (L.), 1996 —
List of animal species used in aquaculture. *FAO Fish.Circ.*, 917: 1-37.

GREENWOOD (P.H.), 1976 —
Fish Fauna of the Nile. *In* RZOSKA (J.), ed.: *The Nile, biology of an ancient river.* Junk Publishers, The Hague.

LEVEQUE (C.), 1990 —
Relict tropical fish fauna in Central Sahara. *Ichth. Explor. Freshw.*, 1: 39-48.

ROBERTS (T.R.), 1975 —
Geographical distribution of African freshwater Fishes. *Zool. J. Linn. Soc.*, 57: 249-319.

SKELTON (P.H.), TEUGELS (G.G.), 1992 —
Neotype designation for the African catfish *Clarias gariepinus* (Burchell, 1822) (Pisces: Siluroidei: Clariidae). *Ichth. Bull.*, J.L.B. Smith Inst. Ichth., 56 : 1-8.

TEUGELS (G.G.), 1982 —
Preliminary results of a morphological study of five nominal species of the subgenus *Clarias* (Pisces; Clariidae). *J. Nat. Hist.*, 16, 3: 439-464.

TEUGELS (G.G.), 1986 —
A systematic revision of the African species of the genus *Clarias* (Pisces; Clariidae). *Ann. Mus. Roy. Afr. Centr.*, 247 : 199 pp.

Comparison of growth performances of the Niger and Bouaké strains of *Clarias anguillaris*

Sebastino Da Costa
Hydrobiologist

Introduction

Clarias anguillaris Linnaeus, 1758 is a teleost fish of the Clariidae family. It belongs to the subgenus *Clarias* (*Clarias*) which has another species of considerable interest to aquaculture in Africa: *Clarias gariepinus* Burchell, 1822 (*Syn.*: *Clarias lazera* Valenciennes, 1840). The only difference between these two species is the number of branchiospines on the first branchial arch which is greatly reduced in *C. anguillaris* : 16-50 *versus* 24-110 in *C. gariepinus* (TEUGELS, 1992).

Clarias anguillaris is also of interest to aquaculture (VOLCKAERT *et al.*, 1994). This species is used in aquacultural production systems in Africa (SYLLA, 1994). In fact, it is often used in aquaculture in place of *Clarias gariepinus* because of the close resemblance with the latter. Because of its wide distribution, it is necessary to study growth performances of this species. The current study uses two strains of *C. anguillaris* : those of Bouaké and the Niger River.

Materials and Methods

Fish from Niger River were brought to the Idessa (l'Institut des Savanes) aquacultural station in May 1993 by the CRO (Centre de Recherches Océanologiques) of Abidjan. This wild population, made up essentially of juveniles, was raised to sexual maturity. Then, a systematic identification was made and a stock of *C. anguillaris* brooders was created. *Clarias gariepinus* was excluded because of the insufficient numbers of brooders. The Bouaké strain of *C. anguillaris* was choosen from the adult stocks available at Idessa and coming from the Loka farm of the rural aquacultural development project.

Artificial reproduction was carried out at the CRO in August 1995. The sex ratio used was 3:3 for each strain. Oocyte maturation was induced using HCG at a dose of 1500 IU/kg of live weight. Two females ovulated for the Bouaké strain and only one for the Niger strain. The egges from each female were kept in separate plastic containers. The *C. anguillaris* Niger sperm was collected and cryopreserved for a period of six months beforehand. That *of C. anguillaris* from Bouaké was collected after sacrificing the males.

The milt was mixed by strain and diluted in physiological serum. An equal weight of ovules (7.5 g) from each of the ovulating females was fertilized with 11.25 ml of diluted sperm. After rinsing with water, the eggs were incubated in sieves previously placed in 220 l tanks at 30°C.

From hatching, the fry were fed intensively with decapsulated *Artemia salina* eggs for two weeks. Fingerlings of mean weights ranging from 0.633 and 0.733 g for *C. anguillaris* Niger and between 0.413 and 0.633 g for *C. anguillaris* Bouaké were transfered to Idessa for pre-grow out, which lasted two months. According to the VIVEEN *et al.* (1985) feeding chart (table 1), the fingerlings were fed with crumbled feed M2GE (table 2) made by Fabrique d'Aliments de Côte d'Ivoire (FACI),

Weeks	1	2	3	4	5	6	7-12	13-20	21-24
Mean weights (g)	1	3	6	10	15	19	24-55	62-140	160-200
feed ratio (%)	25	10	7	4,5	4	3,5	3	2,5	2

Table 1
Feeding chart for the intensive culture of *C. gariepinus* in ponds (Pelleted feed with 30% protein) (VIVEEN *et al.*, 1985)

The growth trials of the Niger and Bouaké strains of *C. anguillaris* was carried out in a series of 15 m3 concrete tanks with a usable water volume of 11.2 m3. The water flow through these structures was 0.14 l/s. The assignment of tanks was random, with 3 tanks per strain. The tanks were stocked with fingerlings at a density of 30 individuals per tank, 2.7 fish per m3. The mean weight at stocking varied between 51.08 and 54.37 g for *C. anguillaris* Niger and between 55.17 and 55.46 g for *C. anguillaris* Bouaké .

Ingredient	%
Crude protein	45
Crude fat	5
Crude fiber	4.5
Calcium	1.5
Phosphorous	1
Na	0,7
Vit.A	9.000 UI/kg
Vit.D3	1.200 UI/kg
Vit.E	50 mg/kg
Vit.B1, 2, 3, 6, 12	100 mg/kg
BHT	
Lysine, methionine	

Table 2
Composition of the Faci feed M2GE.

The fish were fed with the Faci feed M2GE (table 2) in pelleted form at 45% crude protein. The same feed ration was distributed to all fishes. This corresponded to 3% of the highest mean weight observed in the different populations, all strains included. Growth measurements and the adjustment of the feed ration were carried out after each monthly samplings. All fishes were weighed and measured individually. At draining, the same morphometric measurements were taken. Survival rates were noted. The temperature in the experimental tanks ranged on the average between 24 and 28.8°C.

The analysis of the variance of the culture parameters was carried out with an Anova (single classification) for samples of equal or unequal sizes, and the TUKEY-KRAMER test for mean pairs comparison for unequal sample sizes (SOKAL et ROHLF, 1995).

Résults and discussion

The growth performances of the Bouaké and Niger strains of *C. anguillaris* were compared during the grow-out phase. Results are presented in Figure 1.

The juvenile populations of the two strains used for stocking the experimental tanks had similar weights ($p>0.05$ and $p>0.01$). The proportions of males and females in each population studied were 47% and 53% for *C. anguillaris* Bouaké and 43% and 57% for *C.anguillaris* Niger. This gives a balanced sex ratio of approximately 1 :1. This mixed composition (1 : 1), as HENKEN *et al.* (1987), indicate, seems to favor better growth and nutrition for a species of the same genus : *Clarias gariepinus*.

The survival rates recorded at the end of the culture cycle were almost the identical for the two strains, varying between 76.6 and 90% for *C. anguillaris* Bouaké and between 76.6 and 96.6% for *C. anguillaris* Niger. Also, the two strains had fairly close growth performances. However, they were significantly different

(P<0.05and p<0.01). *C. anguillaris* Niger moved ahead of the Bouaké strain from the second month, lasting through the end of the culture period (Fig. 1).

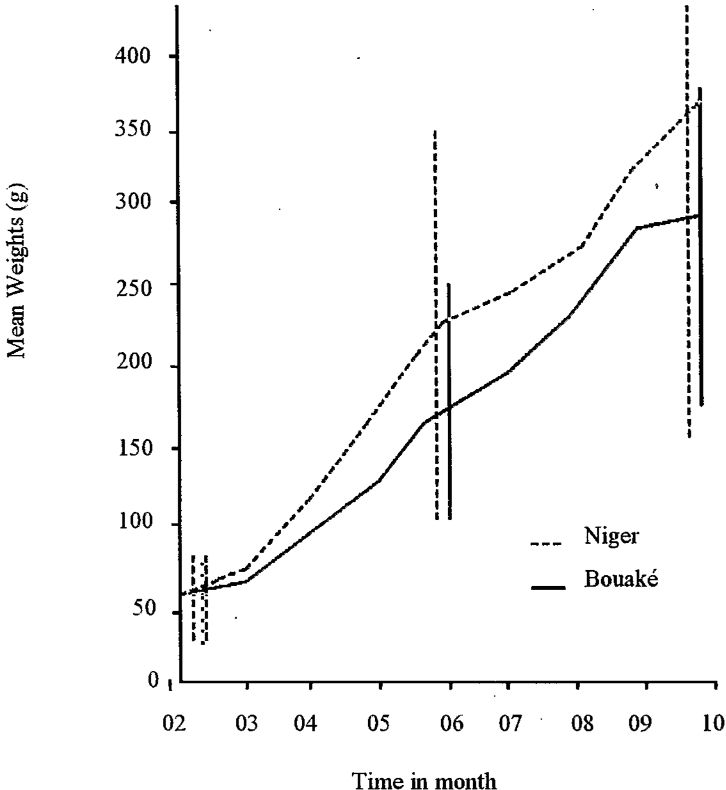


Figure 1
Evolution of the mean weights of individuals of the Bouaké and Niger strains of *C. anguillaris*. Vertical barscorrespond to confidence intervals.

The regression equations relative to the growth of the two strains are as follows :

- PB=36.92 + 1.11x, for *C. anguillaris* Bouaké
- PN=42.26+1.36x, for *C. anguillaris* Niger

where P = weight in grams and x = time in days. The difference in growth observed between the two strains was elatively small and could mainly be explained by the heterogenous mixture of sizes in the populations of each strain tested and not by the sex ratios. Despite the important ratio of males, the Bouaké strain had a lower growth rate. The overall mean daily growth rate was 1.0+0.5 g/d for the Bouaké strain and 1.23+0.5 g/d for the Niger strain. The respective mean weights in the second month of culture were 94.03+45.2 g for the first strain and 113.39+62.4 g for the second. In the eighth month, the values obtained were 301.58+87.31 g and 371.07+182.12 g respectively. Also, the Niger strain offered a better feed conversion for the feed M2GE, 4.4+0.2 *versus* 6.0+0.4 for the Bouaké strain. The observed difference was statistically significantly ($p<0.05$ and $p<0.01$).

Conclusion

The results of the experiment show that *C. anguillaris* Bouaké and *C. anguillaris* Niger are two close strains, at least concerning the zoo-technical parameters studied. They present similar growth and feed conversion rates. The Niger strain has a better growth rate, but the statistical analysis suggests that the differences in growth recorded may not appear if homogeneous populations (size and weight) were used. This suggests that the Niger and Bouaké strains could be used interchangeably for a culture cycle of eight months. In the interest of preserving the biodiversity of aquacultural species, the culture of these two strains should be carried out in the areas of their respective natural distributions.

Acknowledgements

This work as part of the GENETICS program, was made possible thanks to funding from the European Union (contract ERBTS3*CT920079)

References

HENKEN (A.M.), BOON (J.B.), CATTEL (B.C.), LOBEE (H.W.J.), 1987 — Differences in growth rate and feed utilization between male and female African catfish, *Clarias gariepinus* (Burchell, 1822). *Aquaculture*, 63 : 221-232.

COULIBALY-SYLLA (F.), 1994 — *Performances zootechniques de* Clarias anguillaris *(Clariidae) dans les mares artificielles du Delta Central du Niger.* BASIL 94. Abstracts. Session Systèmes de production. Communication effectuée/Poster. p : 157.

TEUGELS (G.G.), 1992 — *Clariidae. In* (eds): LEVEQUE (C.), PAUGY (D.), TEUGELS (G.G.), Faune des poissons d'eaux douces et saumâtres de l'Afrique de l'Ouest. Tome 2. Editions Orstom/Mrac. Coll. *Faune Tropicale* n° 28 : 468-482.

VIVEEN (W.J.A.R.), RICHTER (C.J.J.), VAN OORDT (P.G.W.J.), JANSSEN (J.A.L.), HUISMAN (E.A.), 1985 — *Manuel de pisciculture du poisson-chat africain (*Clarias gariepinus*)*, 108 p.

VOLCKAERT (F.), GALBUSERA (P.), GUYOMARD (R.), 1994 — *Différenciation génétique des populations de* Clarias gariepinus *(Burchell, 1822) et* Clarias anguillaris *(Linnaeus, 1758). In* (ed) : AGNESE (J.F.), Comptes rendus de l'Atelier Biodiversité et Aquaculture, Abidjan 21/25 novembre 1994. CRO/CEE/Orstom. pp : 97 - 100.

SOKAL (R.R.), ROHLF (S.J.), 1995 — *Biometry*. Editions Freeman and Co. New York, 3ème édition. 887 p.

Sensitivity to inbreeding and sperm cryopreservation in the catfish *Heterobranchus longifilis* Valenciennes, 1840

Ziriga Josué Otémé
Biologist

Introduction

The African Clariidae, *Heterobranchus longifilis* Valenciennes 1840, is characterized by a high fecondity (up to 120.000 ovules per kg of body weight). Its growth rate (8 to 12 g per day) makes it a fish of the future for aquaculture. *Heterobranchus longifilis* is being reproduced at the Centre de Recherches Océanologiques (CRO) since 1984, and its reproductive cycle is now fully understood in captivity. This species has been the object of an electrophoretic study (TEUGELS *et al.*, 1992) carried out on 13 loci using six wild and six domestic individuals from the third generation (F3), which showed that the domestic individuals lost a large part of the genetic variability present in the wild population from which they are descended (three polymorphic loci in the 13 studied).

Numerous studies carried out on other fish species, showed that domestication could lead to an important decrease in genetic variability (TANIGUCHI *et al.*, 1983; VUORINEN, 1984; KRIEG and

GUYOMARD, 1985). This loss of variability can sometimes be observed from the first domestic generation (TANIGUCHI *et al.*, 1983). It can, in certain cases, lead to a modification in rearing performances (FERGUSSON, 1992; DANZMANN *et al.*, 1985; DANZMANN *et al.*, 1987).

Species with high fecundity, for which a single pair can beat the origin of a domestic population, risk firstly a decrease ingenetic variability (due to the small necessary effective size needed to maintain a culture population), and secondly a decrease in the heterozygosity rate (due to inbreeding). Numerous studies have shown the relationship existing between the number of heterozygous loci of an individual and certain of its biological characteristics: growth rate and oxygen consumption (KOEHN and SHUMWAY, 1982), weight loss rate during starving (RODHOUSE and GAFFNEY, 1984; DIEHL *et al.*, 1985). Generally speaking, heterozygotes have a more efficient metabolism than do homozygotes (KOEHN and SHUMWAY, 1982).

In aquaculture the availability of gametes throughout the year is important to ensure a constant supply of fish. From that point of view, *H. longifilis* presents a definite interest for aquaculture since its gametogenesis is continuous once sexual maturity is reached. However, the males have to be sacrificed and the testes dissected out to collect the sperm as the semen cannot be easily obtained by stripping unlike the ovules in females.

Several means, including long term storage of gametes maybe used to improve fish farm stocks. Cryopreservation of sperm can facilitate artificial insemination and allow a better brood stock management.

Two consecutive studies were carried out. The objective of the first one was to determine whether or not the loss of polymorphysm resulting from successive consanguine crosses in this species is accompanied by differences in zootechnical performances (fecundity, larval survival and growth) of farmed individuals. The aim of the second study was to assess the fertilizing capacity of the sperm after cryopreservation, and to show if this technique can be used to restore genetic variability in farmed strains.

Material and methods

Biological material

The domestic brood stock of *H. longifilis* used were collected at the Layo Station (CRO). They were 3 years old and sexually mature since the age of 1 year. These fish are descendants of a wild stock that had spontaneously colonized the station ponds in 1982 (LEGENDRE, 1983) and were raised in lagoon pens. These domestic individuals (F3), are descendants of three consanguine crosses of one female and one male. Wild broodstock were aged from 2 to 3 years and were captured at the approximate age of 6 months (25 cm) in the lagoon or the neighboring swampy areas and raised at the Layo Station in lagoon cage-pens.

Study of genetic variability

Two samples of 50 individuals from F1 and F4 descending respectively from wild broodstock and from broodstock hatched incaptivity were studied. For each individual, an eye, and a piece of liver and muscle (1 cm^3) were taken. Tissues were preserved at -30°C for several weeks then ground just before analysis. Isoenzymatic variations at 23 loci were examined (AGNESE *et al.*, 1995).

