Fish Genetics Research in Member Countries and Institutions of the International Network On Genetics in Aquaculture

Edited by
Modadugu V. Gupta
Belen O. Acosta

INGA

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CONTENTS

FOREWORD

With a growing world population, especially in developing countries, achieving and improving food security calls for well-targeted actions. Fish are an important food, and fisheries provide employment for many. Declining fish stocks in oceans, rivers and lakes pose a threat to those who depend on catching fish for daily sustenance, and who depend on employment in fisheries, particularly artisanal fisheries.

Aquaculture is one sector that contributes significantly to world food production. As natural fish stocks decline, it is crucial to improve production from aquaculture, to improve production efficiency, and to improve aquaculture species by genetic enhancement.

The International Network on Genetics in Aquaculture (INGA) was established in 1993 to increase the quantity of fish protein consumed by low-income rural and urban people in tropical developing countries through efficient breeding and selection programs that will improve aquaculture production and the income of resource-poor fish farmers. Thirteen developing countries, nine advanced scientific institutions and three international and regional organizations make up INGA's membership.

The INGA network has been a catalyst for substantial progress in genetics research in member countries. These 13 countries produce 83 per cent of world aquaculture output. Some of them are already reaping the benefits from their investment in genetics research in the form of more productive strains of fish.

This proceedings provides a detailed and valuable insight into current aquaculture genetics. The papers were prepared for the Fifth INGA Steering Committee meeting held in March 1999 in Kuala Lumpur, Malaysia. The meeting, kindly hosted by the Malaysian Department of Fisheries and the University of Malaya, was significant because it brought together scientists from both developing and developed countries, permitting a wide exchange of the latest advances in genetics research in aquaculture.

Meryl J. Williams
Director General
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PREFACE

This publication incorporates the information drawn from aquaculture genetics research undertaken or in progress in Member Countries and Associate Member Institutions of the International Network on Genetics in Aquaculture (INGA). INGA has a membership of 13 countries in Asia, the Pacific and Africa, and 12 advanced scientific institutions. The Steering Committee of INGA consists of senior research planners from member countries and institutions as well as ICLARM. The Committee meets at regular intervals to discuss progress in aquaculture genetics, identify areas for possible collaboration, and share information.

The Fifth Steering Committee of INGA hosted by the Malaysian Department of Fisheries and the University of Malaysia was held in Kuala Lumpur, from 3-5 March 1999. The meeting was attended by all members and associate members of the network. The papers presented at this meeting were informative and contained a wealth of information on the state of aquaculture genetics research in the respective countries and institutions . Hence, INGA/ICLARM undertook to publish the papers presented at the meeting. Papers presented at the meeting in 1999 were revised by their respective authors to incorporate the developments that have taken place since then.

Aquaculture genetics research is in its infancy in many developing countries and there is an urgent need for collaboration among institutions in the developing and developed countries for synergy in developing better breeds of fish that can contribute to increased production and food security while ensuring the conservation of aquatic biodiversity. All the members and associate members of INGA have been playing an active and important role in this and in supporting network activities. Close collaboration among network members is evident from the exchange of germplasm following material transfer agreements and quarantine protocols and collaborative research projects that have been developed. On behalf of INGA I thank all those who have contributed to this volume.

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NETWORKING IN AQUACULTURE GENETICS RESEARCH[a]

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ABSTRACT

Aquaculture genetics research is in its infancy compared to that of crops and livestock. Multidisciplinary research coupled with networking are effective tools in genetics research especially in developing countries.

Variability in fish species, farming systems and production environments made it imperative for genetic enhancement of aquaculture species to be done through regional and international cooperation. Realizing this, ICLARM-the World Fish Center initiated the International Network on Genetics in Aquaculture (INGA) in 1993. At present, the network has a membership of 13 countries from Asia, the Pacific and Africa and 12 advanced scientific institutions.

In the short time since its inception, the network has made significant progress in: assisting member countries in developing national breeding programs; initiating two regional research programs for genetic improvement of carps and Nile tilapia; assisting the transfer of germplasm between member countries for research and to disseminate improved strains; assisting the formation of national genetics networks; assisting in capacity building among developing country scientists; and providing information on genetics research and policy issues in biodiversity conservation, intellectual property rights, etc.

Background

About a billion people, mostly in developing countries, depend on fish as a primary source of animal protein. Global fish production reached 122 million t in 1996, of which only 90 million t were used for human consumption (FAO 1999). FAO estimates that by the year 2010, demand for food fish would increase by 13.5-18.0% or to about 105-110 million t. Annual growth in capture fisheries production decreased from 6% in the 1950s and 1960s to 0.6% in 1995 and 1996 (FAO 1999), suggesting an increase in dependence on production from aquaculture.

New (1991) estimated that the world's projected requirement for aquaculture products would increase from 19.6 million t in the year 2000 to 62.4 million t in 2025. Based on FAO statistics (1998), the 13 member countries (Bangladesh, China, Côte d'Ivoire, Egypt, Fiji, Ghana, India, Indonesia, Malaysia, Malawi,

[a] *ICLARM Contribution No. 1612*

Philippines, Thailand and Vietnam) of the International Network on Genetics in Aquaculture (INGA) collectively produced 25.7 million t of fish from aquaculture in 1998, accounting for 83.3% of world's aquaculture production.

Concomitant with technological advances in aquaculture are the problems associated with environmental degradation, genetic deterioration of cultured stocks and decline in aquatic biodiversity limiting options for new species and strains for aquaculture and diminishing natural productivity and resilience of aquatic ecosystems. A review of world's fisheries indicate that the potential contribution of aquaculture could only be realized if a number of issues, including genetic improvement of aquaculture species and biodiversity, are addressed (FAO 1995).

Remarkable progress has been made in improving the productivity of crops and livestock during the last 3-4 decades through breeding and selection. However, in spite of the long history of fish farming, it was only in recent years that efforts have been made to harness the benefits of genetic enhancement in fish. Norwegian scientists have amply demonstrated the possibility of increasing productivity of farmed salmon and trout through an efficient breeding program. In 1995, salmon farming produced a combined output greater than the sum of the country's meat production from swine, cattle and poultry (Gjoen and Bentsen 1997).

It was ascertained, however, whether the same benefits could be obtained in the case of tropical and subtropical finfish which contribute about 90% of the global aquaculture production. This uncertainty posed a challenge to scientists to develop techniques for the genetic improvement of tropical finfish used in aquaculture.

The collaborative research project for the genetic improvement of Nile tilapia (*Oreochromis niloticus*) implemented by ICLARM in collaboration with the Philippine and Norwegian institutions, indicated the potential of increased production through selective breeding. In its sixth generation of selection, the GIFT strain had demonstrated 77% faster growth and 60% higher survival compared with farmed strains in the Philippines (Eknath and Acosta 1998; Eknath et al. 1998). Subsequent evaluation of the second generation GIFT strain in Bangladesh, China, Thailand and Vietnam showed that the GIFT strain had higher growth rates than local strains, the difference ranging from 18% in China to 66% in Bangladesh. Analysis of overall potential impact of improved fish in the five countries indicated that the adoption of improved strain would result in reduced cost of production and increased fish production (Dey 2000).

Overall, the genetic gains per generation and per year of coho, rainbow trout, Atlantic salmon, channel catfish and tilapia was estimated at about 15% and 5%, respectively (Gjedrem 1997). Gjedrem's assumption was that if efficient breeding programs were introduced throughout the aquaculture industry, the aquaculture production by the year 2025 would be higher than the projected requirement for aquaculture products.

In Asia, evidences of inbreeding and unintentional selection among hatchery-based and established farmed stocks have been found (Pullin and Capili 1988; McAndrew et al. 1993; Eknath 1995). In Africa, freshwater species are on the verge of extinction, despite it being considered as the world's repository of diverse freshwater fish fauna. There are also uncontrolled fish transfers and introduction of exotic fish in many countries, threatening local aquatic resources.

Realizing that multidisciplinary research is an effective tool in genetics and breeding and networking is a proven mechanism for international cooperation, national institutions in developing countries in Asia, Africa and ICLARM formally established the International Network on Genetics in Aquaculture (INGA) in 1993 (Seshu et al. 1994). At present, INGA has a membership of 13 countries from Asia, Africa and the Pacific (Bangladesh, China, Côte d'Ivoire, Egypt, Fiji, Ghana, India, Indonesia, Malawi, Malaysia, Philippines, Thailand and Vietnam) and 11 advanced scientific institutions (Fig. 1). The following is a brief report on activities being undertaken by INGA and the important role it has been playing through consolidation of strengths of its members.

Network Activities

INGA acts as catalyst for genetics research being undertaken by national programs. In 1997, the 13 member countries reviewed the declining fish catches from natural resources and came up with the "Manila Resolution" (Box 1) stressing the need for concerted regional and international efforts for advancing fish breeding and genetics through cooperation (The INGA Planning Meeting 1997). To accomplish this, the

following major activities have been and are being carried out.

Development of national breeding programs

INGA has been assisting member countries in the development of plans and strategies for the implementation of national fish breeding programs based on their respective needs and resources. The network and the Norway's Institute of Aquaculture Research (AKVAFORSK) have assisted the Aquaculture Research Institute Nos. 1 and 2 in Vietnam in developing breeding plans for: (1) Nile tilapia (*O. niloticus)* through combined multitrait selection; (2) silver barb (*Barbodes gonionotus*) through individual (mass) selection to improve growth; and (3) mrigal (*Cirrhinus mrigala*) through individual selection to improve growth rate.

Assistance was also provided to the Research Institute for Freshwater Fisheries, Indonesia, in developing breeding plans for: (1) common carp (*Cyprinus carpio)* using multitrait selection; (2) Nile tilapia (GIFT strain) using individual selection; and (3) milkfish (*Chanos chanos*) using individual selection.
The breeding programs in member countries are also regularly reviewed by the network (Table 1).

Initiation of regional research programs

Genetic improvement of carps in Asia

In carp culture, diverse farming systems and socioeconomic scenarios prevail in various countries in Asia. About 20 carp species are extensively cultured and for each species, several traits have been identified as having improved (Gupta et al. 1998). Since many of the carp species are common to aquaculture systems in Asian countries, the six major carp-producing member countries of INGA (Bangladesh, China, India, Indonesia, Thailand and Vietnam) recognized that a collaborative effort would be better than each country trying to improve all their species. This has resulted in the implementation of the regional project for genetic improvement of 4 species of carps (*C. carpio, M. amblycephala, Labeo rohita, B. gonionotus*) with funding from the Asian Development Bank and ICLARM.

Collaborative tilapia genetic research in Africa

In Africa, INGA/ICLARM with start-up funds provided by the International Development Research Center (IDRC), Canada, initiated a regional collaborative

Fig. 1. Member countries and associate member institutions of INGA.

Box 1. Manila Resolution adopted by INGA members.

The participants of the Planning Meeting of INGA resolve to encourage the INGA member countries, donors and other institutions/organizations to give due emphasis for conservation and sustainable use of aquatic genetic resources and biodiversity by:

- organizing national aquaculture genetics networks and providing support for coordination of activities;
- providing support to national genetics research programs;
- strictly adhering to the germplasm transfer and quarantine protocols approved by the network members which conform to wider international standards and rigorously implementing the guidelines;
- sharing knowledge and methodologies;
- sharing germplasm, with prior informed consent and on mutually agreed terms;
- providing technical and policy inputs to national and international efforts to maintain aquatic biodiversity and genetic resource conservation;
- assisting in the implementation of the Convention on Biological Diversity (CBD); and
- providing support to INGA activities.

research project aimed at documentation of tilapia genetic resources, including indigenous knowledge, genetic characterization of tilapia strains and initiation of genetic improvement of tilapias. Institutions in Côte d'Ivoire, Egypt, Ghana and Malawi are implementing the research.

Strengthening of national research capacity

Since genetics research started only in recent years in member countries of the network, emphasis is being laid in capacity building through organization of training programs in quantitative genetics by ICLARM/ INGA in collaboration with AKVAFORSK.

Member countries of INGA do not have the same level of scientific training and expertise. Aquaculture and genetics research is generally more advanced in Asia than in Africa. In view of this, INGA has been organizing cross-country visits for senior scientists of participating countries.