Reproduction

Oocyte maturation and ovulation were induced by a single intramuscular injection of human chorionic gonadotropin (hCG) at adose of 1.5 I.U. per g of body weight. The females used were selected firstly on the basis of their stoutness and the softness of their belly, and mainly on the basis of their oocyte diameter. Oocyte diameters from selected females were measured from a sample of 30

to 40 oocytes per female collected by intra ovarian biopsy before hormonal inducement.

After ovulation, each female was stripped of the maximum of her ovules. For each female, all ovules were weighed, then 300 to 400 ovules were weighed and counted to determine fecundity (number of ovules collected). Male broodstock received no hormonal treatment. The sperm collected after sacrificing the males and dissecting the testicles was kept on ice after a one-tenth dilution with 0.9% NaCl. For the strain comparison, six wild females and four wild males on one hand and four F3 females and four F3 males on the other hand were used.

Evaluation of zootechnical performances

Each female was fertilized using pooled sperm obtained from a mixture of the milt from the different males of the same generation. The quality of ovules harvested was evaluated using hatching percentages on lots of 200 to 300 ovules fertilized with 200 µl of diluted sperm. At hatching (24 hours after fertilization), the proportions of normal and deformed fry obtained from each lot were determined by observation and counting on a light table. Modal oocyte diameter, hatching percentage and the proportion of normal and deformed fry were determined for each female.

Early fry growth was followed for a period of 14 days from D1 (one day after hatching). The experiment was carried out in Two PVC tanks subdivided into six compartments of a working capacity of 50 l and attached to a closed circuit. For the different groups of broodstock (domestic and wild) six lots of 300 fry (after yolk absorption) were taken from a pool made proportionally using the percentage of normal fry from each female. In the comparison of performance between the F1 and F4 strains, three replicates of 300 fry from each strain were placed in six compartments of 50 l (six fry per liter).

Fry were fed *ad libitum* at a rhythm of 6 meal per 24-h period. From the second day after hatching and until the eighth, the fry were fed only *Artemia salina* nauplii. From the 9th to the 11th day

the nauplii were progressively replaced by artificial feed (Trouvit), which became the sole feed until the 14th day.

To follow the evolution of fry weight of the two generations, 24 fry were taken from each compartment and weighed after draining on the 5th, 8th, 11th and 14th day. At the end of the experiment, a count of all individuals was made to establish the survival rate in each compartment.

Cryopreservation of sperm

The fish and the sperm

The study was conducted with gametes collected from sexually mature 3 to 5 year-old individuals coming from a F1generation.

Five males of *H. longifilis* were sacrificed and the sperm was collected by dissecting the testes. The sperm from all the males was pooled and a sample of the pooled milt was deep-frozen in liquid nitrogen for 8 months prior to the beginning of the breeding experiment.

The milt from a second group of five males was collected and pooled using the same procedure as described above. A sample of this milt was deep-frozen in liquid nitrogen for 1 hour prior to insemination. Another sample of the same milt was stored in a glass tube kept sealed on crushed ice until use as fresh milt.

Cryopreservation techniques and sperm quality

The diluent tested in this study was based on that of MOUNIB'S (1978) (125 mM sucrose, 100 mM potassium bicarbonate, 6.5mM reduced glutathion) to which were added 5 % DMSO (Dimethylsulfoxide), 5 % Glycerol and 10 % hen Egg yolk.

The sperm was mixed with the diluent at a ratio of 1:3 and placed in 5 ml straws and allowed to freeze 3 cm above the level of liquid nitrogen for 20 mn before transfer into liquid nitrogen (OTEME *et al.*, 1996). The motility of the sperm was evaluated before and after freezing.

The fertilizing ability of the sperm was evaluated using hatching percentages on batches of 200 to 300 ovules collected from one female *H. longifilis* and artificially inseminated respectively with fresh sperm, sperm (from the same pool) thawed after 1 hour of cryopreservation and with sperm cryopreserved in liquid nitrogen for 8 months, at a ratio of 200 µl of milt (diluted 1:10 in 0.9 % NaCl solution) for 400 mg of ovules.

Results and discussion

Genetic variability

Of the 23 loci studied, only two were revealed as polymorphic : Mdh-1 and Pgm. At locus Mdh-1, two alleles with almost identical frequencies were observed in the two populations (F1 and F4) : Mdh-1 f (fast) at a frequency of 75% and Mdh-1 s (slow) at a frequency of 25% in the F1 population, Mdh-1 f at a frequency of 73% and Mdh-1 s at a frequency of 27% in the F4 population. At locus Pgm, two alleles were observed only in the F1 population, the allele Pgm s at the frequency of 95% and the allele Pgm f at the frequency of 5%. The observed rate of polymorphism (average heterozygosity), H, is equal to 2% for the F1 strain and 1.7% for the F4 strain.

The results obtained can be compared to those of TEUGELS *et al.* (1992). The enzymatic activities of nine additional loci were observed (Aat-2, Adh, Ak, Es-1, Es-2, Fbp, Icdh, Iddp-2, Ldh-3) as compared to this study, but none of these loci were shown to be polymorphic. At locus Mdh-1, these authors observed two alleles in the wild population, Mdh-1 100 and Mdh-1 75. These two alleles most certainly correspond to the observed alleles Mdh-1 f and Mdh-1 s. The frequencies of these two alleles are very similar in the F1 (75%/25%) and F4 (73%/28%) populations. These values are also comparable to those observed by TEUGELS *et al.* (1992) for the wild

population (60%/40%). However, these results show that the F3 population studied by TEUGELS *et al.* (1992) was not monomorphic for the allele Mdh-1 100 as their results seem to suggest since the two alleles were found in the F4 population.

At locus Pgm, TEUGELS *et al.* (1992) observed three alleles only in the wild population : Pgm 100 (30%), Pgm 85 (60%) and Pgm 60 (10%), the F3 population being monomorphic for the allele Pgm 85. Only two of these alleles were observed in the F1 population of our study, Pgm f (fast) and Pgm s (slow) and one single one in the F4 population, Pgm s. It is very likely that the allele Pgm s present in the heterozygous state in the F4 population is the allele Pgm 85 present in the homozygous state in the F3 population (TEUGELS *et al.*, 1992). The allele Pgm f observed in our study is, most probably, the allele Pgm 100.

The F4 population differs from the F1 population by an absence of polymorphism at the locus Pgm. The F1 population differs from the wild population by lower number of alleles at the locus Pgm (2 instead of 3). In all cases, a loss over successive generations of the least frequent alleles, namely Pgm 60 (10% in the wild population) and the Pgm 100 (30% in the wild population) is observed. Only the locus Mdh-1 of which the alleles are both at high frequencies in the wild population (60% and 40%) continues to be represented by two alleles at high frequencies in domestic populations. The heterozygosity rate observed for the wild population is equal to 8.5%. If H values for the F1 and F4 populations are calculated considering only the 12 loci common to both our study and that of TEUGELS *et al.* (1992), we obtain 3.9% and 3.2% respectively. The main cause of this loss of polymorphism is the small necessary effective size (½ effective population size) of stocks, which is to say, the real number of broodstock used to create a new generation.

Evaluation of zootechnical performances

Table I summarizes the results obtained during artificial fertilization. No significant differences (Duncan test at a fixed significance of 5%) were observed between the two strains in egg diameter (P = 18.5%), hatching percentage (P = 61%) and

percentage of normal fry (P = 58%). Only comparison of the percentages of deformed fry showed a significant difference (P = 3%). Deformed fry were more numerous in the F4 population; however, percentages remained small (less than or equal to 2%).

Results of the comparison of the body weight are shown in table II. At the 14th day (D14), the survival of the F1 population was very significantly higher than that of the F4 population (P =1%). Mean weight also were significantly higher for the F4 population than for the F1 generation (P = 2%).

Femelle	Weight (g)	ED (mm)	H (%)	DF(%)
Wild	4050	1.52	87.3	1.5
Wild	8100	1.49	93.5	1.8
Wild	4600	1.56	95.2	1.4
Wild	6400	1.67	87.9	1.6
Wild	5250	1.45	94.9	1.5
Wild	4250	1.50	99.0	1.6
F3	5100	1.51	97.7	1.7
F3	5600	1.47	74.6	1.7
F3	3000	1.47	93.3	1.8
F3	6450	1.44	95.9	2.0

Table I
Results of artificial fertilization to obtain F1 and F4 populations of *H. longifilis*. ED, egg diameter ; H, hatching percentage ; DF, deformed fry.

Because of the very different survival rates of the two generations, the corresponding densities in the culture tanks were not the same. Because of this, the observed differences in growth rates may be a genotype result (if one exists) or an effect of the density.
The body weight which appeared greater, of F4 individuals may

therefore be explained in part (or perhaps in totality) by the difference in rearing density resulting from a higher mortality observed in the F4 population. The correlation determined between initial rearing density and final body weight suggests that the mortality observed in the F4 population occurs very early during rearing.

Génération	D1	D5	D8	D11	D14	IN	FN
F1	2	22	60	97	129	300	282
F1	2	20	55	86	131	300	246
F1	2	23	59	93	150	300	261
F4	2	21	63	109	174	300	149
F4	2	18	54	99	181	300	171
F4	2	23	63	88	174	300	174

Table II
Results of comparison of growth between three lots of generation 1 (F1) individuals and three lots of generation 4 (F4) individuals of *H. longifilis*. D5, D8, D11, D14, weight at the 5th, 8th, 11th, 14th day ; IN, initial number of fry ; FN, final number of fry.

Sperm cryopreservation

Sperm motility

The sperm motility measured before and after cryopreservation showed that the motility was altered by the freezing-thawing process. Fresh sperm exhibited a percentage of motile spermatozoa ranging from 70 to 80 % ten sec after dilution, while cryopreserved sperm only showed motility percentages comprised between to 20 and 30 %.

Hatching of fertilized eggs

Total hatching rates obtained were 78.9%, 81.1%, and 83.4% respectively for the fresh sperm, the sperm cryopreserved for one

hour in liquid nitrogen and the sperm cryopreserved for 8 months in liquid nitrogen.

The observed hatching rates of deformed fry were 6.2%, 5.8% and 6.0% respectively for the fresh sperm, the sperm cryopreserved for 1 hour and 8 months in liquid nitrogen.

These results show that the cryopreserved sperm was as effective as the fresh sperm in fertilization trials and that *H.longifilis* sperm can be cryopreserved for at least several months with no effect on its fertilizing ability. Similar results were obtained with *Clarias gariepinus* by STEYN and VAN VUREN (1987) who reported that the sperm of this species could be cryopreserved for 28 months with no deterioration of its fertizing ability.

Conclusion

The results show that *H. longifilis* is a species highly sensitive to domestication. In four generations, a significant decrease in fry survival rate appears. The origin of this higher mortality must be determined. Several hypotheses can be proffered: the existence of lethal genes which might be expressed shortly after hatching, the influence of individual heterozygosity rates, an emphasized cannibalism phenomenon (behavior modification), a greater variability in growth rates favoring cannibalism (without modification of intrinsic population behavior). The apparent increase in the rate of deformed fry in the F4 population is very small, the maximum percentage observed being 2%. Considering the very high fecundity of this species, this rate is insignificant. This species is at once very prolific and possesses very high growth rate (about 10 g per day). For these reasons, its culture is developing in Africa and in Europe. Growers need to pay particular attention to the necessary effective size of their populations. A loss of genetic variation via a reduction of the number of broodstock (genetic drift), or an increase in the number of homozygotes

(inbreeding), may noticeably alter the performances of cultured populations. The fertilizing ability of *H. longifilis* sperm is not affected by cryopreservation. This offers the possibility not only of limiting the quantity of male individuals sacrificed or operated for reproduction, but also of constituting a gene bank in order to limit inbreeding, to maintain, and if necessary to improve the quality of broodstocks through a selection programme.

Acknowledgements
This work was made possible thanks to funding from Orstom (l'Institut français de recherche scientifique pour le développement en coopération).

Reference

AGNESE (J.-F.), OTEME (Z.J.), GILLES (S.), 1995 — Effects of domestication on genetic variability, fertility, survival and growth rate in a tropical siluriform: *Heterobranchus longifilis* Valenciennes 1840. *Aquaculture*, 131: 197-204.

DANZMANN (R.G.), FERGUSON (M. M.), ALLENDORF(F. W.), 1985 — Does enzyme heterozygosity influence developmental rate in rainbow trout? *Heredity*. 56: 417-425.

DANZMANN (R.G.), FERGUSON (M. M.), ALLENDORF (F. W.), 1987 — Heterozygosity and oxygen-consumption rate as predictors of growth and developmental rate in rainbow trout. *Physiol. Zool.* 60(2): 211-220.

DIEHL (W.J.), GAFFNEY (P.M.), MCDONALD (J.H.), KOHEN (R.K.), 1985 — Differences in weight standardized oxygen consumption, growth and weigt loss between low and high growth Mytilus edulis (Mollusca). *Physiologist*, 28:366-371

FERGUSSON (M.M.), 1992 — Enzyme heterozygosity and growth in rainbow trout: genetic and physiological explanations. *Heredity* 68, 115-122

KOEHN (R.K.), SHUMWAY (S.R.), 1982 — A genetic/physiological explanation for differential growth rate among individuals of the American oyster *Crassostrea virginica (Gmelin). Mar. Biol. Lett.* 3: 35-42.

KRIEG (F.), GUYOMARD (R.), 1985 — Population genetics of French brown trout (Salmo trutta L.) : large geographical differenciation of wild populations and high similarity of domesticated stocks. *Génét. Sél. Evol.* 17 (2): 225-242.

LEGENDRE (M.), 1983 — Examen préliminaire des potentialités d'un silure africain *H. longifilis*

(Valenciennes, 1840)
pour l'aquaculture en milieu lagunaire. *Doc. Scient. Cent. Rech. Océanogr.* Abidjan, 14 (2) 97-107.

MOUNIB (M.S.), 1978 —
Cryogenic preservation of fish and mammalian spermatozoa. *Journal of Reproduction and Fertility*, 53: 13-18.

OTEME (Z.J.), NUNEZ RODRIGUEZ (J.), KOUASSI (C.K.), HEM (S.), AGNESE (J.F.), 1996 —
Testicular structure, spermatogenesis and sperm cryopreservation in the African clariid catfish *Heterobranchus longifilis* (Valenciennes, 1840). *Aquaculture Research*, 27: 805-813.

RODHOUSE (P.G.), GAFFNEY (P.M.), 1984—
Effect of heterozygosity on metabolism during starvation in the American oyster *Crassostrea virginica*. *Mar. Biol.* 80: 179-187.

STEYN (G.J.), VAN VUREN (J.H.J.), 1987 —
The fertilizing capacity of cryopreserved sharptooth catfish (*Clarias gariepinus*) sperm. *Aquaculture*, 63: 187-193.

TANIGUCHI (N.), SUMANTADINATA (K.), IYAMA (S.), 1983 —
Genetic change in the first and second generations of hatchery stock of black seabream. *Aquaculture*, 35: 309-320.

TEUGELS (G.G.), GUYOMARD (R.), LEGENDRE (M.), 1992 —
Enzymatic variation in African clariid catfishes. *Journal of Fish Biology*. 40: 87-96.

VUORINEN (J.), 1984 —
Reduction of genetic variability in a hatchery stock of brown trout, *Salmo trutta* L.
Journal of Fish Biology, 24: 339-348.

Allozyme comparisons of fish used in aquaculture in South Africa

Characterization, conservation, selection based on molecular markers and selection as a result of cryopreservation of semen

Herman van der Bank
Geneticist

I Introduction

Inland aquaculture in South Africa is effectively limited to two species, rainbow trout and sharptooth catfish (Table 1). In a non drought year the annual output of trout would peak 1000 tons, and 1500 tons for catfish. Aquaculture research in South Africa decreased drastically since the demise of the National Aquaculture Programme of the Foundation for Research Development in 1989/90. This is the reason why recent production statistics for many species are not available. However, it is heartening that interest in aquaculture is once again flaring, and that the private sector has been funding some research projects. Research on triploidy, heat tolerant trout and cold tolerant tilapia strains were undertaken at the University of Stellenbosch, and the aquaculture potential of a catfish hybrid (*Heterobranchus longifilis* X *Clarias gariepinus*) was evaluated at Rhodes University. Although

the hybrid was reported to be imminently suited to aquaculture, no commercial production has commenced.

Species:	1988	1989	1990	1991	1992	1993
African catfish (*C. gariepinus*)	137	203	1500	1150	n.a.	n.a.
Rainbow trout (*O. mykiss*)	600	620	950	1220	990	1000
Tilapia (*O. mossambicus*)	20	20	30	40	n.a.	n.a.
Carp (*C. carpio*)	5	10	20	35	n.a.	n.a.
Ornamental fish	2.5	3.5	3.9	4.2	5.5	4.8

Table 1
South African aquaculture production statistics in metric tons (HECHT and BRITZ, 1993; BRITZ, personal communication, 1997).
n.a. = not available.