Exchange of fish germplasm

INGA has been assisting the member countries in exchange of germplasm for evaluation, direct use in aquaculture or utilization in breeding programs. Protocols and quarantine procedures formulated by the network based on international codes of practice and material transfer agreements (Box 2) are being followed in transfer of germplasm through the network.

The network has coordinated the following exchanges of genetic materials:

- Improved *O. niloticus* (GIFT strain) from the Philippines to Bangladesh, China, Fiji, India, Indonesia, Thailand and Vietnam;
- *O. niloticus* strains from Côte d'Ivoire and Kenya to Egypt;
- *O. aureus* and *O. niloticus* from Egypt to China;
- Improved *C. carpio* strain from Vietnam to Bangladesh, India, Philippines and Thailand;
- Diverse stocks of *B. gonionotus* from Indonesia and Thailand to Bangladesh;
- *C. mrigala* from India to Vietnam; and
- Improved *L. rohita* strain from India to Thailand.

Table 1. Progress of breeding programs in member countries of INGA.

Country	Species	Activity
Bangladesh	*B. gonionotus*	Selective breeding, line crossing and development of monosex female population
	C. catla, L. rohita	Selective breeding and line crossing experiments for genetic improvement
China	*C. carpio, M. amblycephala*	Mass selection for improvement of growth
Côte d'Ivoire	*O. niloticus*	Evaluation of local strains for selective breeding
Egypt	*O. niloticus*	Evaluation of local strains for selective breeding
Fiji	*O. niloticus*	Performance evaluation of Fijian tilapia and GIFT strain
Ghana	*O. niloticus*	Evaluation of local strains for selective breeding
India	*L. rohita*	Combined family and within family selection for further genetic improvement
	C. carpio	Production of sterile and monosex population
Indonesia	*C. carpio*	Diallele crossing experiment of gynogenetic common carp
Malawi	*O. shiranus*	Mass selection for genetic improvement
Malaysia	Red tilapia, *O. niloticus*	Selective breeding
Philippines	*O. niloticus*	Continuation of multitrait (growth and frequency of spawning females) selection
Thailand	*B. gonionotus*	Mass selection for improvement of growth
	C. carpio	Mass selection for improvement of growth
Vietnam	*C. carpio*	Family selection for improvement of growth
	B. gonionotus	Establishment of base population for selective breeding

Box 2. Material Transfer Agreement.

A country planning to import new or exotic species has to sign a Material Transfer Agreement which states that the recipient agrees:

- to abide by the provisions of the Convention on Biological Diversity;
- to preclude further distribution of germplasm to locations at which it could have adverse environmental impact;
- to not claim ownership over the material received; nor seek intellectual property rights over the germplasm or related information;
- to ensure that any subsequent person or institution to whom they make samples of germplasm available is bound by the same provision;
- that the responsibility to comply with the country's biosafety and import regulations and any of the recipient country's rules governing the release of genetic materials is entirely its own;
- to follow the quarantine protocols suggested by ICLARM/ INGA; and
- to abide by the International Code of Transfer of Germplasm in case germplasm is transferred beyond the boundaries of the country.

Formation of national networks

INGA assisted in the formation of national networks, e.g., in India, Indonesia, Malawi, Malaysia and the Philippines. The international network also provides its local counterparts opportunities and linkages necessary for research and development.

Information dissemination

There is a fairly large amount of information available on genetics research. However, many of the institutions in member countries do not have access to this information. Hence the network has been assisting the members with dissemination of information. Through the network, member countries can have access to reports, reprints and other materials on fish genetics research. The network has also been publishing its Newsletter in Naga, ICLARM quarterly.

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AQUACULTURE GENETICS RESEARCH IN BANGLADESH

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ABSTRACT

Aquaculture productivity has declined in recent years in Bangladesh. The reasons for this are habitat degradation, loss of genetic diversity and stock deterioration in hatchery populations due to poor broodstock management and inbreeding. In consonance with the government's plans to rehabilitate inland fisheries in the country, the Bangladesh Fisheries Research Institute through its Freshwater Station, initiated fish genetics research in 1986.

Making effective breeding plans for commercially important carps and other fish species is an important research area for the development of genetically improved broodstock. A new dimension of this research has begun with the involvement of international agencies such as the International Center for Living Aquatic Resources Management, Australian Centre for International Agricultural Research/Commonwealth Scientific and Industrial Research Organization and Institute of Aquaculture, University of Stirling, Scotland.

The major research programs being implemented are: genetic stock improvement of endemic (*Catla catla* and *Labeo rohita*) and exotic (*Barbodes gonionotus*) carps, catfish (*Heteropneustes fossilis*) and genetically improved GIFT strain Nile tilapia (*Oreochromis niloticus*); population genetics of an anadromous clupeid, hilsa shad (*Tenualosa ilisha*); and genetic conservation of some endemic threatened fish species. This paper presents the highlights of these research programs in Bangladesh and future plans.

Introduction

Stock deterioration in hatchery populations caused by poor broodstock management and inbreeding has been observed in recent years in Bangladesh. Retarded growth, reduction in reproductive performance, morphological deformities, increased incidence of diseases and mortalities of hatchery-produced seed have been reported. This has resulted in deterioration of carps and barb seed quality. There is widespread concern that stocking of such genetically poor quality seed in floodplains and related open water bodies for fishery enhancement might cause serious feral gene introgression in the wild stocks.

To avoid loss of genetic diversity and inbreeding depression in hatchery populations, the development of improved broodstocks through implementation of effective breeding plans for commercially important carps and other fish species has been identified by

the Bangladesh Fisheries Research Institute (BFRI) as an important area for research. Research was initiated with technical and financial support from the International Center for Living Aquatic Resources Management (ICLARM); Australian Centre for International Agricultural Research (ACIAR)/Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia; and Institute of Aquaculture (IOA), University of Stirling, Scotland. Genetic evaluation of improved GIFT strain Nile tilapia (*Oreochromis niloticus*) was undertaken during 1994-1996. Genetic stock improvement of two selected carp species, silver barb (*Barbodes gonionotus*) and catla (*Catla catla*), has been in progress since January 1997 under the auspices of ICLARM. A collaborative research program with IOA to produce all-female population of *B. gonionotus*, through gynogenesis and sex reversal, has been initiated. The population genetic structure of hilsa shad (*Tenualosa ilisha)* in Bangladesh is being investigated in collaboration with ACIAR/CSIRO, Australia. Other programs being implemented include the selective breeding of rohu (*Labeo rohita*), further genetic selection and development of all-male population of GIFT strain *O. niloticus*, genetic manipulation in catfish (*Heteropneustes fossilis)* and genetic conservation of endangered fish species like *Labeo gonius, Cirrhinus reba, Channa striata* and others.

Freshwater Fish Species Used for Artificial Seed Production

Endemic carps and other fish species

There are at least 13 species of carps and other species under six genera inhabiting Halda and Padma-Brahmaputra River systems. Of these species, eight species of carps and barbs are used for artificial seed production in hatcheries (Table 1). Breeding techniques have been developed for the other five species: *Clarias batrachus, Ompok pabda, Mystus cavasius, Anabas testudineus,* and *H. fossilis*.

Exotic carps and other fish species

Several exotic fish species have been introduced in Bangladesh since 1960. Such introductions were not properly recorded. Table 2 lists the introduced fish species which are bred in hatcheries for seed production.

Genetics Research in Progress

Carp species

Breeding plans for stock improvement of catla and rohu

Land races of *C. catla* and *L. rohita* were collected from Halda, Jamuna and Brahmaputra river systems, and were investigated for differences in extrinsic genetic traits by means of morphological and growth assessment. During the spawning season of 1999, two wild *L. rohita* stocks (from Jamuna and Brahmaputra Rivers) were mated with existing hatchery stocks to produce three crossbreds (i.e., hatchery♀ x Jamuna♂; Jamuna♀ x Brahmaputra♂; and Brahmaputra♀ x Jamuna♂) and three purebred lines (i.e., hatchery♀ x hatchery♂; Jamuna♀ x Jamuna♂; and Brahmaputra♀ x Brahmaputra♂). With all these lines, selective breeding and line crossing program will be continued, which will result in the development of genetically improved strain,

Table 1. Endemic fish species of Bangladesh (Hussain 1998).

Family	Species	Common name	Local name
Cyprinidae	*Labeo rohita*	Rohu	Rui
	Catla catla	Catla	Katla
	Cirrhinus mrigala	Mrigal	Mrigal
	Labeo calbasu	Calbashu	Kalibaush
	Labeo bata	Bata	Bata
	Labeo gonious	Gonious	Gonnia
	Puntius sarana	Barb	Sarpunti
	Tor putitora	Tor mahseer	Mahashoal
Claridae	*Clarias batrachus*	Asian catfish	Magur
Siluridae	*Ompok pabda*	Pabda	Pabda
Bagridae	*Mystus cavasius*	Gulsha	Golsha
Anabantidae	*Anabas testudineus*	Climbing perch	Koi
Heteropneustidae	*Heteropneustes fossilis*	Asian catfish	Shingi

Table 2. Exotic fish species used for artificial seed production in Bangladesh (Hussain 1998).

Family	Species	Common name	Country	Year introduced
Cyprinidae	*Ctenopharyngodon idella*	Grass carp	Hongkong	1966
			Nepal	1979
			Japan	1970
	Mylopharyngodon piceus	Black carp	China	1983
	Hypophthalmichthys molitrix	Silver carp	Hong Kong	1969
	Aristichthys nobilis	Bighead carp	Nepal	1981
	Cyprinus carpio var. communis	Common carp	China	1960
			Vietnam	1995
	Cyprinus carpio var. specularis	Mirror carp	Nepal	1979
			Hungary	1996
	Barbodes gonionotus	Silver barb	Thailand	1987,1994
	Tor putitora	Mahseer	Nepal	1991
Schilbedae	*Pangasius sutchi*	Thai pangas	Thailand	1990
Claridae	*Clarias gariepinus*	African catfish	Thailand	1991
Cichlidae	*Oreochromis niloticus*	Nile tilapia	Thailand	1974
				1987
	O. mossambicus x O. niloticus	Red tilapia	Thailand	1988
	O. niloticus	GIFT strain	Philippines	1994, 1996

with better cultivable traits. Genetic evaluation in terms of growth and other relative performances will be undertaken in nursery and grow-out systems.

Phenotypic differences among wild stocks of L. rohita

The wild stocks of *L. rohita* from different river systems of Bangladesh were compared with hatchery stocks for phenotypic differences. Variations were observed in some meristic characters (Table 3). There were similarities between hatchery and Halda River stocks, particularly in the number of pectoral, anal and ventral fin rays, lateral line scales and vertebrae. Allozyme analysis and comparison of growth performance are in progress.

Interspecific hybridization between endemic and exotic barbs

Interspecific hybridization between two barb species, *P. sarana* (endemic) and *B. gonionotus* (exotic) was undertaken by Begum (1996). Fertilization (55-62%) and hatching (19-25%) of reciprocal hybrids were significantly lower ($P<0.01$ and $P<0.05$, respectively) than those of the control groups' fertilization (73-

Table 3. Meristic characters (range and average) of *L. rohita*, collected from hatcheries and different river systems of Bangladesh (figures in parentheses are the coefficient of variation).