The following discussion is a summary of results obtained for the characterization, conservation, selection based on molecular markers or selection as a result of cryopreservation of semen of trout, catfish and other species used in inland aquaculture in South Africa.

Rainbow Trout

Rainbow trout is an alien species, first imported for angling purposes from England in 1897. This species can be regarded as a success from angling and aquaculture points of view, but it had a detrimental effect on a number of indigenous species. Trout

products include fresh, smoked whole, sliced smoked fillets, gravelax, paté, traviar and terrine. Eyed trout eggs are also exported to the Northern Hemisphere during their summer months (local winter), and proved to perform exceptionally well. The demand for South African trout eggs abroad exceeds the current production thereof.

It is interesting that trout production figures increased by 49% between 1989 and 1991, but hardly changed since 1992 when 990t were produced (Table 1). Trout farmers use between 720 and 750t of feed to produce 430t of trout; the gate selling price average R 12/kg and the total value is estimated at R 11.6 million.

Genetic variation studied in nine rainbow trout populations in South Africa showed average heterozygosity values of 4.5-7.5% and these relatively high levels can be attributed to the historical mixing of strains (VAN DER BANK *et al.,* 1992b). Growth rate differences related to genotypic variation, whilst food conversion rate and survival performance results did not seem to be related to heterozygosity values. The trout were obtained from Brink (in HECHT and BRITZ, 1993), who was able to improve the production performance of local trout strains (compared to control groups) to 6.5-14%, average 10.2%, in a subsequent study. Trout normally cannot tolerate summer temperatures higher than 20°C, but we have an eastern Cape strain which tolerates temperatures as high as 26°C.

Sharptooth Catfish

Catfish products include fresh and smoked fillets, and tinned catfish. The waste (gut, *etc.*) is used as an additional protein source for farmed catfish and the bones are used to produce bone meal to supplement their diets. In addition, the skins are used to produce leather for wrist watch straps, gloves, handbags, *etc.*, and the

pituitary gland (hypophysis) is used to induce spawning of various fish species.

There was an 87% increase of catfish production from 1989 to 1990, followed by a 23% drop over the period 1990 to 1991 and it can be expected that there has been little development since the last survey (Table 1). Drought and marketing problems were responsible for the decrease in production. However, a potential increase to ca. 5,000-6,000t per annum can be achieved (projection based on established production capacity of the present catfish farming community) and rapid progress can be anticipated because hatchery techniques have been mastered, good rainfall has occurred since 1996, and market constraints have reduced (HECHT and BRITZ, 1993).

Genetic variation in two commercially used (domesticated) and a wild population of catfish were compared and we obtained an average mean heterozygosity value of 5% for the latter population, but very little (0.3%) and much more (7.6%) for the two domesticated populations respectively (VAN DER BANK *et al.*, 1992a).

Overcompensation for a loss of genetic variation was achieved at the latter population (since the owner uses crosses between various wild and domesticated stocks) and the opposite holds for the other domesticated population (*i.e.* he uses the progeny to produce the next batch, thereby inducing inbreeding). The use of domesticated stocks to start new hatcheries may have negative implications for conservation because the escape of an access number of domesticated catfish into the wild (*e.g.* due to dam walls destruction as a result of heavy rains) could detrimentally affect the survival of progeny after introducing uncommon alleles.

GROBLER *et al.* (1992) have determined that a significant difference exists between the frequencies of some alleles in fast and slow growing groups of catfish. The feasibility of genetic selection for rapid growth in *Clarias gariepinus* was tested by VAN DER WALT *et al.* (1993b), who found noticeable differences between various genotypes. For example, one group increased its initial mass

advantage over another group from 105% at 30 days to 115% at 90 days.

A study by VAN DER WALT *et al.* (1993a) confirmed that the alleles which correlated to growth increase were similar between those obtained for the selected South African population and that of the population from The Netherlands, which were subjected to many generations of mass selection.

The important difference between the results from these two studies is that the South African catfish were less inbread, and are therefore better suited as candidates for aquaculture (to combat morphological irregularities associated with inbreeding). This was confirmed by GROBLER and VAN DER BANK (1994), who concluded that phenotypic variation is positively correlated to heterozygosity for different weight and length groups of catfish.

The effect of cryopreservation and various cryodiluents on allozyme variation in F_1 progeny of African catfish were demonstrated by VAN DER BANK and STEYN (1992). They have shown that significant differences of allele frequencies from expected Hardy-Weinberg proportions occurred in offspring obtained by using cryopreserved milt, compared to the control group produced by using fresh semen. These differences related to different cryodiluents and fertility as a result thereof. These selective qualities of cryopreservation techniques may have far reaching implications. For example, VAN DER BANK and STEYN (1992) used similar cryodiluents and freezing rates used by commercial institutions for livestock and humans, and selection of specific catfish genotypes were favoured by using these cryodiluents. Thousands of women annually make use of sperm banks and if the technique used for cryopreservation is found to favour specific sperm in humans also, it could have obvious ethical implications. However, VAN DER WALT *et al.* (1993c) have shown that the freezing rate used to induce cryopreservation is an important factor to reduce selection for specific allele combinations because an appropriate freezing rate minimises this effect. Therefore, an optimal freezing rate would be ideal to conserve the natural resources for future utilisation.

Other Inland species

VAN DER BANK (*in press*) reported results obtained for tilapias. Tilapias are very popular as table fish; tilapia production increased from 11 to 40 metric tons from 1988 to 1991 (Table 1) and currently the demand exceed the supply thereof in southern Africa. Furthermore, due to the pressure on marine stock, it is expected that the demand for freshwater fish would increase. Despite these facts, very few fish farms have been developed.

Isozyme studies were predominantly used to assess genetic variation and differentiation of tilapia species in southern Africa (VAN DER BANK and FERREIRA, 1987a,b; LIZEMORE *et al.*, 1989; VAN DER BANK *et al.*, 1989; OOSTHUIZEN *et al.*, 1993, *in press*; VAN DER BANK, 1994). We are now also involved in studies regarding allozyme variation in domesticated nile crocodile (*Crocodylus niloticus*), and VAN DER BANK (1995) and VAN DER BANK and VAN DER BANK (1995) studied allozyme variation in two freshwater mussel species. These results were obtained to characterize populations, and it can be used in subsequent studies for selection of suitable stocks for aquaculture. This need was identified because alternate protein sources should be investigated due to the ever increasing human population growth world wide. In addition to the food sources mentioned above, aquaculture holds other benefits. For example, in South Africa one person is employed per 2,9 tons of trout and catfish (HECHT and BRITZ, 1993).

Conclusion

No sensible long-term management or conservation plan can be implemented without a proper, initial understanding of the amount and pattern of genetic variation within the species. For instance, it would not have been possible to improve production characteristics

and to maintain high performance levels without the above-mentioned studies to characterize the taxa and to monitor effects of cryopreservation, management, directed selection and conservation efforts.

References

GROBLER (J.P.), VAN DER BANK (F.H.), 1994 — Allozyme polymorphism and phenotypic variation in African catfish (*Clarias gariepinus*). *Comp. Biochem. Physiol.* 107B: 111-116.

GROBLER, (J.P.), DU PREEZ (H.H.), VAN DER BANK (F.H.), 1992 — A comparison of growth performance and genetic traits between four selected groups of African catfish (*Clarias gariepinus* BURCHELL 1822). *Comp. Biochem. Physiol.* 102A: 373-377.

HECHT (T.), BRITZ (P.J.), 1993 — Aquaculture '92. *Proc. Aquacult. Assoc. sthn Afr.* 1, 198 pp.

LIZEMORE (E.), FERREIRA (J.T.), VAN DER BANK (F.H.), 1989 — An electrophoretic and morphometric evaluation of two subspecies of *Tilapia rendalli* from southern Africa. *Madoqua*, 16(1): 35-39.

OOSTHUIZEN (J.S.), VAN DER BANK (F.H.), FERREIRA (J.T.), 1993 — Genetic distances between and evolutionary relationships for *Tilapia guinasana*, *T. rendalli* and *T. sparrmanii*. *Biochem. Syst. Ecol.* 21(3): 351-362.

OOSTHUIZEN (J.S.), VAN DER BANK (F.H.), FERREIRA (J.T.), *in press* — A protein electrophoretic study to determine the amount and pattern of allozyme variation in *Tilapia guinasana*. *Madoqua*.

VAN DER BANK (F.H.), 1994 — Molecular systematics of fifteen Old world cichlid species using allozyme data. *Biochem. Syst. Ecol.* 22(8): 791-801.

VAN DER BANK (F.H.), 1995 — Allozyme variation in a freshwater mussel population (*Coelatura kunenensis* Mousson, 1887) from southern Africa. *Water S.A.* 21(2): 153-157.

VAN DER BANK (F.H.), in press — Tilapia biodiversity, genetic resources conservation and sustainable use: A South African perspective. *Proceedings of the Workshop on the Characterization of Ghanaian Tilapia Genetic Resources for Use in Fisheries and Aquaculture*, 4-7 June, 1996, Accra Ghana.

VAN DER BANK (F.H.), FERREIRA (J.T.), 1987a — Electrophoretic analysis of serum transferrins and serum esterases of three morphometrical different populations of *Oreochromis mossambicus* (Peters). *S. Afr. J. Wildl. Res.*, 17 (1): 13-15.

VAN DER BANK (F.H.), FERREIRA (J.T.), 1987b — Morphometric and electrophoretic analyses of three wild populations of *Oreochromis mossambicus* (Peters). In: *Proc. World Symp. on Selection, Hybridization*

and Genetic Engineering in Aquaculture. K. TIEWS (ed.) Bordeaux, France. 27-30 May 1986, Berlin, 1987 (I): 103-111.

VAN DER BANK (F.H.), STEYN (G.H.), 1992 — The effect of cryopreservation and various cryodiluents on allozyme variation of glucose phosphate isomerase in the F_1 progeny of African catfish (*Clarias gariepinus*). *Comp. Biochem. Physiol.* 103B(3): 641-643.

VAN DER BANK (F.H.), VAN DER BANK (M.), 1995 — Enzyme polymorphism in the freshwater mussel species, *Aspatharia pfeifferiana* (Mollusca, Lamellibranchiata) from southern Africa. *Animal Genetics* 26: 31-35.

VAN DER BANK (F.H.), GRANT (W.S.), FERREIRA (J.T.), 1989 — Electrophoretically detectable genetic data for fifteen southern African cichlids. *J. Fish Biol.* 34: 465-483.

VAN DER BANK (F.H.), GROBLER (J.P.), DU PREEZ (H.H.), 1992a — A comparative biochemical genetic study of three populations of domesticated and wild African catfish (*Clarias gariepinus*). *Comp. Biochem. Physiol.* 101B: 387-390.

VAN DER BANK (F.H.), SWART (M.K.J.), FERREIRA (J.T.), 1992b. — Genetic variation in nine rainbow trout (*Oncorhynchus mykiss*) populations in South Africa. *Comp. Biochem. Physiol.* 103B: 495-499.

VAN DER WALT (L.D.), VAN DER BANK (F.H.), STEYN (G.J.), 1993a — Allozyme variation in domesticated African catfish (*Clarias gariepinus*) from the Netherlands. *Comp. Biochem. Physiol.* 104B: 15-18.

VAN DER WALT (L.D.), VAN DER BANK (F.H.), STEYN (G.J.), 1993b — An association between glucose-6-phosphate isomerase phenotypes and rapid growth in the African catfish (*Clarias gariepinus*). *Comp. Biochem. Physiol.* 104B: 765-768.

VAN DER WALT (L.D.), VAN DER Bank (F.H.), STEYN (G.J.), 1993c — The suitability of using cryopreservation of spermatozoa for the conservation of genetic diversity in African catfish (*Clarias gariepinus*). *Comp. Biochem. Physiol.* 106A: 313-318.

Morphologic and genetic differentiation of natural populations of *Chrysichthys nigrodigitatus* (Siluroidei, Claroteidae)

Béatrice Adépo-Gourène
Geneticist

Guy G. Teugels
Ichthyologist

Jean François Agnèse
Geneticist

Introduction

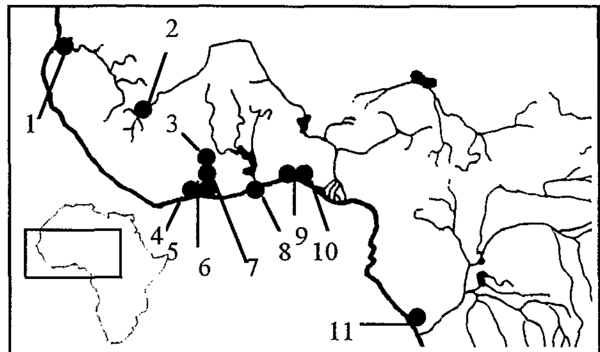
Chrysichthys nigrodigitatus (Lacépède, 1803) is a silurid found in most of the West African hydrographic basins from Senegal to Zaire. It is an economically important species whose culture in lagoons has been developed in certain countries like Côte d'Ivoire, where the annual production is 350 to 400 t (OTEME, 1993). But as for most fish species of aquacultural interest, the research programs which studied the biological cycle, the production conditions and the commercialization of the species did not take into account the genetic resources of the natural populations. However, the biological characteristics of the populations including their reproduction, depend in part on their genetic patrimony. The knowledge of genetic characteristics of fish species of aquacultural

interest is necessary to characterize the strains and the populations and also to show introgressions (hybridization between close species). It also allows the determination of management schemes (maintenance, study and restoration of the genetic variability of strains, reconstitution of stocks in the natural environment) and improvement plans (comparison of performances of genetically differentiated strains) and finally to create new strains by crossing. The first genetic studies of *C. nigrodigitatus* populations were carried out by AGNESE in 1989 during a study on the genetic differentiation of several West African siluriform species of interest to fisheries and aquaculture. These first works showed that the population from the Niger river (Mali) is very differentiated from those coming from rivers in Côte d'Ivoire. The current work studies the diversity of natural populations of *C. nigrodigitatus* over a larger portion of its distribution range. Two techniques were used to achieve this goal: morphological analysis of samples and enzymatic protein electrophoresis. The main objectives of this study were: to establish reference data which could be used for the management and the protection of natural populations and culture strains, to enable the comparison of zoo-technical performances of the most morphologically and genetically differentiated samples and to propose possible applications for aquaculture.

Materials and methods

Genetic and morphological studies were carried out using eleven samples of *C. nigrodigitatus* from different basins along the West African coast (Fig. 1). The Jacqueville strain is made up of domesticated fish (fifth generation) taken from a fish farm for comparative purposes. Because of preservation problems, certain samples could only be analyzed using one technique.

The morphological analyses were performed by the Ichthyology Laboratory of Tervuren in Belgium. Fifteen metric characteristics and eight meristic characteristics were measured on each specimen.



I Fig 1
Collecting sites of *Chrysichthys nigrodigitatus* samples: 1, Dagana, 2, Selingue, 3, Abengourou, 4, Layo, 5, Jacqueville, 6 Bonoua, 7, Koutoukro, 8, Bator, 9, Abobo, 10, Guezin, 11, Bas Kouilou.

These were: (1) total length, (2) standard length, (3) head length, (4) snout length, (5) width of the premaxillary band, (6) length of the occipital process, (7) width of the occipital process, (8) length of the nasal barbels, (9) predorsal distance, (10) preadipose distance, (11) prepectoral distance, (12) prepelvic distance, (13) preanal distance, (14) dorsal-adipose distance, (15) dorsal length, number of branchiospines on the epibranchial, number of branchiospines on the cerato- and hypobranchial, number of soft rays in the dorsal fin, number of soft rays in the pectoral fin, number of simple rays in the pelvic fin, number of branched rays in the pelvic fin, number of simple rays in the anal fin, number of branched rays in the anal fin. Statistical analyses of the data were carried out using CSS: Statistica software (Statsoft, version 3.3). The genetic diversity was studied in the Genetics Laboratory at the Centre de Recherches Océanologiques in Abidjan. The electrophoretic analyses studied 19 loci and 8 populations. The genetic variability was evaluated using two indices: i) the polymorphism rate P which corresponds to the number of polymorphous loci compared to the total number of loci studied, ii) the mean heterozygosity (H) calculated using NEI's formula (1978).