Stock	No. of					
	Dorsal fin rays	Pectoral fin rays	Ventral fin rays	Anal fin rays	Lateral line scales	Vertebrae
Hatchery	13 - 15	14 - 17	8 - 10	6 - 8	40 - 43	31 - 33
(n=75)	14.2 ± 0.5	15.5 ± 1	9.0 ± 0.2	6.9 ± 0.3	41.4 ± 1	32.9 ± 0.4
	(3.2)	(6.5)	(2.3)	(4.8)	(1.4)	(1.3)
Brahmaputra R.	13 - 15	17 - 18	9	7 - 8	41 - 42	33 - 34
(n=25)	14.1 ± 0.5	17.8 ± 0.4	9.0 ± 0	7.2 ± 0.4	41.6 ± 0.5	32.2 ± 0.4
	(3.5)	(2.1)	(0.0)	(5.7)	(1.2)	(1.3)
Halda R.	13 - 15	16 - 18	8 - 10	6 - 8	40 - 43	30 - 35
(n=50)	13.9 ± 0.4	16.8 ± 0.5	8.9 ± 0.3	7.2 ± 0.7	41.6 ± 0.6	33.0 ± 0.5
	(3.0)	(2.9)	(3.5)	(1.9)	(1.5)	(1.6)
Jamuna R.	14 - 15	17 - 18	9	7	41 - 42	33
(n=25)	14.6 ± 0.5	17.9 ± 0.3	9.0 ± 0	7.0 ± 0	41.8 ± 0.4	33.0 ± 0
	(3.4)	(1.7)	(0.0)	(0.0)	(0.9)	(0.0)

81%) and hatching (49-56%). Both control groups produced significantly higher ($P<0.05$) percentage of normal embryos (52-59%) compared with both hybrids (23-24%). No variations were found between the two control and hybrid groups.

Genetic improvement of B. gonionotus

B. gonionotus was first introduced from Thailand in 1977, and subsequently in 1994 wild germplasm was introduced from Thailand and Indonesia with the help of ICLARM. Studies are in progress to develop a genetically improved and all-female population of *B. gonionotus* using these three founder stocks through selective breeding and genetic manipulation techniques under projects supported by ICLARM and the Department for International Development (UK), respectively (Hussain 1997).

For genetic improvement of *B. gonionotus* through selective breeding and line crossing techniques, germplasm obtained from Thailand and Indonesia and existing local stocks were used. The representative breeders of F_1 generations of the three unrelated stocks were crossed through 3 x 3 diallele crossing to produce nine genetic groups. The rationale of this crossing scheme is to form a heterogeneous outbred base population for the breeding program. For each of the reciprocal crosses, five to eight pairs were mated separately and the best three progeny (larvae) groups were selected to make 18 F_1 full sib progeny families which were then communally stocked by mixing 125 and/or 695 larvae from each family. Subsequently, they were transferred to communal grow-out ponds where stocking density was maintained at 0.75 to 1.0 fish/m^2 (Fig. 1). A separate growth trial experiment was conducted for eight months with communal F_1 crossbred and existing purebred (Bangla x Bangla) strain in four chambered earthen ponds with two replicates at a stocking density of 3 fish/m^3. At harvest, the maximum and minimum average weight gains of communal crossbred groups were 77.26 g and 49.21 g, respectively, whereas those for the control groups were 61.47 g and 56.13 g, respectively (Table 4). The data of monthly mean weight showed superior growth rate in crossbreeds but was not significantly different ($P>0.05$).

During the spawning season of 1998, 20% of largest females (at least 150 pairs) were selected from mature communal F_1 crossbreeds and used in the production of F_2 generation (Fig. 2). A comparative growth evaluation of F_2 crossbred and control groups (Bangla x Bangla) in nursery and grow-out systems indicated that the former had higher

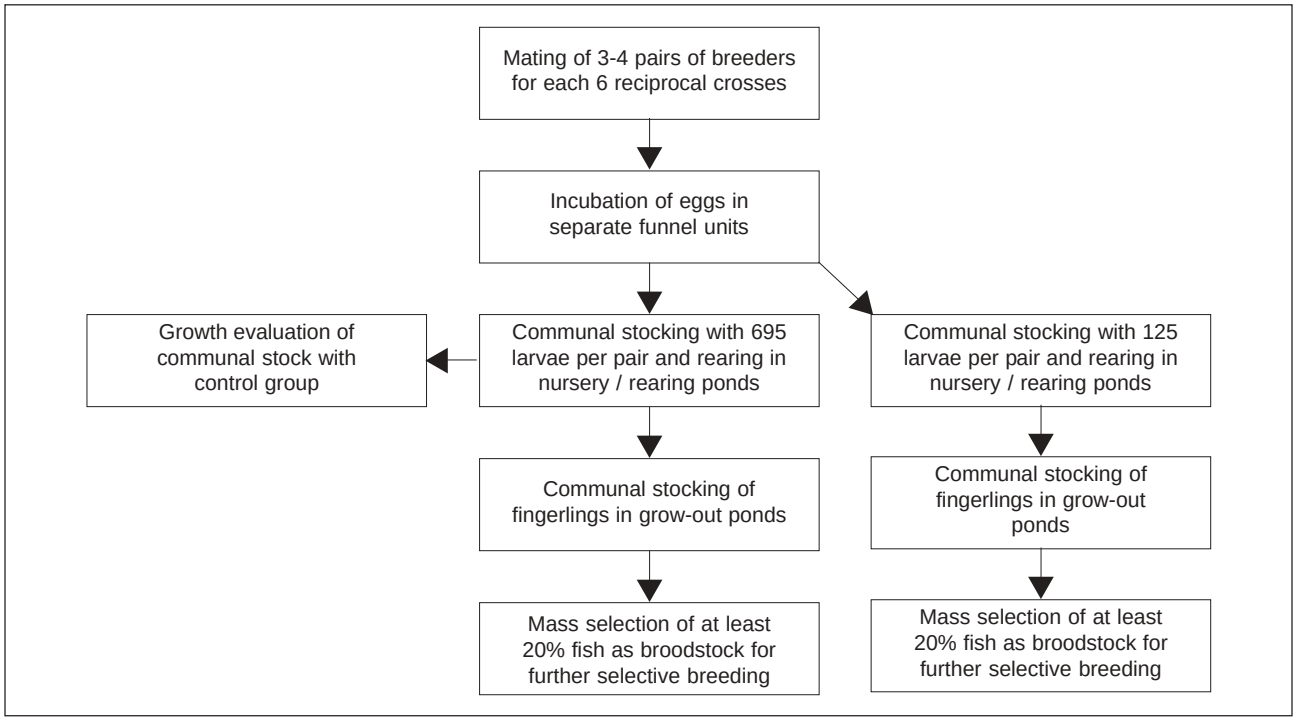

Fig.1. Design for mating and production of F_1 broodstock from the base population derived from reciprocal cross of *B. gonionotus*.

Table 4. Comparison of performance between crossbred and control groups of *B. gonionotus* .

Month	Average Weight (g)			
	Communal F_1 hybrid		Control group	
	Group 1	Group 2	Group 1	Group 2
Initial	3.86 ± 1.04	3.86 ± 1.04	3.49 ± 0.88	3.49 ± 0.88
October	17.41 ± 3.70	16.58 ± 4.09	20.01 ± 3.94	13.43 ± 4.01
November	25.17 ± 6.40	21.53 ± 4.14	24.02 ± 4.77	17.68 ± 3.33
December	32.50 ± 17.89	23.79 ± 7.60	28.48 ± 5.11	21.92 ± 8.51
January	40.97 ± 10.13	33.42 ± 9.60	36.18 ± 11.00	32.07 ± 9.24
February	43.56 ± 10.06	36.11 ± 8.89	40.23 ± 9.75	35.58 ± 11.22
March	46.25 ± 14.57	39.76 ± 12.10	42.56 ± 13.45	39.37 ± 12.18
April	55.24 ± 12.47	45.40 ± 11.62	45.60 ± 14.49	48.80 ± 11.71
May	77.26 ± 29.49	49.21 ± 13.56	61.47 ± 14.05	56.13 ± 13.96

growth in terms of weight at an early age than the latter, but this was not statistically significant. In grow-out ponds, the crossbred group showed significantly higher growth (7-8%).

During the spawning season of 1999, 15% of largest females and males were selected from the mature F_2 crossbred progenies and used them (182 pairs) for the generation of F_3 progenies. At the same time, 78 pairs of nonselected control (existing Bangla x Bangla) breeders were separately used to produce control progeny group. The on-station comparative growth evaluation trial data revealed that the average genetic gain in terms of body weight achieved by the selected group (F_3) was 22-25% over nonselected control group.

Like other cyprinids, female *B. gonionotus* has better growth than males; therefore, a program for mass production of all female population through gynogenesis and sex reversal was initiated in 1995 in collaboration with the University of Stirling, Scotland, and the National Aquaculture Genetic Research Institute (NAGRI), Thailand. The protocol for such approach was to produce neomale (phenotypic male having XX genotype)

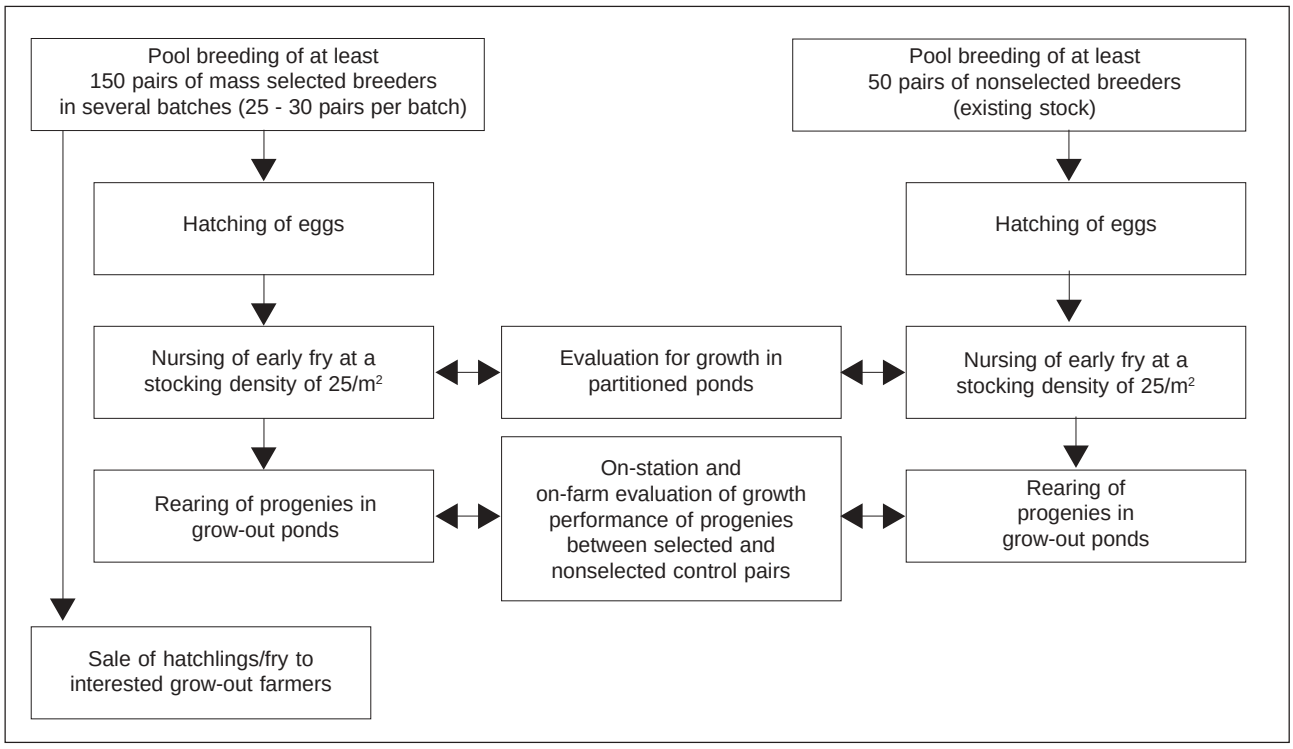

Fig. 2. Design for mating of selected broodstock and production of F_2 generation of *B. gonionotus* and their evaluation for growth.

through feeding androgen hormone treated feed to the gynogenetic fish and then crossing neomales with normal females for mass production of all female seeds.

Neomales of *B. gonionotus* were imported from NAGRI for mating them with females belonging to wild germplasm of Thailand and Indonesia (introduced in 1994 for selective breeding program). Production of additional batches of neomale through gynogenesis and sex reversal was initiated (Pongthana et al. 1998).

The putative monosex female progeny groups produced were tested on-station and on-farm. Putative monosex females produced from newly produced neomales and mixed sex control fish attained weights of 55-105 g in on-station trials, while 40-80 g in on-farm trials. The sex ratio in the harvested neomale progenies varied from 50% to 90% female. The male individuals present in the neomale progenies could be of second generation neomale or there might have been some complexities in the sex determination, XY systems.

Sexual dimorphism for weight in *B. gonionotus* was initially done through sampling of different populations from different culture systems. The sexual dimorphism indices (ratio of mean weight of females to mean weight of males, which is expressed as percentage) for weight in *B. gonionotus* at the age of one year class ranged between 1.1 and 1.7 in different populations and culture systems. Proximate composition of the carcass of female and male fish indicated insignificant differences ($P>0.05$) in moisture, protein and ash content, except fat (Azad 1998).

Catfish species

Genetic manipulation in H. fossilis

Meiotic gynogenesis was induced in *H. fossilis* by application of cold shock to the eggs that were fertilized with UV-irradiated sperm. Optimal irradiation was achieved when the sperm suspension with a concentration of 1×10^8 sperm ml^{-1} in a physiological saline (0.085) solution was exposed for 2.5 min to an intensity of UV at 250 $\mu W\ cm^{-2}$. Cold shock was applied to eggs 3-7 min after fertilization for a duration of 5-25 min at a temperature range of 2-6°C (Table 5). Apart from gynogens, haploid, triploid and normal diploid control groups were maintained simultaneously to ascertain the functions of irradiation, cold shock and quality of germ cells used. The haploids were produced by using irradiated sperm but without cold shock and the triploids were produced through cold shock of eggs fertilized with normal sperm. The status of ploidy-haploid, diploid normal/gynogen and triploid were assessed through karyological investigation which revealed the chromosomal number (N=28, 2N=56 and 3N=84). Diameter and volume of erythrocytes along with nucleus from normal diploid, gynogen and triploid individuals of two months of age were compared to assess their ploidy status. All values of these measures in triploid were found to be

Table 5. Effect of cold shock to a developing embryo at various temperatures and duration on ploidy manipulations in *H. fossilis*. (Sperm irradiated at 250 µW cm^{-2} with a concentration of 1.10^8 sperm ml^{-1}).

Temperature/ Duration	Observation	Meiotic gynogens (%)	Haploid control (%)	Triploid control (%)	Diploid control (%)
2°C for 10 min; 3-7 min after fertilization	Fertilization	85 - 96	83 - 97	91 - 98	- -
	Hatching	33 - 69	78 - 100	45 - 79	87 - 100
	Survival (3 days)	59 - 90	0	58 - 84	27 - 68
	Induction rate	94 - 100	100	94 - 100	0 - 83
2°C for 15 min; 3-7 min after fertilization	Fertilization	83 - 95	83 - 97	82 - 97	
	Hatching	32 - 56	78 - 100	40 - 73	87 - 100
	Survival (3 days)	36 - 76	0	23 - 40	27 - 68
	Induction rate	95 - 100	100	22 - 41	0 - 83
4°C for 10 min; 3-7 min after fertilization	Fertilization	73 - 90	83 - 97	91 - 97	
	Hatching	9 - 14	78 - 100	24 - 63	87 - 100
	Survival (3 days)	0 - 1	0	7 - 11	27 - 68
	Induction rate	0 - 3	100	0	0 - 83
4°C for 15 min; 3-7 min after fertilization	Fertilization	72 - 91	83 - 97	86 - 96	
	Hatching	11 - 23	78 - 100	22 - 56	87 - 100
	Survival (3 days)	15 - 28	0	16 - 28	27 - 68
	Induction rate	21 - 32	100	17 - 22	0 - 83

significantly higher (P<0.05) than that of corresponding normal diploid and gyno-genetic diploid, but insignificant differences (P>0.05) were found between the last two groups. A slow development of embryo and also delayed hatching were observed in gynogenetic and triploid groups (Gheyas 1998).

Production of all-male population of GIFT strain *O. niloticus*

Administration of androgen hormone 17 alpha-methyltestosterone (MT) orally to the fry of *O. niloticus* from first feeding stage to 40 days at different doses ranging from 60 to 100 µg/g of feed in hapas, pond and aquarium resulted in 75-95% males. Seven to 10-day old fry of GIFT strain *O. niloticus*, when administered orally with MT hormone at a dose of 60 and 100 µg/g of feed and SRT-99 (MT 10%) feed obtained from the Philippines for 3 weeks resulted in 95-100% males (n=50) in the batch that were administered with a dose of MT 100 µg/g feed whereas 85% males were obtained in the batch fed with MT 60 µg/g feed. SRT-99 hormone treated feed also resulted in 95-100% males (n=50).

Population genetic structure of hilsa shad, *Tenualosa ilisha*

The population genetic structure of *T. ilisha* which constitutes 25% of the total fish production in Bangladesh was investigated using starch gel electrophoresis. The research was done in collaboration with ACIAR/CSIRO, Australia. Fish samples were collected from different geographical areas within and beyond Bangladesh (Table 6). Starch gel electrophoresis of different enzymes of samples of various tissues was initially attempted to investigate allozyme variation. Five loci - Idh-l, Idh-m, Mdh-l, Mdh-m and Pgm - were polymorphic over the six locations studied. Levels of variation were low but significant differences in allele frequencies were detected among Kuwait, Bangladesh and Indonesia (Table 7). However, allozyme data, so far, cannot discriminate genetically distinct stocks of hilsa within Bangladesh (Hussain et al. 1998).

Conservation of endangered carp species

The International Union for the Conservation of Nature, Bangladesh (1998) documented 11 cyprinid species as critically or fairly endangered in the country (Table 8), indicating a need for the development of artificial breeding techniques for the conservation of the carp's "gene pool". Attempts were made to develop artificial propagation techniques for *Tor putitora* and *Puntius sarana,* and very recently for *L. gonius* and *C. reba.*

Future Research Plans

Following are plans for aquaculture genetics research in Bangladesh:

- Genetic characterization of wild land races of *C. catla* and *L. rohita*;
- Initiation of genetic stock improvement of *C. catla* and *L. rohita* using selective breeding and

Table 6. Locations and sample sizes used in the electrophoretic study.

Location	Number
Kuwait	47
Bangladesh	65
Khulna	
Aricha Ghat	70
Chandpur	69
Cox's Bazar	82
Indonesia	33

Table 7. Results of G statistics tests (similar to contingency X^2 tests) for homogeneity of alleles. H_o: all alleles are drawn from the same homogeneous population.

Comparisons	P	Significant loci
Within Bangladesh	NS	None
Kuwait vs. Bangladesh vs. Indonesia	P<0.001	Mdh-m, Pgm, Idh-l
Kuwait vs. Indonesia	P<0.001	Idh-1
Kuwait vs. Bangladesh	P<0.001	Mdh-m, Pgm
Indonesia vs. Bangladesh	P<0.001	Mdh-m, Pgm, Idh-l

Table 8. List of endangered carp and barb species of Bangladesh.

Scientific name	Local name	Critically endangered	Fairly endangered
Labeo nandina	Nandina	X	
L. boga	Bhangan	X	
L. gonius	Ghonia		X
L. bata	Bata		X
L. pangusia	Ghora maach		X
L. calbasu	Kalbaus		X
Cirrhinus reba	Laachu, bata		X
Puntius sarana	Sarpunti	X	
P. ticto	Tit punti		X
Tor tor	Mahashol	X	
T. putitora	Mahashol	X	

line crossing techniques;

- Evaluation of growth performance of F_4 putative genetically improved *B. gonionotus*;
- Genetic sex determination and production of all-female population of *B. gonionotus* through gynogenesis and sex reversal;
- Continuation of selection of GIFT strain *O. niloticus* for further genetic improvement;
- Optimization of sex reversal technique for GIFT strain *O. niloticus*;
- Continuation of genetic manipulation in *H. fossilis*, particularly production of all-female population through inducing homogametic neomales; and
- Continuation of population genetics study of *T. ilisha*.

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AQUACULTURE GENETICS RESEARCH IN CHINA

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ABSTRACT

China is the largest aquaculture country. The total output of fisheries in 1998 reached 38.2 million t, in which the share of aquaculture was 57%. This paper gives a brief review of the aquaculture genetics research in China, including genetic characterization, breeding for genetic improvement and conservation. The major achievements were breeding of common carp *(Cyprinus carpio)* (such as red carp, jing carp) and crucian carp *(Carassius auratus)* (such as allogynogenetic crucian carp) in early 1980s; gene engineering in late 1980s; and genetic conservation efforts in 1990s. These efforts contributed to the development of aquaculture in China.

Introduction

Fish production in China has increased rapidly since 1979. It reached 38 million t or about 30% of the world production in 1998. Developing more rapidly than capture fisheries, aquaculture's contribution to total fish production increased from 26% in 1978 to 57% in 1998.

Since marine capture fisheries has maintained zero growth since 1999, the increase in fish production comes entirely from aquaculture. Thus, genetic improvement of aquaculture species is expected to play a major role in the coming century. This paper summarizes the status of genetic research in China.

Genetic Characterization

Chinese carps

The genetic characterization of silver carp (*Hypopthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*) and black carp (*Mylopharyngodon piceus*) has been studied in detail since 1980s (Li et al. 1990; Li 1998). The grass carp, silver carp and bighead carp from three major rivers of China, i.e., Yangtze River, Pearl River and Amur River, were studied. Results indicated that fish in Yangtze River, grow fastest. The growth differences in wild populations of silver carp from Yangtze River and Pearl River and hatchery populations from Yangtze River and Pearl River basins are presented in Fig. 1. This finding has led to understanding of selection.

The diploid number of chromosomes in Chinese carps is 48, but the composition of chromosomes is different (Table 1). By biochemical genetic study, the LDH is coded by two loci, Ldh-A and Ldh-B, and shows five bands in muscle, eye, heart and brain. Besides loci Ldh-A and Ldh-B, there is Ldh-C in liver showing one major band. The relative activity and mobility of bands are different in Chinese carps (Table 2). The average heterozygosity

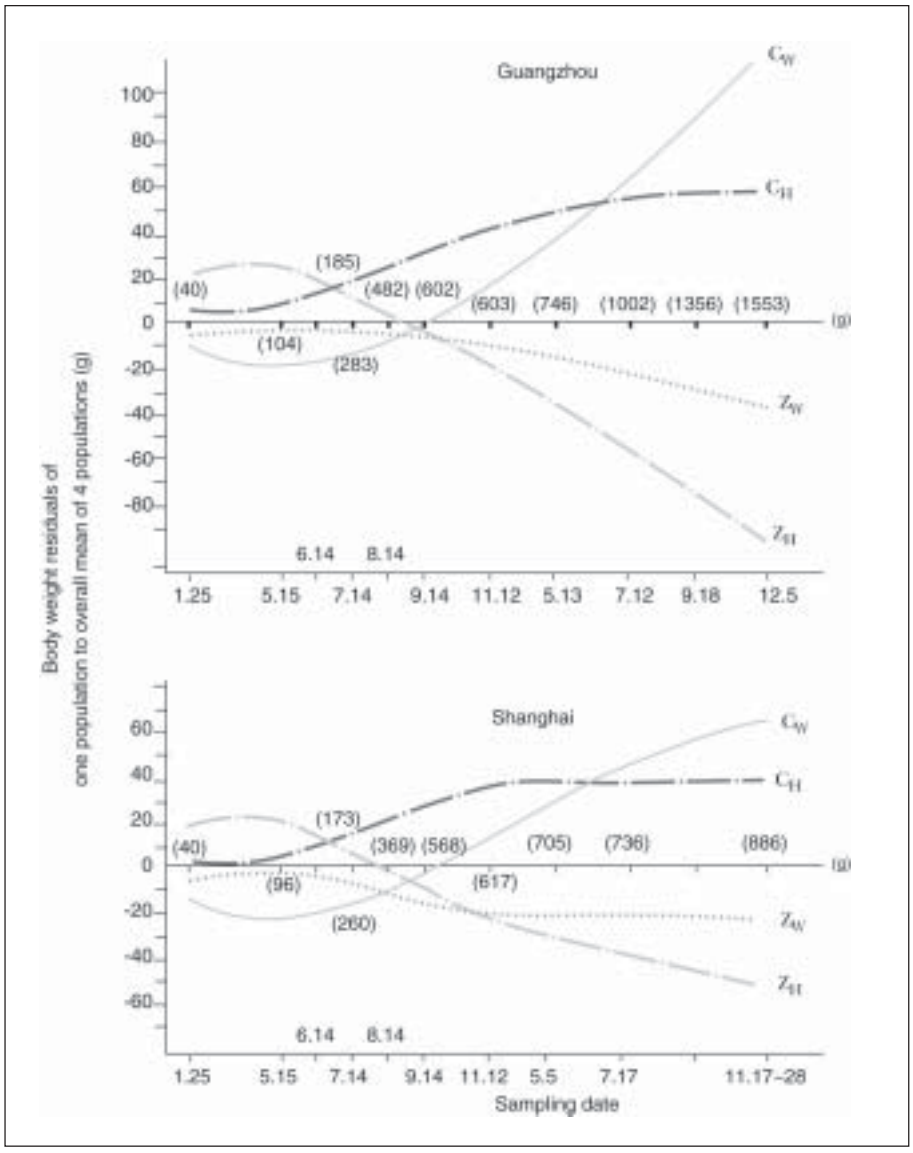

Fig. 1. The residual curves of body weight among four populations of silver carp, from two to three years old, in experimental stations of Shanghai (Yangtze River basin) and Guangzhou (Pearl River basin) by communal stocking. The numbers in parentheses are the overall means of four populations (Li et al. 1990). C_H = hatchery fish from the Yangtze River ; C_W = wild fish from the Yangtze River; Z_H = hatchery fish from the Pearl River ; Z_W = wild fish from the Pearl River

and the mean proportion of polymorphic of Chinese carps from the three major rivers are presented in Table 3.