Results and discussion

The morphological data obtained for fourteen characteristics, excluding the total length, were subjected to an analysis of principal components; the populations were divided into four groups based on their country of origin (Fig. 2). The nasal barbel length is the most discriminating characteristic on the second axis. The dorsal length, the premaxillary band width and the occipital process length are the most discriminatory on the third axis. The first axis was not taken into account because it was highly influenced by specimen size. Samples from Congo and Côte d'Ivoire were clearly separated from those of Senegal and Mali. There is a great deal of overlapping in the zone occupied by the Congo sample, taken from brackish water near the mouth of the Kouilou, and samples from Côte d'Ivoire. This overlapping is seen particularly with populations from Ebrié Lagoon (Layo, Jaqueville and Bonoua).

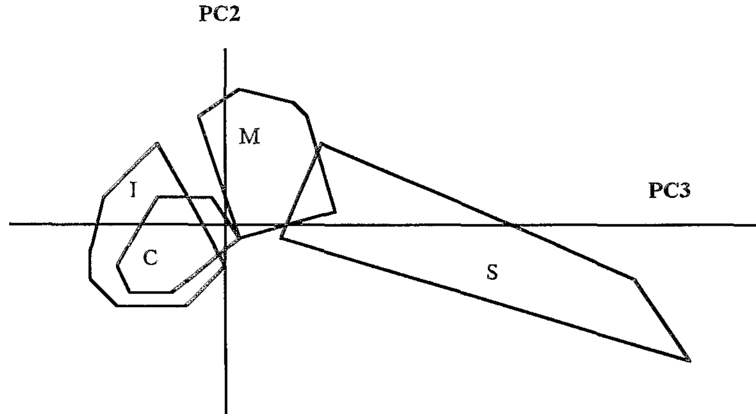


Figure 2
Plot of a principal component analysis using 14 log-tranformed metric variables of *Chrysichthys nigrodigitatus* specimens arranged in groups based on their country of origin: I, Côte d'Ivoire, M, Mali, C, Congo, S, Senegal.

Populations from Mali and Senegal overlap slightly. Concerning meristic characterisitics, only the number of branchiospines on the lower part of the first branchial arch and the number of branched rays in the anal fin showed some variation. The Congo population distinguishes itself from the others by a greater number of branchiospines (15 to 18) and branched rays.

Genetically, of the 19 loci analyzed by enzymatic electrophoresis, 6 were shown to be polymorphic in the *C. nigrodigitatus* samples. The Congo and Senegal samples were monomorphic for all loci studied (Table 1). Those from Layo and Jacqueville showed private alleles EST-1*A and PROT*B. The polymorphism rate, P99, determined for all populations varied from 0.0 (Dagana, Congo) to 15.7 (Layo, Jacqueville) with a mean of 10.5. This rate is comparable to that estimated by AGNESE *et al.* (10.3) in 1989 in the same species and in *C. johnelsi*. The mean heterozygosity rate was 4.1%. This rate was comparable to those found in the literature. AVISE and AQUADRO (1982), estimated it to be 5.4% for all fish in general. Certain African silurids have the following values: 4.7% for *Clarias gariepinus* (VAN DER BANK *et al.*, 1992), 11% in *Heterobranchus longifilis* (TEUGELS *et al.*, 1992).

population	1	2	4	5	8	9	10	11
$P_{99\%}$	0.0	15.7	26.3	21.0	10.5	15.7	15.7	0.0
$H_{\%}$	0.0	5.8	6.3	6.0	4.5	5.3	4.7	0.0

Table 1
Summary of the polymorphism and heterozigosity values observed for the 8 populations of *C. nigrodigitatus* studied.

The highest mean heterozygosity rates were observed from Layo (6.3%) and Jacqueville (6.0%). The Jacqueville population is made up of domestic specimens (fifth generation in captivity) issued from several hundred brooders some of which are taken from the wild each year (Ebrié Lagoon) near Layo. Therefore, the culture technique used avoids loss of genetic variability. In effect, the high

number of brooders used but also the systematic introduction of new wild brooders helps maintain the genetic variability of the original population.

The results obtained using these two techniques show certain similarities. All the *C. nigrodigitatus* populations, with the exception of those from Côte d'Ivoire and Congo, were different morphologically. This differentiation was ordained geographically. In effect, the populations the most differentiated are those which were the most geographically separated (Senegal-Mali and Congo).

Concerning the genetic differentiation, the most polymorphic populations were those from Côte d'Ivoire and the monomorphic populations were located at the limits of the species' distribution (Senegal and Congo).

Conclusion

Knowledge of the genetic diversity of natural populations allows the monitoring of natural stocks the conservation of which may become necessary due to manmade environmental alterations.

From an aquacultural viewpoint, knowledge of the genetic diversity of wild populations allows for appropriate choices in sampling sites and eventual crosses in order to obtain strains with high genetic variability. For example, the Senegal and Congo strains being monomorphic sometimes for different alleles, it would be interesting to perform crosses between them in order to obtain an eventual heterosis effect.

Crosses could be performed between specimens from different populations in order to obtain a synthetic strain possessing the majority of the variability of the species. So that a cross between individuals from Côte d'Ivoire and Niger would produce a strain possessing most of the alleles of the species. Such a synthetic strain would be likely to have zoo-technical advantages (growth rates,

resistance to disease as well as to other environmental aggressions, etc...) because of its high genetic variability. In effect, different studies (DAZMANN *et al.*, 1986, 1987, 1988, 1989; MITTON and GRANT, 1984; ALLENDORF and LEARY, 1986; ZOUROS and FOLTZ, 1987; 1990; AGNESE *et al.*, 1994) of the relationships between the genetic variability and zoo-technical aptitudes have shown the existence of a correlation between these two types of factors. These works have shown that heterozygous specimens often have zoo-technical performances (growth, viability and fecundity rates; egg size; disease and environmental stress resistance, etc...) much higher than homozygous specimens. This strain may also be capable of a greater adaptive ability to captive conditions.

Knowledge of the genetic variability of cultured strains would allow the monitoring of these stock in time and space and notably to confirm the absence of introgression. As an example, this study shows that the domestic strain (Jacqueville) shows a polymorphism rate as high as that of the natural population it originates from. Therefore, there has been no loss of variability, contrary to what has been shown for *Heterobranchus longifilis* (AGNESE *et al.*, 1994). In effect, in the domestic strain of this species, a loss of genetic variability, in comparison to the natural population, has been observed in fourth-generation captive specimens. This loss is accompanied by a strong decrease in larval survival rates. On the contrary, in *C. nigrodigitatus*, no new alleles have been observed among domestic strains, which indicates the absence of introgression in these stocks.

Finally, it would be interesting to test the zoo-technical performances of specimens from the most polymorphic populations (Côte d'Ivoire, Ghana, Togo, Benin).

Acknowledgements

This work was supported by the European Community (contract n° ERBTS3 *CT920079) and Orstom (l'institut français de recherche scientifique pour le développement en coopération).

References

AGNESE (J.F.), 1989 — *Différenciation génétique de plusieurs espèces de Siluriformes ouest-africains ayant un intérêt pour l'aquaculture.* PhD Thesis, Université de Montpellier-II, France.

AGNESE (J.F.), PASTEUR (N.), LEVEQUE (C.), 1989 — Différenciation génétique de quelques populations de *Chrysichthys nigrodigitatus* et de *C. johnelsi* (Pisces, Bagridae) de Côte d'Ivoire et du Mali. *Rev. Hydrobiol. trop.*, 22 (2): 101-106.

AGNESE (J.F.), OTEME (Z.J.), GILLES (S.), 1994 — Effects of domestication on genetic variability, fertility, survival and growth rate in a tropical siluriform: *Heterobranchus longifilis* VALENCIENNES 1840. *Aquaculture*, 131 (1995): 197-204

ALLENDORF (F.W.), LEARY (R.F.), 1986 — Heterozygosity and fitness in animals. *In* SOULE (M.E.), ed.: Conservation Biology. Sunderland, MA : Sinauer, p 57-76

AVISE (J.C.), AQUADRO (L.F.), 1982 — A comparative summary of genetic distances in the vertebrates. Pattern and correlations. *Evol. Biol.*, 15: 151-185.

DANZMANN (R.G.), FERGUSON (M.M.), ALLENDORF (F.W.), 1986 — Does enzyme heterozygosity influence developmental rate in rainbow trout? *Heredity*, 56: 417-425.

DANZMANN (R.G.), FERGUSON (M.M.), ALLENDORF, (F.W.), 1987 — Heterozygosity and oxygen consumption rate as predictors of growth in developmental rate in rainbow trout. *Physiol. Zool.*, 60 (2): 211-220.

DANZMANN (R.G.), FERGUSON (M.M.), ALLENDORF (F.W.), 1988 — Heterozygosity and components of fitness in a strain of rainbow trout. *Biol. Journ. Linn. Soc.*, 33: 285-304.

DANZMANN (R.G.), FERGUSON (M.M.), ALLENDORF, (F.W.), 1989 — Genetic vartiability and components of fitness in a hachery strain of rainbow trout. *J. Fish. Biol.*, 35 (Supplement A): 313-319.

MITTON (J.B.), GRANT (M.C.), 1984 — Associations among protein heterozygosity, growth rate and developmental homeostasis. *A. Rev. Ecol. Syst.*, 15: 479-499.

OTEME (Z.J.), 1993 — Reproduction et fécondité du mâchoiron *Chrysichthys nigrodigitatus* en élevage. *J. Ivoir. Océanol. Limnol.*, 2: 53-59.

TEUGELS (G.G), GUYOMARD (R.), LEGENDRE (M.), 1992 — Enzymatic variation in African Clariid catfishes. *J. Fish Biol.*, 40: 87-96.

VAN DER BANK (F.H.), GROBLER (J.P.), DU PREEZ (H.H.), 1992 — A comparative biochemical genetic study of three African Catfishes (*Clarias gariepinus*). *Comp. biochem. Physiol.*, Vol. 1013, 3: 387-390.

ZOUROS (E.), FOLTZ (D.W.), 1987 — The use of allelic isozyme variation for the study of heterosis. *In* RATAZZI (M.C.), SCANDALIOS (J.G.), WHITTH (G.S.), ed.: *Isozymes*. New York: Liss, Vol. 13, pp 1-59.

Microsatellite variation in the african catfish *Chrysichthys nigrodigitatus* (LACEPEDE, 1803) (Siluroidei, Claroteidae)

Giorgos Kotoulas
Geneticist

Jean-François Agnèse
Geneticist

Eleftherios Zouros
Geneticist

Variation at three microsatellite loci was examined in four natural populations of the West african catfish *C. nigrodigitatus*: Senegal (n=46), Selingue Dam, Niger (n=54), Ebrie lagoon, Ivory Coast (n=32) and Volta Lake (n=28). One farmed population, Sial (Société ivoirienne d'aquaculture lagunaire) in Ebrie Lagoon, created with 700 founders and farmed for five generations, was also examined (n=85). Summary statistics are given in Table 1.

The number of alleles was high in all three loci (29 to 30) and the number of the average number of alleles per population varied from 22.0 to 6.67. Heterozygosities were also high, but interestingly the higher mean heterozygosity was exhibited by the locus with the smaller number of alleles. Smaller heterozygosities were observed in Selingue dam and Sial farm, whereas the three natural populations had higher and comparable heterozygosities. Between 17% to 60% of alleles were « private », that is they were observed only in one population. The number of private alleles across loci

varied among populations from 16 to zero. The farmed population had no private allele, an observation consistent with the history of the population. The Dam population has also a small number of private alleles, but the low number of Volta is unexpected. On the basis of neutrality and random mating, one can compare effective population sizes. The five populations appear to fall into three categories in this respect: Niger Dam and Sial Farm are the smaller, followed by Volta, followed by Senegal and Ebrie. It is interesting to note that the levels of polymorphism in the natural populations of species living in rivers can be as high as marine species, suggesting that these populations have a long history and have mantained large sizes.

	Microsatellite Locus			Population				
	Cn1	Cn2	Cn3	Niger	Senegal	Ebrie	Volta	Sial
N	231	251	254	-	-	-	-	-
Na	-	-	-	54	46	32	28	85
A	38	30	29	-	-	-	-	-
Aa	-	-	-	12.7	20.3	22.0	14.0	6.67
np	13	18	5	4	13	16	3	0
Ha	0.88	0.68	0.92	0.74	0.89	0.89	0.85	0.76
FIS	-0.008	0.097	0.050	0.057	0.162	0.058	0.016	-0.062
Ne,i	-	-	-	1400	4000	4000	2800	1600
Ne,s	-	-	-	3500	20500	20500	10800	4100

Table 1
Summary statistics of microsatellite variation at three loci in five populations of the West African catfish *Chrysichthys nigrodigitatus*. Note: N =Total number of animals scored, Na = number of animals scored averaged over loci, A= total number of alleles observed, Aa= number of alleles observed averaged over loci, np= number of private alleles, Ha = average heterozygosity over populations (first set of columns) or over loci (second set of columns), Nei = effective population size (averaged over loci) under the step-wise mutation model. In both estimates of Ne, mutation rate was assumed to be $u = 5x10^{-5}$.

Note that the farmed population suffered an important reduction of polymorphism within five generations, relatively to the levels of the population of origin, despite an aquaculture practice aiming at keeping high levels of polymorphism. A dam effect can be also seen, but a sample from the Niger river should be analyzed to test for levels of polymorphism outside the Selingue Dam.

Most populations are in Hardy-Weinberg equilibrium. An exception is the sample from Senegal river, at Dagana, where a trend for excess of homozygotes is present in all loci, with significant values for two out of three loci. Given the high levels of polymorphism, inbreeding seems not a probable cause of this excess. One possibility is that the excess is artifactual (*e.g.* variation in the efficiency of PCR). Another possibility is a temporal or ethological Walund effect, whereupon non-random mating is a consequence of differences in the time or behavior of spawning. If this type of breeding structure is shown to be true, it will be of basic importance for management decisions and conservation policy for this species.

A highly significant heterogeneity was observed in all pair-wise comparaisons of samples for all three loci. It is of interest that this holds even for the farmed population relatively to the population of its origin. Random drift can be highly effective in causing allele frequency changes even when the founder population is large (700 animals) and the time since establishement of the farm is small (five generations). Large differences between wild populations, on the other hand, imply a long history of effective, if mot absolute, isolation.

Based on these three loci a factorial analysis of correspondence provides a relatively good assignment of individuals to populations. For the most polymorphic samples (Ebrie lagoon and Senegal river) there is considerable overlap. In a pilot experiment involving a small number of animals scored for five instead of three loci, the assignment was infallible. Thus, a relative small increase in the number of microsatellite loci (provided they are as polymorphic as the three reported here) and estimates of frequencies based on larger sample sizes will suffice for the assignment of individuals to their population of origin.

These results show that microsatellite is the tool of choice for population monitoring, preservation of genetic diversity and breeding programs in aquaculture. The same individuals used in a study of allozyme and morphological variation (ADEPO-GOURENE *et al.*, 1997). Allozymes showed a relatively high variability in populations from the middle of the geographic distribution of the species (Ivory Coast), relatively to almost complete monomorphism in the Northern (Senegal) and Southern (Congo) extremes. This may mean that the species has originated in the middle of the distribution and extended its presence in both directions, North and South. The present study shows that microsatellites are as variable in Senegal as in Ivory Coast (Ebrie Lagoon). The difference between allozymes and microsatellites is most likely due to the difference in mutation rate, which is much higher in microsatellites. This means that microsatellites have much « shorter memory » of the evolutionary history of the population and can be used most profitably to read the recent history of the species.

Acknowledgements

This work as part of the GENETICS program, was made possible thanks to funding from the European Union (contract ERBTS3*CT920079)

References

ADEPO-GOURENE (B.), TEUGELS (G. G.), RISCH (L. M.), HANSSENS (M. M.), AGNESE (J.-F.), 1997 — Morphological and genetic differentoation of 11 populations of the African catfish *Chrysichthys nigrodigitatus* (Siluroidei; Claroteidae), with consideration of their biogeography. *Can. J. Zool.* 75: 102-109.

Résumés

Béatrice Adépo-Gourène, Laurent Pouyaud, Guy G. Teugels, Marc M. Hanssens, Jean-François Agnèse : « Différenciation morphologique et génétique des populations naturelles de *Sarotherodon melanotheron* Ruppel 1852 (Teleostei, Cichlidae) »

Une étude génétique et morphologique a été entreprise sur trente-neuf échantillons représentant plusieurs populations d'Afrique de l'Ouest de *Sarotherodon melanotheron*. La différenciation morphologique permet de séparer les populations en trois groupes et concorde avec la description des trois sous-espèces faite par Trewavas en 1983. La différenciation génétique observée entre les populations pourrait être accompagnée de différences dans les performances zootechniques des individus. Cette étude ouvre de nouvelles perspectives pour la caractérisation des performances zootechniques des populations de *Sarotherodon melanotheron* dans le but de sélectionner les meilleures souches aquacoles.