The difference in allelic frequency variation expressed by genetic similarity as observed among populations distributed in three major rivers of China are presented in Fig. 2. Lu et al. (1997) performed a polymerase chain reaction-restriction fragment length polymorphism analysis on 365 juvenile fish of silver carp, bighead carp, grass carp and black carp from three representative nursery grounds in the Yangtze River to provide first assessment of the mtDNA diversity in Chinese carps and test the hypothesis that they are composed of more than one genetic stock. The mtDNA diversity was high in silver carp, bighead carp and black carp, but much less in grass carp (Table 4).

Common carp *(Cyprinus carpio)*

The large diversity in pigmentation is characteristic of all subspecies of common carp living in nature and of different domesticated strains. Several red carp varieties, e.g., Xingguo red common carp (*C.carpio* var. *singuonensis)*, purse red common carp (*C. carpio* var. *wuyuanensis)* and glass red carp (*C. carpio* var. *wananensis)* were produced by Chinese fish breeders through selection.

Table1. Composition of chromosomes of Chinese carps (Li 1998).

Species	Metacentrics	Submetacentrics	Subtelocentrics
Silver carp	10	9	5
Bighead carp	12	9	3
Grass carp	13	7	4
Black carp	14	4	6

Table 2. Relative activity (RA, %) and relative mobility (RM, %) of LDH in muscle of Chinese carps (Li 1998).

Species		Band				
		A4	A3B1	A2B2	A1B3	B4
Silver carp	RA	45.0	32.3	18.6	2.8	1.4
	RM	46.8	61.0	72.2	87.0	100.0
Bighead carp	RA	54.1	26.0	15.9	2.0	1.9
	RM	46.7	57.9	68.8	85.7	100.0
Grass carp	RA	47.2	19.1	13.5	5.2	1.9
	RM	7.1	36.3	47.5	77.8	100.0
Black carp	RA	41.9	20.2	11.5	2.9	2.6
	RM	25.3	41.4	61.6	79.8	100.0

Table 3. Average heterozygosity and mean proportion of polymorphism in Chinese carps from three major river systems of China (Li 1998).

Species	Heterozygosity	Proportion of polymorphism (%)
Silver carp	0.0484 - 0.0591	11.8 - 23.5
Bighead carp	0.0772 - 0.1133	15.8 - 29.4
Grass carp	0.0454 - 0.1076	20.0 - 33.3
Black carp	0.0010 - 0.0350	5.9 - 11.8

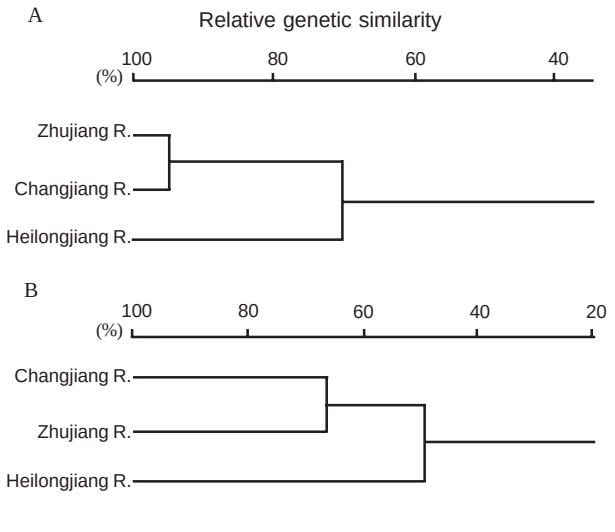

Fig. 2. Dendrogram showing genetic similarity among populations of silver carp (a) and grass carp (b) in Yangtze (Changjiang), Pearl (Zhujiang) and Amur (Heilongjiang) Rivers (Li et al. 1990).

The biochemical genetic study indicated that the average heterozygosity and the mean proportion of polymorphism of Chinese carps of the above varieties are slightly different (Table 5).

Crucian carp (*Carassius auratus*)

Crucian carp is the fifth most important species in freshwater aquaculture in China. In 1996, its production reached 690 000 t. Silver crucian carp (*C. auratus gibelio*) and Penzhe crucian carp (*C. a.* var *pengzenensis)* are widely used in aquaculture production and genetic studies in China. In silver crucian carp, the average heterozygosity and mean proportion of polymorphism are zero (Li 1998).

Blunt snout bream *(Megalobrama amblycephala)*

Blunt snout bream (Wuchang fish) was first recorded in 1960s from the natural fauna of Liangtze Lake in the middle-reach of Yangtze River. Subsequently, it was introduced into large areas as a principal cultured species. Its production reached 380 000 t in 1996. Its average heterozygosity is 0.756 and mean proportion of polymorphism is 19.6% (Li 1997).

Tilapias

The tilapias cultured in China are the Sudan strain (introduced in 1978), GIFT strain of Nile tilapia (*Oreochromis niloticus*) (1994, from the Philippines), and blue tilapia (*O. aureus*) (1983, from USA). The average heterozygosity and mean proportion of polymorphism of the above strains are different (Table 6).

Breeding for Genetic Improvement

Hybridization

Artificial hybridization among cyprinids of different families and genera was carried out extensively during the last three decades in China. Available information indicates that 112 crosses were carried out among fish of three orders, five families, and 32 species. Of these, hybrids among populations of common carp usually showed some heterosis. Some of these hybrids with valuable characteristics for aquaculture, and have been certificated by the

Table 4. Number of mitochondrial, overall nucleon diversity and nucleotide diversity of Chinese carps from Yangtze River.

Species	No. of haplotypes	Nucleon diversity	Nucleotide diversity
Silver carp	28	0.681	0.018
Bighead carp	19	0.584	0.008
Grass carp	7	0.231	0.002
Black carp	27	0.890	0.011

Table 5. Average heterozygosity and mean proportion of polymorphic Xingguo red carp and glass red carp.

Fish	Heterozygosity	Proportion of polymorphism(%)
Xingguo red carp[1]	0.0738	15.79
Glass red carp[2]	0.0962	21.05

[1]Li (1998).

[2]Inspection report on the Wanan glass red carp origin farm, Jiangxi province, 22 January 1999; made by the Aquatic Seed Inspection Center, Shanghai Fisheries University.

Table 6. Average heterozygosity and mean proportion of polymorphism of strains of Nile tilapia and blue tilapia.

Fish	Heterozygosity	Proportion of polymorphism(%)
Sudan strain Nile tilapia[1]	0.040	11.8
GIFT strain Nile tilapia[2]	0.035-0.047	10.5
Blue tilapia 1983[1]	0.000	00.0
Blue tilapia[3]	0.042	10.5

[1]Li (1998).

[2]Inspection report on the tilapias of Nanjing Tilapia Seed Farm, 1998; made by Aquatic Seed Inspection Center, Shanghai Fisheries University.

[3]Inspection report on the tilapias of Guangzhou Tilapia Seed Farm, 1998; made by Aquatic Seed Inspection Center, Shanghai Fisheries University.

The blue tilapia in this farm was introduced from Thailand in 1994.

Ministry of Agriculture in China, are:

- Yin common carp - scattered mirror common carp (female) x (nucleo-transfer purse red common carp [nuclear donor] x crucian carp) (male).
- Feng common carp - Xingguo red common carp (female) x scattered mirror common carp (male).
- Heyuan common carp - red purse common carp (female) x *C. carpio* var. *yuankiang* (male).
- Yue common carp - red purse common carp (female) x *C. carpio* (from the Xiangjiang River, male).
- Tricrossed common carp - (*C. carpio* var. *wuyuanensis* x *C. carpio* var. *yuankiang*) (female) x scattered mirror carp (male).
- Furong common carp - scattered mirror common carp (female) x Xingguo red common carp (male).
- Cold-tolerant red common carp – through hybridization of purse red common carp (low cold tolerance) with local common carp (high cold tolerance) and subsequent selective breeding.
- High-catchability common carp – through hybridization of beishi common carp (easy to catch) with local common carp (difficult to catch) and subsequent selective breeding.
- Jian carp (*C.carpio* var. *Jian*) - most hybrids have serious separation problem in F_2 generation and hence only F_1 is good for aquaculture. This necessitates production of F_1 generation every year and careful maintenance of their parents. To resolve this problem, based on hybridization, Zhang and Sun (1994) undertook a set of new combined breeding techniques of family selection, family crossing and gynogenesis. This resulted in a new variety, Jian carp (*C. carpio* var. *Jian*) which has stable genetic properties and has spread nationwide.

Selection

The best examples of selection of common carp in China are:

- Xingguo red carp - produced by nine generations of selection during 1972-1984. Growth rate has improved by 12.7%; full red color individuals were 86.6%; and average ratio of body length to height was 3.38, with a spindle shape.
- Purse red carp - produced by 10 years of continued selection for body shape and red color. Full red color individuals were 89.8%; ratio of body length to height was 2.0-2.3, with a purse shape (ARI 1973; JFS 1982).
- Glass red carp - produced by six generations of selection (1973-1983) for the transparent (gut and gills in larval stage) character. Full red color individuals were 84% (Wanan Fish Farm and Jiangxi University 1984).

Meanwhile, the blunt snout bream has undergone serious degeneration because of overexploitation of natural stocks, massive stocking, widespread transplantation and artificial breeding, and poor management of broodfish in the last 30 years. The degeneration is reflected in: (1) decreasing growth rate; (2) body shape becoming thin and

long; (3) decrease in proportion of edible part; and (4) early maturity. In 1986, a selection program was started, which aimed at protecting this fish's genetic resource, preventing degeneration and improving performance in aquaculture. The objective is to produce a new strain with better growth and body shape.

The foundation population of bream was obtained from Yuli Lake in Hubei Province in 1985 and 1986. From this population, two lines were established, and mass selection was undertaken. As a result, the fifth generation from the two lines showed 29.1% higher growth than the base population. The selection response was 35.8% for each generation. The ratio of standard length to height of body was 2.27, similar to the original strain.

A study indicated that improper management and breeding result in decreased growth rate. For example, a blunt snout bream inbred in three consecutive generations had a growth rate 16% lower than the original fish. Heterozygosity also decreased.

Genome manipulations (chromosomal engineering)

Polyploid breeding

In China, polyploid breeding research was initiated in the middle 1970s. Hydrobiology Institute, Academia Sinica, was the first to use cold shock and chemical induction to obtain triploids of grass carp and grass carp x blunt snout bream hybrid. Subsequently, heat shock and hydropressure were applied to induce triploids but very few were used in production. There is interest in the production of tetraploid fish. Crossing tetraploid fish with diploid fish results in triploid fish that could be used in aquaculture production. So long as tetraploid fish could mate with each other and get tetraploid progeny, the former could continue generating. Wu et al. (1981, 1988) produced a tetraploid carp by crossbreeding Xingguo red carp (female) x grass carp (male), then backcrossing the hybrid and grass carp. The resultant tetraploid carp showed strong disease resistance. Gui et al. (1990,1991) produced tetraploid crystal-colored crucian carp by hydropressure and cold shock. This was a creative idea in polyploid induction. However, the two carps were not used in aquaculture production. Liu Yun and his colleagues (pers. comm.) found several tetraploid crucian carps during their selection study, which were used to produce the triploid crucian carp, commercially called the Xiangyuan crucian carp.

Haploid breeding

Since the 1970s, China has produced more than 10 gynogenetic species, including grass carp, silver carp, common carp, loach (*Misgurnus anguillicaudatus)*, crucian carp, rainbow trout (*Oncorhynchus mykiss*), etc.

The crucian carp, *C. auratus gibelio,* is a natural gynogenetic fish. Jiang et al. (1982) used sperm of Xingguo red common carp to "fertilize" the ova of silver crucian carp, and obtained allogynogenetic crucian carp, with fast growth and good shape. This variety is highly preferred by producers and consumers in China. This practice of gynogenesis proved successful.

Androgenesis is a more valuable technique in theory, but produces little success in practice. Liu et al. (1987) used nucleo-transfer in loach and got pure diploid mature fish from androgenesis. Yi et al. (1990) used gamma rays to irradiate ripe egg, mated with normal sperm, and got diploid common carp-grass carp fish via androgenesis.

Sexual control

China started research on sex control in the 1970s. Several methods were used:

- Interspecific crossbreeding - such as *O. niloticus* (female) x *O. aureus* (male), resulting in about 95% males.
- Sex reversal hormone treatment - sex reversed *O. mossambicus*, Nile tilapia, red tilapia, common carp, grouper, *Clarias leather*, crucian carp and black porgy (*Sparus macrocephalus*) have been produced successfully by feeding the fry with estradiol or methyltestosterone treated feed. However, sex reversed fish are seldom used in commercial production in China because of food safety considerations.
- YY supermale technique - Yang et al. (1980) used estradiol benzoate to turn XY *O. mossambicus* into XY female, mated it with XY fish, selected YY super female from progenies and then mated YY with normal XX, producing 100% male fish. These fish have significant growth superiority with 35% increase in yield.
- Gynogenesis and sex reversal technique - Wu et al. (1981) used methyltestosterone or

testosterone propionate to change gynogenetic common carp into XX physiological female fish. This female was mated with XX male, resulting in gynogenetic inbred strain of carp. Using F_1 of gynogenetic Xingguo red carp as female parent and male gynogenetic mirror carp, an all-female hybrid with higher production was produced.

Cell engineering

Nucleo-transfer

Fish nucleo-transfer technique was used first in China by Tong Dizhou and his team (1963, 1973a, 1973b, 1973c). They succeeded in transferring the nucleus of crucian carp vesicular germ cell into the nucleus of the removed egg of *Rhodeus* spp. This technique could bind the different fish's nucleus and cytoplasm, creating a new body. Since 1970s, nucleo-transfer has been applied in breeding common carp and crucian carp, and grass carp and blunt snout bream (Tong 1973a, 1973b; Yan et al. 1984; Yan and Wang 1985). Nuclear cytoplasm hybrids of common carp-crucian carp, crucian carp-common carp, grass carp-blunt snout bream, and blunt snout bream-grass carp, etc., were produced. Of these, common carp-crucian carp nucleo-transfer fish reproduced three generations, and showed 20% faster growth than parental red common carp.

Cell fusion

Tong (1973a, 1973b) used Xiantai virus to induce the cell fusion of crucian carp vesicular germ cells and Ehrlich ascites cells. Yan et al. (1984) tested the main parameters of cell fusion by polyethylene glycol (PEG) in crucian carp. Liu et al. (1988) used electrical fusion method and got a fusion ratio of 47% and a survival ratio of 88.6% in vesicular germ cell of *Paramisgurnus dabryanus*.

Yi et al. (1988), using *P. dabryanus*, *M. anguillicaudatus*, common carp and red crucian carp as materials for electric fusion, produced six fusion fish of *P. dabryanus* and four fusion fish of common carp and red crucian carp. Zhang et al. (1988) used laser to fuse the eggs of *M. anguillicaudatus* and *P. dabryanus* with a fusion rate of 53%.

Gene engineering

Tong et al. (1963) and Tong (1973a, 1973b) found out that injecting mRNA from the ripe egg of crucian carp, or DNA from liver and testis of crucian carp into goldfish fertilized egg, could change the goldfish's genetic properties.

With the development of cloning and DNA recombinant technique, gene transfer research was initiated. Zhu et al. (1985, 1986) recombined human GH gene and mouse MT gene promoter, and then microinjected it into a fertilized egg of goldfish, and observed heterogene integration. Subsequently, many institutes have undertaken studies on gene-transfer using mainly mammalian (human and cow) GH gene. Gene-transferred common carp, crucian carp, blunt snout bream, *Paramisgurnus* sp. and trout were produced. Some of them showed significant growth superiority. The gene-transfer fish model (Zhu et al. 1989) indicated that once the heterogene expresses, it could improve the performance of gene-transfer fish significantly. Heterogene could be passed on by generative propagation and expressed in offspring. With further research, all-fish gene transfer was proposed, i.e., to produce gene-transfer fish with all the gene components from fish themselves. Zhu et al. (1990) successfully cloned the GH from common carp and grass carp and actin gene from common carp, and got functional actin promoter. Sun et al. (1993a, 1993b, 1995) separated and cloned MT gene promoter from northern common carp gene library, and cloned GH gene from salmon gene library. Then they transferred the all-fish gene of common carp MT promoter and salmon GH gene into carp, producing a new common carp strain with faster growth.

While microinjection is the major method used in gene transfer, some new methods such as sperm

Table 7. Gene transfer experiments on cyprinids in China.

Species	Promoter	Gene	IG	References
Carassius auratus	mMT	hGH	+	Zhu et al. 1985
C. auratus	RSV	neo	?	Yoon et al. 1989
C. auratus	ß-act	CAT	?	Liu et al. 1990
Cyprinus carpio	RSV	rtGH	+	Zhang et al. 1990
	RSV	csGH	+	Zhang et al. 1990
	mMT	hGH	+	Zhu et al. 1990
Megalobrama amblycephala	mMT	hGH	?	Xia et al. 1992

Notes:
IG - increased growth ; mMT - mouse metallothionein promotor ; h - human ; GH - growth hormone ; RSV - rous sarcoma virus ; neo - neomycin resistance ß-act - ß-actin ; CAT - chloramphenicol acetyltreansferase ; rt - rainbow trout ; cs - coho salmon

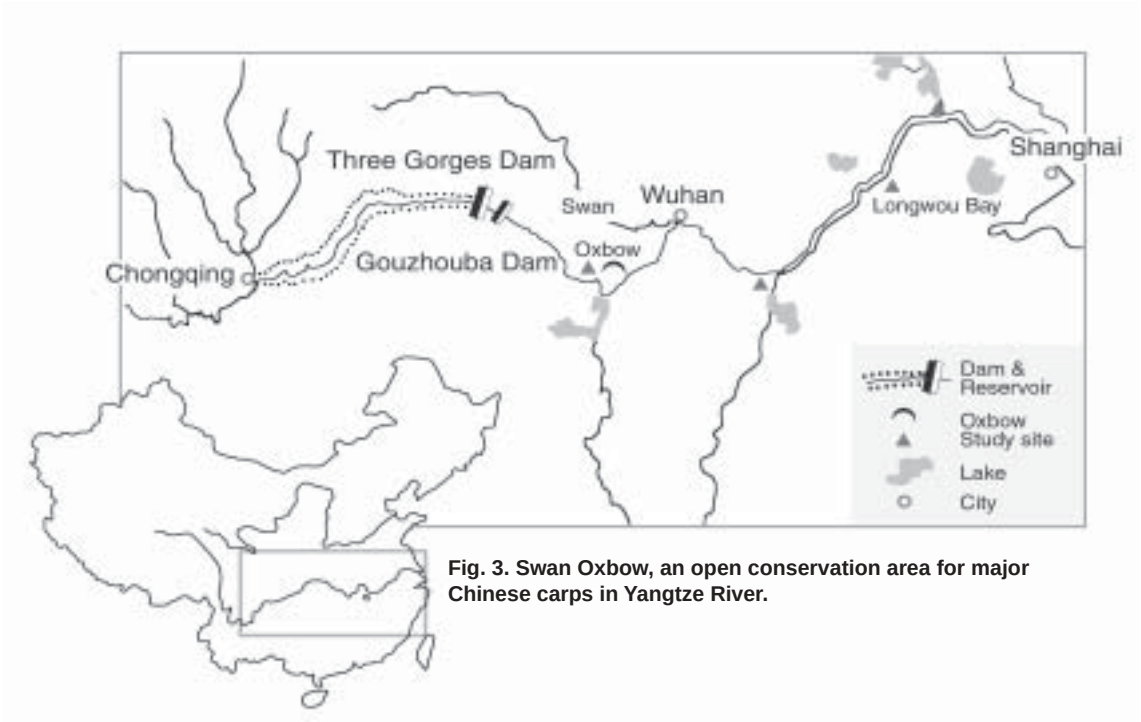

Fig. 3. Swan Oxbow, an open conservation area for major Chinese carps in Yangtze River.

vehicle, electrical pulse, injection method, *in vitro* cultivation, are also being explored. Gene-transferred fish has been investigated by many people (Table 7).

Conservation

Aquatic biodiversity in China is under stress due to high demand for fish and rapid development of aquaculture industry. Hence, genetic conservation of aquatic organisms is essential not only for the country but also for the world. Efforts are being made by the government, fisheries scientists and communes, but these are not enough.

The application of genetic principles to aquaculture is far behind that of agriculture or animal husbandry. There is no single management strategy for conservation of genetic resources. It must be considered on species-by-species and case-by-case basis. For each species, appropriate *in situ* or *ex situ* conservation needs to be undertaken. Some species that need to be conserved in China are listed in Table 8.

In situ conservation

Protecting the genetic diversity at population level involves ecosystem maintenance and species management.

The Yangtze River basin accounts for 60% of the total freshwater fish production in China. It is not only the cradle of Chinese fish culture, but also the prime world site for genetic diversity of Chinese carps and the major source of wild brooders for artificial breeding.

The Swan Oxbow Lake (Fig. 3), formed in the 1970s and has an area of 1 200 ha, remains open to Yangtze River and has been selected for major Chinese carp conservation. The carps migrate between the Oxbow Lake and Yangtze River. It is expected that the Swan Oxbow Lake can keep sufficient fish to supply brooders to hatcheries. A 2 000 ha conservation area for blunt snout bream was set up in Yuli Lake in 1990. A 300 ha natural spawning ground has been rehabilitated, and a fish screen has been built to

Table 8. Priority species for aquatic genetic resources conservation in China.

Family	Species
Cyprinidae	*Cyprinus carpio*
	Carasius auratus
	Ctenopharyngodon idella
	Hypophthalmichthys molitrix
	Aristichthys nobilis
	Megalobrama amblycephala
	Myxocyprinus asiaticus
Cichlidae	*Oreochromis niloticus*
	O. aureus
Acipenseridae	*Acipenser sinensis*
	A. dabryanus
Clupeidae	*Macrura reevesi*
Grapsidae	*Eriocher sinensis*
Trionychidae	*Trionyx sinensis*

Table 9. Live gene banks in China (Li 1996).