Mots clés : *Sarotherodon melanotheron* — Morphométrie — Allozyme — Microsatellite — Aquaculture.

Béatrice Adépo-Gourène, Marc M. Hanssens, Guy G. Teugels, Jean-François Agnèse : « Différenciation morphologique et génétique des populations naturelles de *Chrysichthys nigrodigitatus* (Lacepède,1803) (Siluroidei, Claroteidae) »

L'analyse de la différenciation morphologique et génétique a été entreprise sur onze populations de *Chrysichthys nigrodigitatus*. Ces populations sont morphologiquement et génétiquement différenciées. Cette différenciation est géographiquement répartie. Les populations les plus morphologiquement différenciées sont les deux populations qui sont à la limite de l'aire de répartition de l'espèce. Elles ont également les taux de polymorphisme les plus bas et sont toutes les deux monomorphes (Dagana, Bas Kouilou).

Les populations de Côte d'Ivoire sont les plus polymorphes. La description de la variabilité génétique des populations de *C. nigrodigitatus* constitue une référence qui permettra de suivre et de surveiller la pureté des souches utilisées en aquaculture.

Ces résultats ouvrent également des perspectives sur de futures recherches de variabilité des performances zootechniques associées aux différences génétiques. Des protocoles de croisement peuvent aussi être envisagés pour obtenir des souches synthétiques ayant une plus grande variabilité génétique ou pour rechercher un effet d'hétérosis.

Mots clés : *Chrysichthys nigrodigitatus* — Aquaculture — Morphométrie — Allozyme.

Jean-François Agnèse : « Les introductions de poissons dans les eaux douces d'Afrique: premières données sur leurs impacts génétiques »

L'introduction de n'importe quelle espèce dans le milieu naturel peut avoir des impacts génétiques. Ces impacts sont définis non seulement comme des altérations du génome des espèces autochtones mais aussi comme des altérations du génome des espèces introduites.

Après avoir passé en revue les principaux types d'altération possibles, leurs causes et leurs conséquences, nous avons examinés les introductions d'espèces en Afrique pour lesquelles nous disposons de données sur leurs impacts génétiques : les hybrides entre *Oreochromis niloticus* et *O. mossambicus* dans le lac Itasy à Madagascar; les mêmes hybrides dans le lac Ihema au Rwanda; le cas du lac Victoria avec *O. niloticus*, *O. variabilis* et *O. esculentus*; celui de la souche Bouaké de *O. niloticus* dans les eaux de Côte d'Ivoire et enfin, le cas de *Limnothrissa miodon*, Clupeidae, originaire du lac Tanganyika et introduit dans le lac Kivu.

Mots clés : Introduction — Transfert — Introgression — Afrique — Hybridation — Poisson.

Jean-François Agnèse, Béatrice Adépo-Gourène, Laurent Pouyaud : « Hybridation naturelle chez les tilapias »

Les tilapias forment un groupe d'espèces bien connues pour leurs capacités à s'hybrider. Les hybrides sauvages peuvent être rangés en trois catégories: ceux causés par des transferts d'espèces, ceux causés par des changements de l'environnement induits par l'homme, enfin ceux véritablement naturels. La majorité des cas rapportés d'hybridation naturelle appartiennent à la première catégorie. Dans le lac Naivasha, au Kenya, où *Oreochromis spilurus nigra* et *O. leucostictus* ont été introduits dans les années 1950; dans le lac Bunyoni en Ouganda où *O. niloticus* a été introduit en 1927 depuis le lac Edward et *O. spilurus nigra* en 1932 depuis le lac Naivasha; dans le lac Itasy, à Madagascar, où *O. macrochir* a été introduit en 1958 et *O. niloticus* en 1961; dans le lac Ihema, au Rwanda, où *O. macrochir* a été introduit vers la fin des années 1960, et après l'introduction de *O. niloticus* vers la fin des années 1940; enfin, dans le lac Victoria, où *O. niloticus* a été introduit dans les années 1960 et où *O. variabilis* et *O. esculentus* ont disparu. L'hybridation entre *Tilapia zillii* et *T. guineensis* en Côte d'Ivoire est un exemple d'hybridation qui résulte d'une perturbation de l'environnement créée par l'action de l'homme (construction de barrages hydro-électriques). Le dernier cas d'hybridation présenté ici a lieu à l'aval des chutes de la Koroboué sur la rivière Comoé. Il concerne trois espèces *T. zillii*, *T. guineensis* et *T. dageti*.C'est le seul cas d'hybridation où l'homme ne joue vrai-semblablement aucun rôle.

Mots clés : Hybride — Tilapia.

Jean-François Agnèse, Béatrice Adépo-Gourène, Laurent Pouyaud : « Différenciation génétique et statut subspécifique des populations naturelles de *Oreochromis niloticus* (Teleostei, Cichlidae) »

Nous avons analysé la différenciation génétique de 17 populations naturelles de *Oreochromis niloticus* (Linnaeus,

1758) en utilisant les allozymes, les polymorphismes de fragments de restriction (PLFR) de l'ADN mitochondrial (ADNmt) et les microsatellites. Les populations étudiées, du fleuve Sénégal au lac Tana et du lac Manzalla au lac Baringo représentent toutes les sous-espèces décrites précédemment. Les locus nucléaires variables (16) montrent que les populations peuvent être rangées en trois groupes: 1, les populations d'Afrique de l'Ouest (Sénégal, Niger, Volta et bassin du Tchad); 2, les populations de la vallée du Rift éthiopien (lacs Awasa, Ziway, Koka et la rivière Awash) 3, le bassin du Nil (lac Manzalla, Le Caire, lac Edward) et les populations de la vallée du Rift kenyan (lacs Turkana, Baringo et rivière Suguta). Neuf différents haplotypes d'ADNmt ont été trouvés lors de l'analyse des PLFR dans une portion de 1 Kb de la région de la Dloop. Le réseau obtenu montre également qu'il y a trois groupes de populations géographiquement distinctes. Toutes les populations d'Afrique de l'Ouest et *O. aureus* sont regroupées, à l'autre extrémité du réseau on trouve les populations d'Ethiopie et entre ces deux groupes les populations du Kenya et de l'Ouganda. Les populations du Nil montrent des affinités à la fois avec les populations d'Afrique de l'Ouest et avec celles des lacs Turkana et Tana. L'étude des microsatellites donne également des résultats comparables. Les implications taxinomiques de ces résultats sont discutées.

Mots clés : Allozyme — Taxinomie — ADNmt — Microsatellite — *Oreochromis niloticus*.

Aggrey J.D. Ambali, Roger W. Doyle : « Analyse de la diversité génétique de *Oreochromis shiranus* dans les bassins de retenue au Malawi »

Des études ont été entreprises pour estimer la diversité génétique des populations de *Oreochromis shiranus* dans les bassins de retenue du Malawi. Ces bassins comportent plusieurs espèces de poissons dont *O. shiranus sp*, *Tilapia rendalli*, *Clarias gariepinus*, *Serranochromis robustus*, *Barbus sp* et des haplochromines. *O. shiranus* a été trouvé

dans tous les bassins où l'espèce a été introduite entre 1955 et 1968. Les espèces carnivores comme *C. gariepinus* et *S. robustus* ont été trouvé dans certains bassins, la première étant apparu spontanément à partir des cours d'eau afférents et la seconde ayant été volontairement introduite pour contrôler les populations de tilapia. La longueur standard moyenne de *O. shiranus sp* dans ces réservoirs va de 13,6 à 20,5 cm, ce qui est plus important que la taille moyenne observée dans les populations sauvages. La tendance observée montre que dans les réservoirs où le ratio prédateur/proie est grand, la taille moyenne des tilapias est également grande. Des sondes microsatellites ont été utilisées pour étudier la diversité allélique de deux sous espèces de *O. shiranus*: *O. shiranus shiranus* et *O. shiranus chilwae*. Le nombre moyen d'allèles varie de 3,2 ± 0,37 à 10,2 ± 2,15. La diversité allélique est faible dans les réservoirs avec un ratio prédateur/proie élevé. La distance de Cavalli-Sforza et Edwards (1967) entre les populations des bassins varie entre 0,024 et 0,204. Le coefficient de corrélation normalisé de Mantel entre les distances génétiques et les distances géographiques est faible (0,265) et non significatif (p = 0,8516). Les microsatellites montrent que des croisements entre *O. sh. shiranus* et *O. sh. chilwae* ont eu lieu dans la plupart des bassins.

Mots clés : *Oreochromis shiranus* — Microsatellite — Aquaculture.

Olga Assémien : « Caractérisation zootechnique de quatre souches de *Oreochromis niloticus* »

La vitesse de croissance de trois souches sauvages de *Oreochromis niloticus*, Ghana (GHA), Niger (NIG) et Sénégal (SEN), a été comparée à celle d'une souche domestique, la souche Bouaké (BKE). Cette souche provient d'un croisement entre une souche du bassin de la Volta (Burkina-Faso, arrivée en 1957) et une souche du bassin du Nil (Ouganda, arrivée en 1968). L'ensemble du matériel biologique était constitué de juvéniles, qui ont été

grossis puis mis en reproduction en bacs cimentés pour les comparaisons de croissance. Les différents tests se sont déroulés sur une période de cinq mois. La ration alimentaire, identique pour tous les étangs était basée sur le groupe ayant le poids moyen le plus élevé. Chaque mois, une pêche de contrôle a permis d'ajuster cette ration. Tous les poissons ont été pesés individuellement à la mise en charge et à la vidange. Sur le plan zootechnique, en nous basant sur l'évolution des poids moyens, la croissance journalière individuelle et l'indice de consommation, les souches présentant les meilleures performances de croissance seraient BKE et SEN. Les conséquences sur l'utilisation de ces différentes souches sont discutées.

Mots clés : *Oreochromis niloticus* — Croissance.

Herman van der Bank : « Variation allozymique au sein des espèces utilisées en aquaculture en Afrique du Sud »

Les deux espèces de poissons d'eaux douces les plus importantes économiquement pour l'aquaculture en Afrique du Sud sont la truite arc-en-ciel *Oncorhynchus mykiss* et le poisson chat *Clarias gariepinus*. La truite arc-en-ciel est une espèce introduite, importée pour la première fois d'Angleterre en 1897 pour la pêche sportive. Cette introduction peut être considérée comme un succès du point de vue de la pêche et de l'aquaculture mais elle a eu des effets négatifs sur un grand nombre d'espèces de poissons autochtones. Les truites arc-en-ciel d'Afrique du Sud sont exportées dans l'hémisphère nord en été (localement en hiver), et font preuve de performances exceptionnelles. Des élevages locaux varient en vitesse de croissance et en taux de survie en raison de mélanges de stocks importés de plusieurs pays et de variations génotypiques. Des différences allozymiques entre des populations sauvages et domestiques de *C. gariepinus* ont été observées et reliées à des différences de stratégies dans la gestion des stocks. Cela a des répercussions pour la conservation de l'espèce dans la mesure où les éleveurs achètent leurs stocks de géniteurs à d'autres éleveurs,

parfois éloignés de plusieurs centaines de kilomètres, et parce que ces poissons diffèrent génétiquement des populations locales. Des variations phénotypiques sont affectées par le polymorphisme allozymique; une association a été trouvée entre des caractères génétiques et des performances de croissance chez le poisson chat. On a également montré une action sélective des cryodiluents et des vitesses de congélation sur les spermatozoïdes (sélection des spermatozoïdes présentant certaines combinaisons alléliques). La sélection des stocks de géniteurs pour l'aquaculture et l'utilisation de la cryopréservation pour l'aquaculture ou pour la conservation est discutée sur la base des informations précédentes..

Mots clés : *Oncorhynchus mykiss* — *Clarias gariepinus* — Aqua cultutre — Cryopréservation.

Sébastino Da Costa : « Comparaison des performances de croissance des souches Niger et Bouaké de *Clarias anguillaris* Linné, 1758 »

La croissance des souches Niger et Bouaké de *Clarias anguillaris* (Linné, 1758) a été comparée. Des populations mixtes (1:1) de chaque souche ont été élevées pendant huit mois avec nourrissage à l'aliment M2GE titrant à 45 % de protéine, suivant une ration journalière équivalente à 3 % de leur biomasse. Une série de 6 bacs bétonnés de 15 m^3 chacun, à raison de 3 bacs par souche, pour un volume d'eau utile de 11,2 m^3 a été utilisée. Chaque structure contenait 30 fingerlings avec un poids moyen variant entre 51,08 et 54,37 g pour la souche Niger et entre 55,12 et 55,48 g pour la souche Bouaké. La température de l'eau a variée entre 24 et 28,8 °C. Les souches Niger et Bouaké de *C. anguillaris* se caractérisent par des performances zootechniques presque analogues. Le taux de survie en fin de cycle varie entre 76 et 96 %. *C. anguillaris* Niger a un meilleur indice de conversion de l'aliment. Celui-ci varie entre 4,2 et 4,7 contre 5,5 et 6,6 pour *C. anguillaris* Bouaké. La souche Niger a une meilleure croissance

(1,23 g/j) qui diffère significativement de celle de la souche Bouaké (1,0 g/j) ($p < 0,05$ et $p > 0,01$). Cette différence de croissance est relativement faible et pourrait s'expliquer par l'hétérogénéité en taille et poids des populations de fingerlings en début d'élevage.

Mots clés : Aquaculture — *Clarias anguillaris* — Croissance.

Thomas M. Falk, Eddie K. Abban, Wolfgang Villwock, Lothar Renwrantz : « Hétérogénéité et composition des sous unités de l'hémoglobine de 5 espèces de tilapia (Teleostei, Cichlidae) des genres *Oreochromis* and *Sarotherodon* »

L'hémoglobine de 5 espèces de tilapia des genres *Oreochromis* et *Sarotherodon* (*O. niloticus, O. aureus, O. andersonii, S. galilaeus, S. melanotheron*) a été étudiée. Par filtration sur gel chromatographique, un poids moléculaire de 67-69 kDa a été déterminé pour les molécules tétramériques qui restent stables entre pH 5,0 et 9,1. Quand elles sont soumises à une électrophorèse en gel de polyacrylamide Urée-SDS, les hémoglobines de toutes les espèces se séparent en monomères de 3 différents poids moléculaires allant de 16,3 à 17,6 kDa. Par la suite, les hémolysats de chaque espèce sont séparés par isoélectrophorèse en 23 hémoglobines tétramériques chargées différemment qui ont donné des modèles caractéristiques de chaque espèce. Il a été montré que ces différences résultaient de la présence de différents types de chaînes de globines. Par électrophorèse sur gel de polyacrylamide en présence d'acide urique, un total de 7 α-globines et 5 β-globines a été détecté et les variants caractéristiques des espèces ont été identifiés. De plus, des types d'hémoglobine caractéristiques de sous-espèces ont été trouvés chez *O. niloticus* (*O. n. niloticus, O. n. sugutae*). Pour déterminer la composition en chaînes de globines d'un tétramère, après extraction de chacune des 26 bandes obtenues par isoélectrophorèse, chacune d'entre elle a été analysée par électrophorèse en présence d'urée. Il a été trouvé que les tétramères étaient formés de doublets de chaînes identiques α et β ($\alpha_2\beta_2$, tétramères

symétriques), ou des combinaisons de trois (α_2ßß*, $\alpha\alpha$*$ß_2$) ou quatre ($\alpha\alpha$*ßß*) chaînes distinctes (tétramères asymétriques). Enfin, les chaînes de globines de *O. niloticus* ont été partiellement séquencées à partir de leur partie N terminale. Des différences dans la composition des trois chaînes majeures ß (ß2-, ß4-, ß5) ont été observées (position 9, 12, 21, 29), alors que les chaînes sont bloquées de manière N terminale. L'homologie entre les séquences des parties N terminales des chaînes ß de *O. niloticus* étudié ici et celles des chaînes ß de différentes espèces de poissons confirme le schéma de classification que nous avons utilisé pour les tilapias.

Mots clés : Tilapia — Hémoglobine.

Germain Gourène, Guy G. Teugels : « Aperçu synthétique sur l'aquaculture et la diversité biologique des tilapias (Teleostei, Cichlidae) »

Cette étude est une revue synthétique de l'aquaculture et de la biodiversité des tilapias à travers le monde mais surtout en Afrique, leur continent d'origine. Une présentation sommaire des Cichlidae est donnée. Il en a été de même pour les trois genres de tilapia concernés par la pisciculture : *Tilapia s.s.*, *Sarotherodon* et *Oreochromis*. Une espèce du dernier genre cité, *O. niloticus*, apparaît de toute évidence comme l'espèce la plus polymorphe et la plus utilisée actuellement en pisciculture. Il se dégage également que les espèces à large distribution naturelle sont les plus impliquées dans les diverses activités de pisciculture.