Farm	Province	Species
Hanjiang Yangtze River Origin Chinese Carps Farm	Jiangsu	Chinese carps
Wuhu Aquatic Origin Farm	Anhui	Chinese carps
Ruichang Yangtze River Origin Chinese Carps Farm	Jiangxi	Chinese carps
Laohe Yangtze River Origin Chinese Carps Farm	Hubei	Chinese carps
Laohekou Yangtze River Origin Chinese Carps Farm	Hubei	Chinese carps
Changsa Origin Fish Farm (Xiangjiang River)	Hunan	Chinese carps
Jiaxing Fish Farm	Zhejiang	Chinese carps
Jiujiang Pangze Crucian Carp Farm Fangzheng Crucian Carp Farm Heilongjiang Wild Carp Farm	Jiangxi Heilongjiang Heilongjiang	Pangze crucian carp Silver crucian carp Common carp
Liangzihu Blunt Snout Bream Origin Farm	Hubei	Blunt snout bream
Guangdong Tilapia Farm	Guangdong	Nile tilapia
Nanjing Tilapia Farm	Jiangsu	Nile tilapia
Qingduo Tilapia Farm	Qingduo	Nile tilapia
Mud Carp Farm	Guangdong	Mud carp
Wuyuan Red Purse Carp Farm	Jiangxi	Wuyuan red purse carp
Xingguo Red Carp Farm	Jiangxi	Xingguo red carp
Fangchang River Crab Farm	Anhui	River crab
Panjing River Crab Farm	Liaoning	River crab
Changsa Soft-shelled Turtle Farm	Hunan	Soft-shelled turtle
Shaoxing Soft-shelled Turtle Farm	Zhejiang	Soft-shelled turtle
Yantai Kelp *(Laminaria)* Farm	Shangdong	Kelp
Qidong Zicai *(Porphyra)* Farm	Jiangsu	Zicai
Hainan Aquatic Seed Farm	Hainan	Fish, shrimp

prevent fish from escaping. This conservation can protect the bream at a population level and can produce 1 500 brooder pairs, 1 900 kg of fingerlings and 100 million fry annually.

Ex situ conservation

In China, live gene banks for Xingguo red common carp (4 ha ponds) and purse red common carp (20 ha ponds) have been set up. Each bank can supply hundreds of brooders and thousands of fingerlings of red carp.

The National Committee of Aquatic Varieties Certification was established in 1991 under the Ministry of Agriculture (MOA). By 1995, 24 national farms for wild and domesticated aquatic organisms have been established to maintain and produce better brooders for hatcheries (Table 9, Fig. 4). However, some gene banks are not adequately managed due to lack of financial resources.

Germplasm storage

Even though the technology for long-term storage of fish sperm has been developed successfully, it is not yet applied broadly as a conservation method as in animal husbandry. A fish spermatozoa cryopreservation bank has been set up at the Yangtze Fisheries Institute. The sperms of economically

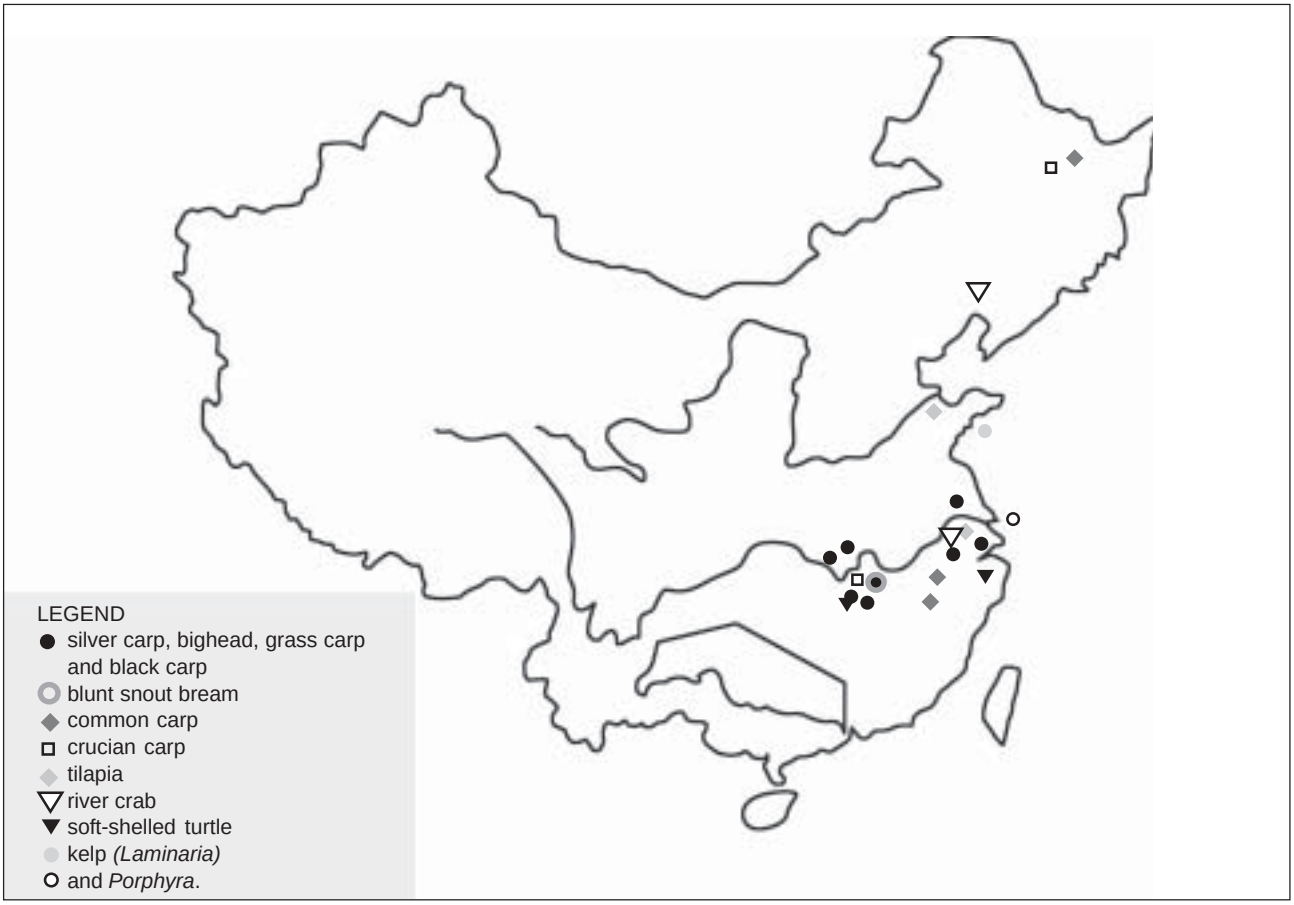

Fig. 4. Locations in China of live fish gene banks.

important species, such as grass carp, black carp, silver carp, bighead carp, blunt snout bream, Xingguo red common carp, mirror common carp and silver crucian carp are stored there.

Current Major Research

Fisheries research in China is organized by the Ministry of Science and Technology, National Foundation for Natural Science and MOA. Currently, research is conducted on the development of fast-growing strains of carps, pure strains of silver carp, bighead carp and grass carp, and conservation of genetic resources.

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FISH GENETICS RESEARCH IN CÔTE D'IVOIRE

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YAPI-GNAORE, V. 2001. Fish genetics research in Côte d'Ivoire, p. 25-28. *In* M.V. Gupta and B.O. Acosta (eds.) Fish genetics research in member countries and institutions of the International Network on Genetics in Aquaculture. ICLARM Conf. Proc. 64, 179 p.

ABSTRACT

Fish genetics research conducted in two research centers and other institutions in Côte d'Ivoire is presented. Most of this research so far conducted focused on population genetics. In addition to electrophoresis characterization, morphometric and meristic characteristics were used to evaluate and describe fish population in Côte d'Ivoire. Recent genetic research tools, such as microsatellites, restriction fragment length polymorphism of mitochondrial DNA techniques have been used. Research activities dealt mainly with tilapias (*Sarotherodon melanotheron, Oreochromis niloticus* and *O. aureus*) and Siluriformes (*Clarias gariepinus, C. anguillaris, Heterobranchus longifilis, H. bidorsalis* and *Chrysichthys nigrodigitatus*) which were mostly used for their potential for aquaculture. One of the research centers, the Fish Station, attached to the Centre National de Recherche Agronomique-Bouaké regional office, is participating in the International Network on Genetics in Aquaculture Project.

Introduction

Dependence on catch from the wild as source of animal protein is a tradition for majority of the population in Côte d'Ivoire. Consequently, overexploitation of fish population, degradation and destruction of fish habitats are major concerns for planners. The need for aquaculture development in the country has become more crucial especially because 60% of the national fish consumption is covered by importation.

For many years, the Ivorian Government has given mandate to two specialized centers to conduct fisheries and aquaculture research: the Centre de Recherche Océanologique (CRO) and the Fish Station of the newly created Centre National de Recherche Agronomique (CNRA), formerly known as the Institut des Savannes (IDESSA) Fish Station. This paper gives a brief overview of the genetics research undertaken and a description of the CNRA structure.

CNRA was created in April 1998 by merging three former research centers: IDESSA, Institut des Forêts (IDEFOR) and Centre Ivoirien de Recherche Technologique (CIRT). The new institution, with headquarters in Abidjan, received mandate to continue activities undertaken by the three centers. It is mainly funded by the private sector. In order to be fully operational and to cover smallholders' concerns in its activities, CNRA partitioned the country into five regions, i.e., Korhogo (northern), Bouaké (central), Gagnoa (west-

central), Man (western) and Abidjan (southern). One or more stations are located in each region. The Fish Station is attached to CNRA Bouaké regional office and is participating in the International Network on Genetics in Aquaculture (INGA) Project.

Genetic Research Tools

Most of the genetics research so far conducted in Côte d'Ivoire focused on population genetics. The main objectives were to characterize wild and cultured stocks of fish in terms of genetic variability between and within populations and differentiation in natural populations; make biogeographical inferences; and identify populations with special potential and of interest to fish farmers. These research activities are important because they can lead to the development of strains for aquaculture or the protection of fish populations which are at risk of extinction.

Genetic tools developed in advanced countries are adapted, or new ones are established for research conducted in Côte d'Ivoire. Species studied include natural populations and their cultured strains from Côte d'Ivoire and other regions in Africa. Enzymatic protein electrophoresis, microsatellites and restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA) techniques have been used. In a very recent collaborative project funded by the European Community, 57 fish populations collected from 23 African countries were evaluated using these three tools. The project called "GENETICS" (GENETic Investigations on Cichlids and Siluriformes) dealt mainly with tilapias (*Sarotherodon melanotheron, Oreochromis niloticus* and *O. aureus*) and Siluriformes (*Clarias gariepinus, C. anguillaris, Heterobranchus longifilis, H. bidorsalis* and *Chrysichthys nigrodigitatus)* which have potential and mostly used for aquaculture in Africa (Adépo-Gourène et al. 1997; Agnèse et al. 1997, 1998; Anon. 1997; and Vreven et al. 1998). The genetic evaluation was extended to some fisheries species - *Ethmalosa fimbriata, Sardinella aurita, S. maderensis, Carcharinus* spp., *Marcusenius ussheri* and *M. furcidens* (Gouréne et al. 1993; Adépo-Gouréne, pers. comm.). In addition to these molecular genetic tools, morphometric and meristic analysis and comparison of zootechnical performances were used.

Electrophoresis characterization

Electrophoresis has been used in Bouaké Fish Station since 1989 on two species, *O. niloticus* (Bouaké and Burkina Faso strains) and *O. aureus* (Israel and Egypt strains). The main objective is to define the genetic identity of the domestic stocks and develop management strategies for these (Rognon 1993). CRO has also recently undertaken electrophoresis, through the GENETICS project. Agnèse (1989 and 1991) and Agnèse et al. (1989) have published electrophoresis evaluations on *C. nigrodigitatus.* For example, 16 populations of *O. niloticus* collected in 17 locations were clustered in three major groups, the West African populations being in the same cluster (Agnèse et al. 1998).

Microsatellites and RFLP of mtDNA

Fish genetics research using microsatellites and RFLP of mtDNA, as part of the GENETICS Project (Anon. 1997), was conducted at CRO in collaboration with the Institut Francais de Recherche Scientifique pour le Developpement en Cooperation, Université de Montpellier, Institut National de la Recherche Agronomique, Musée Royal de l'Afrique Centrale, Institute of Marine Biology-Greece and Université Chatholique de Louvain. The above aquaculture species were evaluated and results from the analyses available, e.g., Agnèse et al. (1998), found that mtDNA of West African *O. niloticus* cannot be distinguished from that of *O. aureus.*

Morphometric and meristic analyses

Recent analyses include those of Adépo-Gourène et al. (1997) on *C. nigrodigitatus* and *C. maurus* and of Vreven et al. (1998) on *O. niloticus*. Similar research is being conducted at both university campuses in Abidjan (Abobo-Adjamé and Cocody). Taxonomic and biogeographical implications were derived from the results of the various morphometric and meristic analyses (Gourène and Teugels 1998; Teugels and Gourène 1998). Production performances (growth, survival rate, fecundity, feed conversion ratio etc.), adaptability to capture breeding or local environments and production systems were compared or evaluated among cultured strains (Otémé 1993; Da Costa and Assémien 1998). These studies provide complementary information to the molecular analysis.