Mots clés : Tilapias — Aquaculture — Biodiversité.

Sylvain Gilles, Jean-Baptiste Amon-Kothias, Jean-François Agnèse : « Comparaison des performances de croissance en eau saumâtre de trois populations de *Sarotherodon melanotheron* (Cichlidae) d'Afrique de l'Ouest »

La survie, la croissance et la maturation sexuelle ont été comparées chez trois populations morphologiquement et

génétiquement différenciées de *Sarotherodon melanotheron*: une population de Dakar au Sénégal, une population de la lagune Ebrié en Côte d'ivoire et une population de Bas Kouilou au Congo. Une première expérience a été menée pendant 176 jours dans des bacs de 4 m^3 à une densité initiale de 33 poissons au m^3. La salinité a varié de 0,6 % au début de l'expérience à 0 % à la fin. Les poissons de la population du Sénégal ont eu les vitesses de croissance les plus grandes et une maturité sexuelle plus retardée que les poissons des deux autres populations. Une deuxième expérience a été menée durant 168 jours sur les populations du Sénégal et de Côte d'Ivoire. Les poissons ont été placés dans des cages situées dans un bassin de 1 ha à une densité initiale de 31 poissons au m^3. La salinité a variée de 1,8 % au début à 3,5 % à la fin de l'expérience. Comme dans la première expérience, les poissons en provenance du Sénégal ont eu une vitesse de croissance supérieure aux poissons de Côte d'Ivoire bien que la maturation sexuelle fût identique dans les deux populations.
La population de *S. melanotheron* provenant du Sénégal est potentiellement intéressante pour l'aquaculture en milieux saumâtres.

Mots clés : *Sarotherodon melanotheron* — Tilapia — Croissance — Aquaculture — Eau saumâtre.

Thomas D. Kocher : « Les cartes génétiques chez les poissons »

Nous avons construit une carte génétique pour le tilapia *Oreochromis niloticus* en utilisant des marqueurs ADN. La ségrégation de 62 locus microsatellites et de 112 AFLP (total = 174) a été étudiée dans 41 embryons haploïdes provenant d'une seule femelle.
Nous avons identifié des liaisons entre 162 (93,1 %) de ces marqueurs. Au total, 95 % des microsatellites et 92 % des AFLP sont liés sur la carte finale. La carte s'étend sur 704 cM en 30 groupes de liaison couvrant les 22 chromosomes de l'espèce. Vingt-quatre de ces groupes de

liaison contiennent au moins un locus microsatellite. A partir de ces marqueurs qui sont à 15 cM, ou moins, les uns des autres, la longueur totale de la carte est approximativement de 100 à 1 200 cM. Les données suggèrent que les marqueurs microsatellites sont légèrement regroupés dans le génome.
Cette carte est le point de départ pour la cartographie de gènes liés à des caractères quantitatifs chez les Cichlidae.

Mots clés : Carte génétique — *Oreochromis niloticus* — Cichlidae — Microsatellite — AFLP — Aquaculture.

Giorgos Kotoulas, Jean-François Agnèse, Elefterie Zouros : « Variation des microsatellites chez *Chrysichthys nigrodigitatus* (Lacepède,1803) (Siluroidei, Claroteidae) »

La variation à trois locus microsatellites a été examinée dans trois populations naturelles de *Chrysichthys nigrodigitatus* : le Sénégal, le barrage de Sélingué (sur le fleuve Niger au Mali), la lagune Ebrié en Côte d'Ivoire et le lac Volta. une population domestique en provenance de la Sial (Société ivoirienne d'aquaculture lagunaire) dans la lagune Ebrié a été également examinée.
Le nombre d'allèles observés dans chacun des trois locus a été important (de 29 à 30) et le nombre moyen d'allèles par population a varié de 6,67 à 22,0. Les valeurs les plus faibles d'hétérozygoties ont été observées dans le barrage de Sélingué et dans la population domestique, alors que les trois autres populations naturelles ont des taux d'hétérozygotie supérieurs et comparables. Selon les locus, de 17 à 60 % des allèles sont privés. Si l'on considère que les allèles microsatellites sont neutres et que les appariement se font au hasard (panmixie), on peut alors comparer les tailles des populations efficaces. Les cinq populations peuvent être rangées en trois catégories : la population du barrage de Sélingué et celle de la Sial ont les tailles efficaces les plus faibles puis vient celle du lac Volta et enfin celles du Sénégal et de la lagune Ebrié. La plupart des populations sont à l'équilibre de Hardy-Weinberg à l'exception de celle du Sénégal où on observe une tendance

à l'excès d'homozygotes. Une analyse des correspondances basée sur ces trois locus permet efficacement d'assigner un individu à sa population.
Ces résultats montrent que les microsatellites sont un outil de choix pour le suivi, la préservation de la diversité génétique et les programmes d'élevage aquacole de cette espèce.

Mots clés : *Chrysichthys nigrodigitatus* — Microsatellites — Aquaculture.

Sébastien Lavoué, Jean-François Agnèse : « L'utilisation de l'ADN ancien pour les études de biodiversité des poissons: l'exemple des Mormyridae »

Les muséums d'histoire naturelle contiennent des millions de spécimens biologiques préservés. Ce travail fait le point sur l'état d'avancement des techniques qui permettent d'utiliser ces spécimens dans des études de génétique.
Après avoir passé en revue les avantages et les problèmes de l'exploitation de l'ADN issu des tissus fixés et en particulier ceux fixés grâce au formaldéhyde, nous présentons les premiers résultats obtenus à partir de Téléostéens de la famille des Mormyridae (Pisces, Osteoglossomorpha). Un fragment de 495 pb du cytochrome *b* a été amplifié et séquencé chez deux spécimens de deux espèces, *Myomyrus pharao* Poll et Taverne, 1967 et *Genyomyrus donnyi* Boulenger, conservés au Musée royal d'Afrique centrale de Tervuren (Belgique) depuis dix-sept ans. Leur position phylogénétique est discutée.

Mots clés : ADN ancien — Formaldéhyde — Poisson — Mormyridae — Biodiversité.

Antonios Magoulas, Giorgos Kotoulas, Kostas Batargias, Elefterios Zouros : « Les marqueurs génétiques en biologie marine et en aquaculture: quand utiliser quoi ? »

Cette communication montre une évaluation comparative des différentes techniques largement utilisées en génétique des populations. Dans un premier temps, nous dressons la

liste des différents types de marqueurs en même temps que nous considérons leurs avantages et désavantages respectifs. Ensuite, nous présentons des exemples de leur utilisation à partir des nos travaux : polymorphisme de réaction antigène anticorps, allozymes, marqueurs nucléaires anonymes, polymorphisme d'amplification aléatoire, minisatellites, microsatellites, variation de l'ADN mitochondrial, RFLP, SSCP, séquençage. Le choix du marqueur dépendra de la question particulière à laquelle on veut répondre : hétérozygotie et sélection *versus* dérive génétique dans les populations naturelles, marqueurs génétiques pour les recherches en aquaculture. Des exemples de nos propres travaux qui illustrent ces points sont présentés.

Mots clés : Aquaculture — Biologie marine — Allozyme — ADNmt — Microsatellite.

Ziriga J. Otémé : « Sensibilité à la consanguinité et gestion des gamètes par cryoconservation chez *Heterobranchus longifilis* Valenciennes, 1840 »

L'aptitude des ovules à être fécondés, le taux de survie et la croissance larvaire ainsi que la variabilité génétique à 23 locus, ont été analysés dans deux populations représentants les générations F1 et F4 du siluriforme africain *Heterobranchus longifilis* Valenciennes, 1840.

Une perte de variabilité génétique a été observée dans la génération F4 par rapport à la génération F1 et dans la génération F1 par rapport à la population sauvage. Ainsi, pour 23 loci étudiés, on observe un seul locus polymorphe avec deux allèles pour la génération F4, deux locus polymorphes avec deux allèles pour la génération F1, alors que la population sauvage est caractérisée par deux locus polymorphes dont l'un est représenté par trois allèles. La souche F1 est caractérisée par un plus faible taux de larves déformées et une plus forte survie au quatorzième jour que la souche F4. Cette différence dans les taux de survie n'a pas permis d'interpréter les différences observées pour les vitesses de croissance.

Ces résultats montrent que *H. longifilis* est une espèce pour laquelle la perte de variabilité génétique entraîne rapidement des baisses de performances sensibles. L'intérêt de la cryoconservation des gamètes comme solution palliative à cette perte de variabilité génétique ou comme moyen de la restaurer à travers une banque de gènes est discuté. Une technique originale de cryoconservation du sperme de *H. longifilis* est présentée.

Mots clés : *Heterobranchus longifilis* — Consanguinité — Cryopréservation — Allozyme.

Maria Lourdes D. Palomares, Christine Marie V. Casal : « Une approche utilisant une base de donnée pour illustrer les tendances génétiques chez les poissons »

Des outils graphiques illustrant les relations entre les variables génétiques ont été appliqués aux données de FishBase, une encyclopédie électronique qui contient des informations biologiques sur les poissons.

Un graphique montrant les valeurs de polymorphisme enzymatique en fonction de l'hétérozygotie attendue pour plus de 800 populations met en évidence une relation de type linéaire. Ce graphique a été utilisé pour identifier les déviations observées à ce modèle linéaire dans certaines études et constitue donc un moyen pour identifier les populations aux caractéristiques particulières.

D'autres figures ont été faites en utilisant la quantité d'ADN par noyau (sur plus de 350 espèces) ou le nombre de chromosomes (sur plus de 1 300 espèces) en fonction de l'ordre phylogénétique basée sur la classification taxinomique publiée par J. Nelson (1994). On observe des tendances à la baisse de la quantité d'ADN par noyau ainsi que du nombre de chromosomes, malgré une grande variation, au sein de l'ordre. Les raisons possibles pour de telles tendances sont discutées.

Mots clés : Fishbase — Base de données — Aquaculture — Génétique — Poisson.

Roger S.V. Pullin : « Les ressources génétiques pour l'aquaculture : propriété et accès »

Les arrangements concernant l'accès et la propriété des ressources génétiques aquatiques sont bien moins développés que ceux concernant les ressources génétiques pour l'agriculture, en particulier en ce qui concerne les plantes. Quoi qu'il en soit, des définitions de propriété et d'accès plus régulés aux ressources génétiques aquatiques sont prévisibles suivant la tendance observée pour les plantes et le bétail. La plupart des pays ont nationalisé leurs ressources génétiques à travers la convention sur la diversité biologique, et les tentatives pour privatiser les ressources génétiques vont croissant à travers la cession de brevets et autres mécanismes de propriété intellectuelle ainsi qu'à travers les droits des fermiers, éleveurs et populations indigènes. Ces développements sont revus avec des références aux recherches et développements en aquaculture incluant les approches prises par l'Iclarm. Les possibilités futures de propriété et d'accès aux ressources génétiques sont discutées.

Mots clés : Ressources génétiques — Poisson — Aquaculture.

Benedict P. Satia, Devin M. Bartley : « Le paradoxe des introductions internationales d'organismes aquatiques en Afrique »

Environ 430 introductions ou transferts d'espèces aquatiques ont été faits dans 42 pays d'Afrique depuis les 150 dernières années. Les introductions ont été réalisées en majorité entre les années 1920 et les années 1970. Ostensiblement, elles ont été considérées comme un moyen efficace d'accroître la production de protéine, de générer des revenus et de produire des emplois, aussi bien que de contrôler des vecteurs de maladie et les mauvaises herbes. Plusieurs introductions ont également été réalisées pour des motifs plus triviaux tels que la nostalgie des personnes déplacées d'avoir autour d'elles une faune familière, spécialement pour la pêche récréative. Certaines introductions d'espèces ont été couronnées de succès et ont

permis de créer des pêcheries. dans beaucoup de cas, les objectifs n'ont pas pu être atteinds ou alors aucune évaluation précise n'a été effectuée. Il y a eu aussi des effets négatifs. Les introductions ont eu des impacts significatifs et sérieux sur les prises de pêche et l'écosystème aussi bien que sur les communautés humaines qui pêchent, élèvent ou vendent les ressources aquatiques. Certains de ces changements étaient inattendus. L'évaluation des introductions d'espèces aquatiques en Afrique devrait être analysée suivant un large spectre qui incluerait des paramètres écologiques aussi bien que socio-économiques, comme le montre l'introduction du Clupeidae pélagique *Limnothrissa miodon* dans les lacs Kivu et Kariba et sa diffusion accidentelle en aval dans le lac Cahora Bassa, ou l'introduction de la perche du Nil *Lates niloticus* dans le lac Victoria. Dans l'un et l'autre cas, le résultat est une pêcherie florissante mais avec d'importants effets socio-économiques. Un autre paradoxe est le Genetic improvement farmed tilapia (GIFT), dont le stock parental a été introduit aux Philippines depuis l'Afrique et qui est développé comme un « super tilapia » par les chercheurs de l'Iclarm. Pendant que cela se réalise, l'Afrique est toujours en train de chercher la meilleure espèce de poisson pour l'aquaculture. Les impacts des introductions d'espèces de poisson sont habituellement difficiles à inverser une fois qu'une espèce a réussi à s'établir et tout aussi difficiles à prévoir précisément. Dans beaucoup de régions du monde, des règles ont été édictées, des protocoles de biosécurité et des codes de procédure sur l'usage responsable des espèces exotiques ont été ou sont en train d'être élaborés, et des approches prudentes concernant l'introduction d'espèces sont poursuivies. En Afrique cependant, un certain laisser faire au sujet des introductions existe toujours et il y a peu, s'il y en a, de mesures de quarantaine efficaces.

Mots clés : Poisson — Introduction — Afrique.

Guy G. Teugels : « Variations morphométriques intra- et interspecifiques chez *Clarias gariepinus* et *C. anguillaris* (Siluroidei, Clariidae) »

Huit populations de *Clarias gariepinus* et six populations de *C. anguillaris*, provenant de l'ensemble de leurs aires de répartition, ont été examinées morphométriquement. Les résultats obtenus confirment que le nombre de branchiospines sur le premier arc branchial est le seul véritable caractère qui permet d'identifier les deux espèces. Chez *C. anguillaris*, une variation intraspécifique limitée a été observée. Chez *C. gariepinus* cependant, les populations d'Afrique de l'Ouest et du Nil sont morphologiquement plus proches entre elles que de celles du lac victoria et d'Afrique du Sud. Différents modèles de colonisation en relation avec les anciennes connexions hydrographiques sont utilisés pour expliquer cette variation intraspécifique.

Mots clés : *Clarias* — Morphométrie — Biogeographie — Afrique.

Guy G. Teugels, Emmanuel J. Vreven : « Résultats préliminaires sur la différenciation morphométrique entre les populations naturelles du tilapia du Nil *Oreochromis niloticus* (Perciformes, Cichlidae) »

Dix-sept populations appartenant à six sous-espèces du tilapia du Nil, *Oreochromis niloticus*, ont été morphométriquement étudiées (vingt-cinq mesures et huit comptages méristiques).

Bien que les résultats obtenus soient encore préliminaires, puisque toutes les sous-espèces connues n'ont pas été examinées et que, pour certaines sous-espèces, très peu de spécimens ont pu être analysés, ils indiquent que la classification sous-spécifique de *O. niloticus* établie par Trewavas (1983) peut être remise en question. Les populations du Nil semblent plus proches des populations de l'Afrique de l'Est que de celles de l'Afrique de l'Ouest. Trewavas (1983) a considéré que les populations d'Afrique de l'Ouest et du Nil appartenaient à la même sous-espèce *O. niloticus niloticus*. A l'intérieur des populations

d'Afrique de l'Est, un degré important de polymorphisme a été observé dans les populations formant la sous-espèce *O. n. cancellatus*. Cependant, nous n'avons pas trouvé d'arguments en faveur des conclusions de Seyoum et Kornfield (1992) qui ont considéré que cette sous-espèce était en fait une nouvelle espèce avec deux sous-espèces.

Mots clés : *Oreochromis niloticus* — Sous-espèce — Taxinomie — Morphologie.

Guy G. Teugels, Germain Gourène : « Bidodiversité et aquaculture des poissons chat africains (Teleostei; Siluroidei): une synthèse »

En Afrique, on connaît 10 familles de poissons chat en incluant les Ariidae et les Plotosidae qui sont marins. Au total elles représentent 58 genres et 465 espèces.
La famille des Mochokidae est la plus spécieuse avec 38 % des espèces.
La famille des Clariidae est sans aucun doute la plus importante économiquement : *Clarias gariepinus* est l'espèce de poisson chat la plus élevée sur le continent africain, et d'autres espèces de Clariidae telle que *Heterobranchus longifilis* sont très prometteuses pour l'aquaculture.
La famille des Claroteidae avec *Chrysichthys nigrodigitatus* est une autre famille actuellement utilisée en aquaculture en Afrique de l'Ouest.
Bien que d'autres famille comme les Schilbeidae et les Bagridae soient importantes dans les pêcheries locales, aucune d'entre elles n'est actuellement utilisée en aquaculture.

Mots clés : Poisson chat — Aquaculture — Biodiversité — Afrique.

Filip Volckaert, Frans Ollevier : « Les poissons transgéniques. Le futur des poissons avec de nouveaux gènes »

Les poissons transgéniques ouvrent des perspectives intéressantes pour les études sur la biologie du développement et l'expression *in vivo* des gènes ; ils sont

utiles comme modèles dans les recherches biomédicales et ils rendent possible l'augmentation de la production aquacole par sélection en manipulant des caractères commercialement importants.

Cette revue s'attache aux buts, aux procédures et aux conséquences environnementales de la fabrication de poissons transgéniques. En général, le matériel génétique provient d'une autre espèce de poisson. Il est constitué d'une séquence promoteur qui contrôle l'expression, d'un transgène qui code pour le caractère désiré, d'un élément régulateur distant, et d'un vecteur plasmidien ou viral. Les transgènes peuvent être subdivisés selon la nature du caractère pour lequel ils codent: gain d'une nouvelle fonction, protéine de régulation, perte de fonction. La méthode de transfert pour une intégration stable dans le génome est concentrée sur le zygote ou le jeune embryon; l'utilisation de massifs cellulaires est également envisagée. Habituellement, on procède par approximation successive pour insérer correctement le gène dans le génome parce que les méthodes de recombinaison homologue ne sont pas encore utilisables. La micro-injection est la principale méthode pour administrer le nouvel ADN. Les implications pour l'aquaculture sont principalement l'augmentation de la croissance, de la résistance au froid et aux maladies.

La biosécurité et les problèmes environnementaux en relation avec les poissons transgéniques devraient être convenablement abordés avant chaque dissémination dans l'environnement naturel.

Les recherches futures porteront très probablement sur la qualité de la construction des transgènes (éléments régulateurs et vecteurs), la méthodologie d'insertion ciblée, la caractérisation biologique des nouveaux caractères, les problèmes environnementaux et les aspects socio-économiques.

Mots clés : Aquaculture — Transgénique — Régulation du gène — Biosécurité — Poisson.

Emmanuel J. Vreven, Béatrice Adépo-Gourène, Jean-François Agnèse, Guy G. Teugels : « Variation morphométrique et allozymique entre les populations naturelles et les populations d'élevage de *Orechromis niloticus niloticus* (Teleostei, Cichlidae) »

Les variations morphométriques et allozymiques de neuf populations naturelles de *Oreochromis niloticus* et de trois populations d'élevage ont été étudiées. Des différences dans la morphologie et les allozymes ont été observées entre les populations naturelles d'Afrique de l'Ouest et du Nil. Bien que toutes ces populations soient rangées dans la sous-espèce *O. n. niloticus*, la population du Nil est plus proche de la population du lac Edward qui appartient à la sous-espèce *O. n. eduardianus*. Des différences morphologiques ont été observées entre les populations naturelles et leurs populations d'élevage. Ces différences sont sans doute écophénotypiques car les populations d'élevage sont génétiquement proches de leurs populations parentales naturelles.

Mots clés : *Oreochromis niloticus* — Morphométrie — Allozyme — Aquaculture.

Abstracts

Béatrice Adépo-Gourène, Marc M. Hanssens, Guy G. Teugels, Jean-François Agnèse: "Morphologic and genetic differentiation of natural populations of *Chrysichthys nigrodigitatus* (Lacepède,1803) (Siluroidei, Claroteidae)"

The analysis of morphologic and genetic differentiation were used on eleven populations of *Chrysichthys*. These populations are morphologically and genetically differentiated. This differentiation is geographically ordained. The populations the most morphologically differentiated are the two which are at the limits of the distribution range. They have the lowest polymorphism rates and are both monomorphic (Dagana, Bas Kouilou). The Côte d'Ivoire populations are the most genetically variable. The description of the genetic variability of *C.. nigrodigitatus* constitutes a reference which can be used to follow strains used in aquaculture. It opens new avenues of research on zoo-technical performances of the most polymorphic populations. Crosses between different populations could also be carried out in order to achieve a heterosis effect or to obtain synthetic strains having higher genetic variabilities.

Key words : *Chrysichthys nigrodigitatus* — Aquaculture — Morphometry — Allozyme.

Béatrice. Adépo-Gourène, Laurent Pouyaud, Guy G. Teugels, Marc M. Hanssens, Jean-François Agnèse : "Morphological and genetic differentiation of west african populations of *Sarotherodon melanotheron* Ruppel, 1852 (Teleostei, Cichlidae)"

Twenty-nine samples representing several West African populations of *Sarotherodon melanotheron* were the subjects of genetic and morphological studies. The morphological and genetic differentiations observed agree with and allow the separation of the samples into three groups corresponding to three of the subspecies described

by Trewavas in 1983. The high genetic diversity observed in the samples may be accompanied by equally high zoo-technical performances. This study opens up new lines of research into the zoo-technical characteristics of *S. melanotheron* populations with the aim of selecting the best aquacultural strain.

Key words : *Sarotherodon melanotheron* — Allozyme — Tilapia — Morphometry— Aquaculture.

Jean-François Agnèse: "Fish introductions in African freshwaters: first data on their genetic impacts"

The introduction in natural environment of any species can have genetic impacts. These can be defined not only as alterations in the gene pool of native species but also as alterations in the introduced populations themselves. After having established the list of possible alterations, their causes and their consequences, the introduced species in Africa for which there are available data on the genetic impact were in reviewed : the hybrids between *Oreochromis niloticus* and *O. mossambicus* in Itasy Lake in Madagascar; these same hybrids in Lake Ihema in Rwanda; the case of Lake Victoria with *O. niloticus*, *O. variabilis* and *O. esculentus*; that of the Bouaké strain of *O. niloticus* in the waters of Côte d'Ivoire, and finally the case of *Limnothrissa miodon*, the Clupeidae originally from Lake Tanganyika and introduced into Lake Kivu.

Key words : Introduction — Introgression — Hybridization — Fish — Africa.

Jean-François Agnèse, Béatrice Adépo-Gourène, Laurent Pouyaud : "Natural hybridization in tilapias"

Tilapias form a group of species which is well known for its abilities to hybridize. The main cases of hybridization in the wild have been cited and analyzed. These wild hybrids are arranged in three categories: those that come from species transfert, those that occur after manmade changes in the environment and finally those that are truly natural.

The majority of the natural hybridization cases reported belong in the first category. In Lake Naivasha in Kenya where *Oreochromis spilurus nigra* and *O. leucostictus* were introduced in the 1950's, in Lake Bunyoni in Uganda where *O. niloticus* was introduced in 1927 from Lake Edward and *O. spilurus nigra* in 1932 from Lake Naivasha; in Lake Itasy, in Madagascar, where *O. macrochir* was introduced in 1958 and *O. niloticus* in 1961; in Lake Ihema in Rwanda, where *O. macrochir* was introduced near the end of the 1960's, after the introduction of *O. niloticus* near the end of the 1940's; finally, in Lake Victoria where *O. niloticus* was introduced in the 1960's and where *O. variabilis* and *O. esculentus* have disappeared. Hybridization among *Tilapia zillii* and *T. guineensis* in Côte d'Ivoire is an example of hybridization following manmade perturbations in the environment (damming). The last hybridization case presented occurs below the Koroboué Falls on the Comoé River. It has happened between three species: *T. zillii*, *T. guineensis* and *T. dageti*. It is the only case of hybridization where the hand of man is not suspected of involvement.

Key words : Hybrid — Tilapia.

Jean-François Agnèse, Béatrice Adépo-Gourène, Laurent Pouyaud : "Genetic differentiation and subspecific status on natural populations of *Oreochromis niloticus* (Teleostei, Cichlidae)"

We analysed the genetic differentiation among seventeen natural populations of the Nile Tilapia *Oreochromis niloticus* (Linnaeus, 1758) using allozymes and Restriction Fragment Length polymorphism (RFLP) of mitochondrial DNA (mtDNA) and microsatellites. Populations studied, from river Senegal to Lake Tana and from Lake Manzalla to Lake Baringo, represent all subspecies which have been previously described. Sixteen variable nuclear loci showed that these populations can be clustered in three groups : 1, West African populations (Senegal, Niger, Volta and Chad

drainages), 2, Ethiopian Rift Valley populations (Lake Awasa, Ziway, Koka and Awash river) and 3, Nile drainage (Manzalla, Cairo, Lake Edward) and Kenyan Rift Valley populations (Lake Turkana, Baringo and River Suguta). Nine different mtDNA haplotypes were found in the RFLP analysis of a 1 Kb portion of the DLoop region. The network obtained showed that there were three geographically distinct groups, all West African populations and *O. aureus* are clustered the two Ethiopian Rift Valley populations are distinct and between these two groups are the Kenyan and Ugandan Rift Valley populations. Nile populations show affinities both with West African populations and with specimens from Lake Tana and Turkana. Microsatellites study give congruent results. Taxonomic and implications of these results are discussed.

Key words : *Oreochromis niloticus* — Allozyme — mtDNA — Taxonomy — Microsatellite.

Aggrey J.D. Ambali, Roger W. Doyle : "Genetic diversity analysis of *Oreochromis shiranus* species in reservoirs in Malawi"

Studies were carried out to assess the genetic diversity of reservoir populations of *Oreochromis shiranus* species in Malawi. It was observed that reservoirs harbored several species including *O. shiranus sp*, *Tilapia rendalli*, *Clarias gariepinus*, *Serranochromis robustus*, *Barbus sp* and haplochromids. *O. shiranus* was found in all the reservoirs where the species was stocked between 1955 and 1968. Carnivorous species like *C. gariepinus* and *S. robustus* were found in some of the reservoirs; the former was stocked naturally through streams while the latter was artificially stocked to control recruitment of tilapias. Mean standard length of *O. shiranus sp* in the reservoirs ranged from 13.6 to 20.5 cm which was higher than the average size in wild populations. The general trend observed was that in those reservoirs where predator/prey was high,

the size range of tilapia was larger than where the ratio was low.

Microsatellite DNA probes were used to study allelic diversity of two subspecies of *O. shiranus*: *O. shiranus shiranus* and *O. shiranus chilwae*. Mean number of alleles ranged from 3.2 ± 0.37 to 10.2 ± 2.15. Allelic diversity was low in reservoirs with high predator/prey ratios. Cavalli-Sforza and Edwards (1967) chord distance between reservoir populations ranged from 0.024 to 0.204. Mantel's normalized correlation coefficient between genetic distance and geographic distance was low (0.265) and not significant (p=0.8516). The microsatellite data showed that genetic hybridization occurred between *O. sh. shiranus* and *O. sh. chilwae* in most of the reservoirs in the country.

Key words : *Oreochromis shiranus* — Microsatellite — Aquaculture.

Olga Assémien : "Zoo-technical characterization of four strains of *Oreochromis niloticus* "

Growth performances of four strains of *Oreochromis niloticus*, a domestic strain, Bouaké (BKE) and three natural strains, Ghana (GHA), Niger (NIG) and Senegal (SEN), have been compared during a five month breeding period. Data analysis show that BKE (2.4g/j±0.2) and SEN (2.3g/j±0.1) strains present the best growth. GHA strain (1.8g/j±0.0) has the smallest growth rate. NIG strain (2.0g/j±0.1), which initial average body weight was inferior to those of the three others, seemed, afterwards, to be more performing than GHA strain.

Key words : *Oreochromis niloticus* — Growth rate.

Herman van der Bank : "Allozyme comparisons fish used in aquaculture in South Africa"

The two economically most important freshwater fish species used in aquaculture in South Africa are rainbow trout, *Oncorhynchus mykiss* abd African sharptooth catfish, *Clarias gariepinus*. Rainbow trout is an alien

species, first imported for angling purposes from England in 1897. This species can be regarded as a success from angling an d aquaculture points of view, but it had a detrimental effect on a number of indigenous species. rainbow trout from South Africa are exported to the nothern hemisphere during their summer month (local winter), and proved to perform exceptionally well. Local strains vary in growth and survival performance due to the mixing of imorted stocks from various countries (as a result of sanctions), and genetypic variation relates to growth performance.
Distinct differences in allozyme variation between wild and domesticated populations of African catfish were found, which relate to differences in management strategies. This is of conservational importance since most new catfish farmers purchase breeding stock from existing farmers (hundreds of kilometres from them), and because these fish differ genetically from local stocks. Phenotypic variation is affected by allozyme polymorphism; an association was found between genetic traits and growth performance in African catfish and selection of semen possessing specific allele combinations were affected by the cryodiluents and freezing rates used. Selection of breeding stocks for aquaculture practices and the use of cryopreservation in aquaculture and/or for conservation based on the above information are discussed.

Key words : *Oncorhynchus mykiss* — *Clarias gariepinus* — Cryopreservation — Aquaculture.

Sébastino K. Da Costa : "Comparison of growth performances of niger and bouaké strains of *Clarias anguillaris* Linné, 1758"

The growth of Niger and Bouaké strain of *Clarias anguillaris* (Linné, 1758) is compared. Sex-mixed populations (1:1) of each strain have been cultured during 8 months and fed with M2GE containing 45 % protein, with a daily ration equivalent to 3 % of their biomass. One serie of 15 m^3 tanks each one, with a 11.2 m^3 of water, have been used for the experiment. Three tanks were used

for each strain. Each structure contained 30 fingerlings with mean weight range from 51.08 to 54.37 g for the Niger strain, and from 55.12 to 55.48 g for the Bouaké strain. Water temperature varied from 24 to 28.8°C. Bouaké and Niger strain of *C. anguillaris* have close zootechnic performances. The survival rate at the end of experiment varied from 76 to 96 %. *C. anguillaris* Niger has a better feed conversion varying from 4.2 to 4.7, against 5.5 to 6.6 for *C. anguillaris* Bouaké. Moreover, the Niger strain has a better growth (1.23 g/j) which is statistically different from that of the Bouaké strain (1.0 g/j) ($p < 0.05$ and $p > 0.01$). This difference is relatively low and might be explained by the heterogenity in lenght and weight of fingerlings populations used at the start of the culture.

Key words : Aquaculture — *Clarias anguillaris* — Growth.

Thomas M. Falk, Eddie K. Abban, Wolfgang Villwock, Lothar Renwrantz : "Heterogeneity and subunit composition of the hemoglobins of 5 tilapiine species (Teleostei, Cichlidae) of the genera *Oreochromis* and *Sarotherodon*"

Hemoglobins of 5 tilapiine species of the genera *Oreochromis* and *Sarotherodon* (*O. niloticus*, *O. aureus*, *O. andersonii*, *S. galilaeus*, *S. melanotheron*) were investigated. By gel filtration chromatography a molecular weight of 67-69 kDa was determined for the tetrameric molecules which remained stable between pH 5.0 and 9.1. When subjected to SDS-Urea-PAGE hemoglobins of all species each were split into monomers of 3 different molecular weights ranging between 16.3 and 17.6 kDa. Subsequently performed isoelectric focusing separated hemolysates into about 23 differently charged tetrameric hemoglobins per species which were arranged in species characteristic patterns. This diversity was shown to result from the occurrence of different types of globin chains. By acidic urea PAGE a total of 7 major α-globins and 5 major ß-globins were detected and species characteristic chain variants were identified. In addition, subspecies

characteristic hemoglobin types were found to exist in *O. niloticus* (*O.n. niloticus, O.n. sugutae*).
To determine the globin chain composition of particular hemoglobin tetramers, after isoelectric focusing of hemolysates 26 bands each were extracted and subsequently analyzed by acidic urea PAGE. Tetramers were found to consisted of doublets of identical α- and ß-chains ($a_2ß_2$, symmetric tetramers), or combinations of three ($\alpha_2ßß^*$; $\alpha\alpha^*ß_2$) or four ($\alpha\alpha^*ßß^*$) distinct chains (asymmetric tetramers). Finally, globin chains of *O. niloticus* were subjected to partial N-terminal amino acid sequencing (pos. 1-40). Differences in the composition of the three major ß-chains (ß2-, ß4-, ß5-chain) could be shown (pos. 9, 12, 21, 29), whereas the α-chains were N-terminally blocked. Homology comparisons between the N-terminal ß-chain sequences of *O. niloticus* studied here and known ß-chains of different fish species supported our classification scheme for the tilapias used.

Key words : Tilapia — Hemoglobin.

Sylvain Gilles, Jean-Baptiste Amon-Kothias, Jean-François Agnèse : "Comparison of brackish water growth performances of *Sarotherodon melanotheron* (Cichlidae) from three West African populations"

Survival, growth and sexual maturation were compared for *Sarotherodon melanotheron* from three morphologically and genetically distinct populations: a population from Dakar in Senegal, a population from Ebrié Lagoon in Côte d'Ivoire and a population from Bas Kouilou in Congo.
A first experiment (176 days) was carried out in concrete tanks (4 m^3), at an initial density of 33 fish/m^3. Salinity ranged from 6 at the beginning of the experiment to 0 at the end. Fish of the Senegalese population were characterized by higher growth rates and later sexual maturation than those of the other two populations.
A second experiment (168 days) was carried out on populations from Senegal and Côte d'Ivoire, at an initial density of 31 fish/m^3, in cages placed in a 1 ha pond.

Salinity ranged from 18 at the start of the experiment to 35. As in the first experiment, fish of Senegalese population had higher growth rates than those of the Côte d'Ivoire population, but sexual maturation was similar en both..
S. melanotheron population from Senegal may have potential for brackish water aquaculture.

Key words : Tilapia — Brackish water — Aquaculture — *Sarotherodon melanotheron* — Growth.

Germain Gourène, Guy G. Teugels : "Overview on biodiversity and aquaculture of tilapias (Teleostei, Cichlidae)"

This paper presents a brief overview of the aquaculture and biodiversity of tilapias in the World and in particular in Africa, their continent of origin. A brief account on the Cichlidae is given. The three tilapiine genera used in aquaculture, *Tilapia s.s.*, *Sarotherodon* and *Oreochromis* are briefly discussed. One species of *Oreochromis*, *O. niloticus*, appears to be the most polymorphic and is presently also the most cultured tilapia. It is also noted that only those species with a large geographical distribution have been retained for aquaculture purposes.

Key words : Tilapias — Aquaculture — Biodiversity.

Giorgos Kotoulas, Jean-François Agnèse, Eleftherios Zouros : "Microsatellite variation in the african catfish *Chrysichthys nigrodigitatus* (Lacepède, 1803) (Siluroidei, Claroteidae)"

Variation at three microsatellite loci was examined in four natural populations of the West african catfish *Chrysichthys nigrodigitatus*: Senegal, Selingue Dam (river Niger in Mali), Ebrie lagoon (Ivory Coast) and Volta Lake. One farmed population, Sial (Société ivoirienne d'aquaculture lagunaire) in Ebrie Lagoon was also examined.
The number of alleles was high in all three loci (29 to 30) and the number of the average number of alleles per population varied from 22.0 to 6.67. Smaller heterozygosities were observed in Selingue dam and Sial

farm, whereas the three natural populations had higher and comparable heterozygosities. Between 17% to 60% of alleles were "private".

On the basis of neutrality and random mating, one can compare effective population sizes. The five populations appear to fall into three categories in this respect: Niger Dam and Sial Farm are the smaller, followed by Volta, followed by Senegal and Ebrie. Most populations are in Hardy-Weinberg equilibrium. An exception is the sample from Senegal river, at Dagana, where a trend for excess of homozygotes is present in all loci, with significant values for two out of three loci.

Based on these three loci a factorial analysis of correspondence of all specimen studied provides a good assignment of individuals to populations.

These results show that microsatellite is the tool of choice for population monitoring, preservation of genetic diversity and breeding programs in aquaculture.

Key words : *Chrysichthys nigrodigitatus* — Microsatellite — Aquaculture.

Thomas D. Kocher : "Genetic mapping in Fishes"

We have constructed a genetic map for tilapia (*Oreochromis niloticus*) using DNA markers. The segregation of 62 microsatellite and 112 AFLP (total = 174) polymorphisms was studied in 41 haploid embryos derived from a single female. We have identified linkages among 162 (93.1%) of these markers. 95% of the microsatellites and 92% of the AFLP polymorphisms were linked in the final map. The map spans 704 Kosambi centimorgans in 30 linkage groups covering the 22 chromosomes of this species. Twenty-four of these linkage groups contain at least one microsatellite polymorphism. From the number of markers 15 or fewer cM apart we estimate a total map length of approximately 1,000 to 1,200 cM. The data suggest that the microsatellite markers are slightly clustered in the genome. This map is a starting

point for the mapping of quantitative traits in cichlid fishes.

Key words : Genedtic map — *Oreochromis niloticus* — Cichlid — Microsatellite — AFLP — Aquaculture.

Sébastien Lavoué, Jean-François Agnèse : "The utilization of ancient DNA to assess fish biodiversity: example of Mormyridae "

The zoological and botanical collections of the world's Natural History Museums by their richness are supports for important systematic studies. Only recently has the molecular exploitation of these collections been envisaged, giving them a new dimension. The *in vitro* chained amplification technique or allows the study of a DNA sequence from a very small quantity of genetic material. The strength of this technique suggests the ability to study rare and/or damaged DNA like that found in fossils or of tissues preserved in museums. After having reviewed the advantages and difficulties of utilizing the DNA of collection specimens during the comparative biology study, and more particularly of those fixed in formaldehyde, we present our results obtained from specimens of the Mormyridae family preserved at the Musée royal d'Afrique centrale (Mrac) in Tervuren and at the Muséum national d'histoire naturelle (MNHN) in Paris..

Key words : Ancient DNA — Biodiversity — Formaldéhyde — Fish — Mormyridae.

Antonio Magoulas, Giorgos Kotoulas, Costas Batargias, Elefterios Zouros : "Genetic markers in marine biology and aquaculture research, when to use what"

This communication provide a comparative evaluation of the various techniques now widely used in population genetics. We first list the various types of markers together with what we consider to be their advantages and disadvantages and then present examples from our own work in a way of illustration : Antibody-antigen reaction polymorphism, Allozymes, Anonymous nuclear DNA

markers, Randomly amplified polymorphic DNA (RAPD), Minisatellites, Microsatellites, Mitochondrial DNA (mtDNA) variation, RFLP, SSCP, Sequencing. Choice of the marker will depend on the particular question one wants to ask : Heterozygosity and fitness Selection *versus* random drift in natural populations, genetic markers in aquaculture research. Examples from our own work which illustrate these points are presented.

Key words : Aquaculture — Genetics — mtDNA — Allozyme — Microsatellite — RAPD — Minisatellite — SSCP.

Ziriga Josué Otémé : "Sensitivity to inbreeding and sperm cryopreservation in the catfish *Heterobranchus longifilis* Valenciennes, 1840"

Fertility, survival rate, larval growth, as well as genetic variability at 23 loci, were analyzed in two populations representing the first and the fourth generations of the African siluriform *Heterobranchus longifilis* Valenciennes 1840. A loss ofgenetic variability was observed in the fourth generation as compared to the first generation, and in the first generation as compared to the wild population. Therefore, for 23 loci studied, only one single polymorphic locus with two alleles was observed for the fourth generation, two polymorphic loci with two alleles for the first generation. The first generation strain is characterized by a lower rate of deformed fry and a greater survival to the 14th day compared to those of the fourth generation strain. The difference in the survival did not allow clear interpretation of the differences observed in growth rates. The results show that *H. longifilis* is a species for which loss of genetic variability rapidly leads to significant decreases in performance. Interest of gamete cryopreservation as a palliative solution to the loss of genetic variability or as a means of restoring it through a gene bank is discussed. An original technique of cryopreservation of *H. longifilis* sperm is presented

Key words : *Heterobranchus longifilis* — Inbreeding — Cryopreservation — Allozyme.

Maria L. D. Palomares, Christine M. V. Casal : "A database approach to illustrate genetic trends in fishes"

Graphical tools illustrating known relationships between genetic variables were applied to data in FishBase, an electronic encyclopedia of biological information on finfish. A plot of polymorphism against expected heterozygosity, for over 800 populations, showed a direct linear relationship. This plot was used to identify the deviations of some studies from the general linear trend and thus provided a tool for finding outliers among populations. Additonal plots were made of DNA content (for over 350 species) and chromosome number (for over 1300 species) of fishes against the phylogenetic order, based on the taxonomic classification published by J. Nelson (1994). Decreasing trends in DNA content and chromosome number were observed, albeit with a high variation within orders. Possible reasons for such a trend are discussed.

Key words : Fishbase — Database — Genetic — Fish — Aquaculture.

Roger S.V. Pullin : "Genetic ressources for aquaculture: ownership and access"

Ownership and access arrangements for aquatic genetic resources are far less developed than are arrangements for genetic resources for agriculture, especially plants. However, defined ownership of and more regulated access to aquatic genetic resources are likely, following the trend for plants and livestock. Most countries have nationalized their genetic resources through the Convention on Biological Diversity, and attempts to privatize genetic resources are increasing, through assignment of patents and other intellectual property mechanisms, and through farmers', breeders' and indigenous peoples' rights. These developments are reviewed with reference to aquaculture research and development, including approaches taken by Iclarm. Future possibilities are discussed with respect to ownership of and access to aquatic genetic resources. An

Appendix of selected works on plant genetic resources is provided, as an indication of the scope of work still needed for aquatic genetic resources.

Key words : Genetic resources — Fish — Aquaculture.

Benedict P. Satia, Devin M. Bartley : "The Paradox of International Introductions of Aquatic Organisms in Africa"

Approximately four hundred and thirty introductions and transfers (briefly introductions) of aquatic species have been made in some forty-two countries in Africa over the last 150 years. The majority of these introductions have been performed between the 1920s and 1970s. Ostensibly, these introductions were considered to be an effective means to increase protein, generate income and provide employment, as well as to control disease vectors and weeds. Several introductions were also performed for trivial motives, for example, the nostalgia of displaced peoples to have around them familiar fauna, especially for recreational fishing.

Successes have followed some introductions of species as a foundation for fisheries. In many cases, the objectives could not be met or no accurate evaluation has been undertaken. There have also been negative effects. Introductions have resulted in significant and serious impacts on capture fisheries and the ecosystem, as well as on the human communities that fish, farm or market aquatic resources. Some of these changes were unexpected.

Assessment of aquatic species introductions in Africa should be analyzed on a broad spectrum which should include ecological as well as socio-economic parameters, as exemplified by the introduction of the pelagic clupeid *Limnothrissa miodon* into Lakes Kivu and Kariba and its accidental diffusion downstream to Lake Cahora Bassa or the introduction of the predatory Nile Perch (*Lates niloticus*) into Lake Victoria. In either case, the result is a flourishing fisheries but with important socio-economic side effects.

Another paradox is the Genetic Improvement Farmed Tilapia (GIFT), the parent stock of which was introduced into the Philippines from Africa and developed into the "super tilapia" by Iclarm researchers.
While this was taking place, Africa was still scouting for the best fish species to be introduced for aquaculture. Impacts of species introductions are usually difficult to reverse once a species become established and difficult to predict accurately. In many other regions of the world regulations have been enacted; biosafety protocols and codes of practice on the responsible use of exotic species have or are being elaborated; and precautionary approaches to species introductions are being pursued. But in Africa a *laisser faire* situation with respect to introductions still exists and there are few, if any, effective quarantine measures.

Key words : Fish — Introduction — Africa.

Guy G. Teugels : "Intra- and interspecific morphometric variation in *Clarias gariepinus* and *C. anguillaris* (Siluroidei, Clariidae)"

Eigth populations of *Clarias gariepinus* and six populations of *C. anguillaris,* originating from allover their distribution range have been examined morphometrically.
The results obtained confirmed that the number of gill rakers on the first bronchial arch is the only reliable feature to identify both clariid species.
Within *C. anguillaris,* only a limited intraspecific variation was observed. Within *C. gariepinus* however, populations from West Africa and the Nile are morphometrically closer to each other than to those from Lake Victoria and southern Africa.
Different colonization patterns, related to earlier hydrographic connections are used to explain this intraspecific variation.

Key words : *Clarias* — Morphometry — Biogeography — Africa.

Guy G. Teugels, Germain Gourène : "Biodiversity and aquaculture of African catfishes (Teleostei, Siluroidei): an overview"

Ten catfish families, including the marine Ariidae and Plotosidae, are presently known from Africa. They include 58 genera and 465 species. The Mochokidae is the most speciose family, containing more than 38% of the species. The Clariidae undoubtedly represents the economically most important family: *Clarias gariepinus* is the most cultured catfish on the African continent and other clariid species such as *Heterobranchus longifilis* are very promising aquaculture candidates. The Claroteidae, with *Chrysichthys nigrodigitatus* is another catfish family presently used in aquaculture in West Africa. Although other families like Schilbeidae and Bagridae are important in local fisheries, no other catfish family is presently used in aquaculture.

Key words : Catfish — Aquaculture — Biodiversity — Africa.

Guy G. Teugels, Emmanuel J. Vreven : "Preliminary results on morphometric differentiation between natural populations of the Nile tilapia *Oreochromis niloticus* (Perciformes, Cichlidae)"

Seventeen populations, belonging to six subspecies of the Nile tilapia *Oreochromis niloticus* have been studied morphometrically (twenty five measurements and eight meristic counts). Although the results obtained are still preliminary because not all known subspecies have been examined and because for some subspecies only few specimens were available for study, they indicate that the subspecific classification of *0. niloticus* as given by Trewavas (1983) can be questioned. Most interestingly, populations from the Nile seem closer to the East African populations than to the West African ones. Trewavas (1983) considered the West African and the Nile populations as belonging to the same subspecies, *0. n. niloticus.* Within the East African populations, a high degree a polymorphism was noted in the *O. n. cancellatus* complex, although we did not find sufficient evidence to

support the conclusion of Seyoum and Kornfield (1992) that this subspecies is a separate species containing two subspecies.

Key words : *Oreochromis niloticus* — Subspecies — Taxonomy — Morphology.

Emmanuel J. Vreven, Béatrice Adépo-Gourène, Jean-François Agnèse, Guy G. Teugels : "Morphometric and allozyme variation in natural populations and cultured strains of the Nile tilapia *Orechromis niloticus niloticus* (Teleostei, Cichlidae)"

The morphometrical and allozyme variation of nine natural populations of *Oreochromis niloticus* and three of their cultured strains has been studied. Differences in external morphology and in allozymes were noted between natural populations from West Africa and from the Nile. Although both are arranged in the same subspecies, *0. niloticus niloticus,* the Nile populations are closer to the population from Lake Edward, identified as *0. niloticus eduardianus.* Morphological differences have been observed between natural populations and their cultured strains. These undoubtedly are related to ecophenotypical influences, because cultured strains are genetically related to their natural parental populations.

Key words : *Oreochromis niloticus* — Morphometry — Allozyme — Aquaculture.

Filip Volckaert, Frans Ollevier : "Transgenic fish. The future of fish with novel genes"

Transgenic fish open interesting perspectives to study developmental biology and the regulation of gene expression *in vivo*, they are useful as a model for biomedical research and make the enhanced selection possible of aquaculture production by the manipulation of commercially important traits. This review focusses on the aims, the procedures and the environmental consequences of generating transgenic fish. Suitable constructs of preferably "all-fish" origin consist of a promoter sequence which controls expression, a transgene which encodes the

desired trait, distal regulatory elements and a viral or plasmid vector. Transgenes can be subdivided according to the nature of the trait: gain-of-function, reporter protein and loss-of-function. The transfer method for stable integration in the genome is targeted to the zygote or the early embryo; the use of embryonic stem cells is under consideration. Trial-and-error for correct insertion in the genome is the standard because methods for homologous recombination are not available yet. Micro-injection is the principal choice of administering the DNA construct. The implications for aquaculture are highlighted with the transgenes promoting growth, lowering freezing temperature and inducing disease resistance. The biosafety and environmental issues related to transgenic fish should be dealt with appropriately before any releases in the natural environment. Future research will likely focus on the quality of the transgene constructs (regulatory elements and vectors), the methodology of targeted insertion, the biological characterisation of the novel trait, environmental issues and socio-economic aspects.

Key words : Aquaculture — Biosafety — Fish — Gene regulation — Transgenic.

achevé d'imprimer sur les presses de :

LAVAUZELLE GRAPHIC
IMPRIMERIE A. BONTEMPS
87350 PANAZOL (FRANCE)
N° Imprimeur : 8036060-98
Dépôt légal : Mai 1998