Comparison of the growth performance of *O. niloticus* from four African countries, Côte d'Ivoire (BKE), Ghana (GHA), Mali (NIG) and Senegal (SEN) was undertaken (Tables 1 and 2). Weights of the fingerlings, initially varying between 60 and 75 g, reached 330-460 g in 150 days of rearing. The Mali

Table 1. Growth performance of male fingerlings of four strains of *O. niloticus* (adapted from Assemien 1998).

	Bouaké (BKE)			Ghana (GHA)		Niger (NIG)			Senegal (SEN)		
Replicate number	1.0	2.0	3.0	1.0	2.0	1.0	2.0	3.0	1.0	2.0	3.0
Initial stocking	110.0	110.0	110.0	110.0	110.0	110.0	110.0	110.0	110.0	110.0	110.0
Final stocking	109.0	107.0	105.0	110.0	101.0	109.0	108.0	103.0	97.0	101.0	104.0
Rearing period (d)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
Initial mean weight (g)	70.4	72.6	70.8	70.5	69.1	59.5	59.1	58.1	72.5	69.7	69.7
Final mean weight (g)	457.9	417.7	404.7	339.7	337.8	360.6	365.7	365.8	406.2	398.5	434.2
Average daily gain (g/d)[1]	2.8	2.2	2.1	1.7	1.7	1.9	1.9	1.9	2.1	2.1	2.3
Feed consumption index[2]	1.6	1.8	1.9	2.2	2.5	2.0	2.0	2.1	1.9	2.0	1.8
Annual production ($kg \cdot ha^{-1} \cdot year^{-1}$)[3]	19 523.0	17 178.0	16 243.0	13 851.0	12 410.0	15 334.0	15 437.0	14 665.0	15 044.0	15 246.0	17 543.0

Notes:

[1]Average daily gain = (final weight – initial weight) / rearing period.

[2]Feed consumption index = (daily ration x rearing period) / (total final weight – total initial weight).

[3]Annual production = (total final weight – total initial weight) x 10 000 m^2 x 365 days / 50 m^2 x 150 days.

Table 2. Mean weight (g) of male fingerlings of the four strains of *O. niloticus* at various periods of rearing (adapted from Assemien 1998).

	Day 0	Day 29	Day 59	Day 118	Day 150
Bouaké (BKE)	71.3 ± 1.3	143.5 ± 6.1	235.1 ± 11.2	379.7 ± 18.0	426.8 ± 22.8
Ghana (GHA)	69.8 ± 2.2	132.8 ± 3.0	201.5 ± 2.4	307.6 ± 11.3	338.8 ± 3.6
Niger (NIG)	58.9 ± 0.1	125.3 ± 4.7	199.2 ± 5.0	315.2 ± 13.9	364.0 ± 9.4
Senegal (SEN)	70.6 ± 1.3	147.5 ± 6.2	230.7 ± 7.3	355.2 ± 11.3	413.0 ± 19.3

strain, which had the lowest initial weight, had a final weight higher than that of the Ghana strain.

Bouake strain showed the highest weight at various periods. The Bouake and Senegal strains appeared to have similar growth performance compared to Mali and Ghana strains. Although the Bouake and Ghana strains are most likely to be related genetically, they differ in growth performance.

Research on Genetic Improvement of Local Tilapia Populations

Through INGA, the project, Genetic Characterization and Improvement of Native Tilapia for Culture, is being undertaken by the CNRA Fish Station in Bouake and its local partners. Preparations are underway for the collection of broodstocks of *O. niloticus* from areas where these were previously introduced such as the Niger Basin in north-west part, Volta Basin in north-east region, dams or small water bodies of the northern region, Buyo Lake and Kossou Lake. The strains collected and *O. niloticus* Bouake strain will be conditioned and bred. The progenies will be used for genetic characterization and strain evaluation experiments prior to initiation of selective breeding work.

Fish Genetics Research in the Future

Despite tremendous efforts devoted to genetics research, the present fish production level is far from being optimal. Selective breeding program aimed at rapid genetic gain on economically important production characters is lacking. One of the major constraints is the absence of qualified personnel in quantitative genetics in various research teams.

Plans for the future include training in quantitative genetics and improvement of infrastructures at the Fish Station. A quantitative geneticist will soon be recruited. The research team will be reinforced with the increase of senior fish scientists to seven by the year 2001.

The CNRA Fish Station intends to contribute, through partnerships with other institutions and competence in the country and elsewhere, to the characterization, evaluation, conservation and utilization of fish genetic resources. Plans are made for CNRA fish scientists to actively participate in national, regional and international fora where these topics are discussed.
A medium-term goal is to develop, through INGA, a selective breeding program for tilapia; test this on-station and on-farm; and fully implement it in CNRA production strategies. In the long-term, the domestication and breeding in captive conditions of inland water fish, such as *Lates niloticus, Labeo coubie, Parachana obscura* and *Distichodus rostratus,* are planned.

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AQUACULTURE GENETICS RESEARCH IN EGYPT

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ABSTRACT

Fish production in Egypt has significantly increased over the last few years reaching 546 000 t in 1998 compared to 306 000 t in 1988. The contribution of aquaculture in the overall fish production has increased as well reaching its utmost share in 1998 (26%). Research on aquaculture topics was conducted in several institutes targeting the increase of productivity as well as working on problems facing this promising sector. However, research on fish genetics in Egypt only started in recent years. The genetic improvement of tilapias and African catfish represents the focus of most genetics research in Egypt. This paper presents the status of genetics research within the International Network on Genetics in Aquaculture and in other national institutes in Egypt.

Introduction

Aquaculture in Egypt has become an increasingly important activity, as an immediate source of animal protein required for the country's growing population. The total fish production in 1998 was estimated at 546 000 t, of which 26% is from aquaculture. Aquaculture is being undertaken at extensive and semi-intensive levels. Most fish farms practice polyculture where tilapia represents about 38% of the total production. Along with tilapia (*Oreochromis niloticus* and *O. aureus*), mullets and carps are also stocked. Fish farms in Egypt include seven government farms (total area of 4 000 ha) and private fish farms (total area of about 50 000 ha) that are located around the lakes. All fish farms rely to some extent on hatcheries for obtaining their seed requirements. Most fish hatcheries belong to the General Authority of Fish Resources Development. The main fish seed produced are common carp, Chinese carps and tilapia. However, new tilapia hatcheries are now emerging. The total production of tilapia fry from hatcheries or fish farms in 1998 was estimated at about 49.9 million fry. *O. niloticus* and *O. aureus* dominate the fry production.

Most consumers in Egypt prefer tilapia compared to other freshwater fish. There are four tilapia species in Egypt: Nile tilapia (*O. niloticus*), blue tilapia (*O. aureus*), white tilapia (*Sarotherodon galilaeus*) and green tilapia (*Tilapia zillii*).

Fish Genetics Research

Fish genetics research in Egypt has a relatively short history concentrating initially on the biochemical identification and strain evaluation. The institutes/

universities working on fish genetics in aquaculture in Egypt are the: Central Laboratory for Aquaculture Research (CLAR), Abbassa; International Center for Living Aquatic Resources Management Regional Research Center for Africa and West Asia, Abbassa; National Institute of Oceanography and Fisheries; Veterinary College-Idfena, Alexandria University; Genetics Department, Faculty of Agriculture, Ain Shams University; Genetics Department, Faculty of Agriculture, Assuit University; Zoology Department, Faculty of Science, Zagazig University; Zoology Department, Faculty of Girls, Ain Shams University; and Genetic Engineering Research Institute, Sadat City, Monofaya University.

Performance evaluation and genetic characterization

CLAR is leading collaborative research along with two schools in Ain Shams University, i.e., Zoology and Genetics. The objectives of the research are to:

- determine whether Nile tilapia originated from a single strain or several strains;
- compare the reproductive performance among different stocks of Nile tilapia collected from three geographical locations: Maryout (MR) - Alexandria at North of Egypt (which has lower temperature); Zawia (ZW) - Kafer El Sheikh at the Delta area (which has saline soil); and Abbassa (AB) middle of delta area as a reference strain;
- compare the growth performances of the three stocks; and
- carry out a biochemical genetic analysis to determine the genetic variations within and among stocks collected from different locations.

This research was undertaken since strain differences, inbreeding and crossings could affect reproductive performance in cultured fishes. Large strain differences influenced reproductive performance in channel catfish (Dunham and Smitherman 1984) and rainbow trout (Kincaid 1976). If similar genetic differences exist for reproductive performance in tilapia, selection of a species or strain of tilapia with lower fecundity might be desirable for minimizing reproduction in rearing ponds. In contrast, strains with higher fecundity may be desirable for enhancing reproduction in hatcheries.

Abbassa stocks with an average weight of 150 g were collected from the production ponds. Maryout stocks were collected as fry (1 g) and then nursed to larger size in earthen ponds. Zawia stocks (150 g) were brought to Abbassa and maintained in ponds until used for spawning. All fish groups were acclimatized to Abbassa conditions upon their arrival.

Spawning activities

The spawning activities of the three strains and their crosses were carried out in circular earthen ponds of 100 m^2 each during 1996 and 1997 (Table 1).There were differences in the total number of fry produced from strains evaluated. During 1996 and 1997 studies, Abbassa strain was highly reproductive, while Maryout and Zawia strains had lower reproduction (Tables 2 and 3). There were differences in the total number of fry produced from each crossing. The cross between Abbassa x Zawia was highly reproductive, while Maryout x Abbassa came intermediate and Zawia x Maryout was the lowest (Table 4). Crossing with Abbassa strain indicated its superiority with regard to fry production.

Growth performance

To evaluate the growth performance of three stocks and their hybrids, the fish were stocked in 0.1 ha earthen ponds at a rate of one fish per square meter. Supplementary feed (25% protein) was given at a rate of 10% of body weight, six days a week for 45 days and later decreased to 5% of body weight. Biweekly samplings were carried out when random samples were individually measured for total length and weight. At the end of the growing season, harvesting was done and individual lengths and weights were estimated. The total yield for each pond was determined.

The growth rate is certainly a key element regardless of the production system. Studies undertaken during

Table 1. Details of spawning to evaluate performance of three strains of *O. niloticus*, 1996-1997.

Spawning	1996 season	1997 season
Period	6 Jun – 30 Sep	20 Jul – 15 Sep
Sex ratio	1.5 : 1	1.5 : 1
No. of females/ponds	66	66
No. of replication	2	2
Harvest intervals	3 weeks	3 weeks
Fish stocks	3 stocks	3 stocks
Crosses	3 crosses	3 crosses
No. of harvest	7	2
Temperature range (°C)	21-25	22-25

Table 2. Fry production of three strains of *O. niloticus* in circular earthen ponds of 100 m² each, 1996.

Strain	No. of female	Mean weight (g)	No. of harvest	No. of fry/ female	No. of fry/g body weight	No. of fry produced
Abbassa	25	144	7	286	1.99	50 000
Zawia	25	265	7	217	0.82	38 030
Maryout	25	282	7	196	0.69	34 230

Table 3. Fry production of three strains of *O. niloticus* in circular earthen ponds of 100 m² each, 1997.

Strain	No. of female	Mean weight (g)	No. of harvest	No. of fry/ female	No. of fry/g body weight	No. of fry produced
Abbassa	66	161.7	2	190	1.17	12 507
Zawia	66	187.0	2	158	0.84	12 507
Maryout	66	268.5	2	159	0.85	10 494

Table 4. Fry production of three crossings of *O. niloticus* in earthen circular ponds of 100 m² each, 1997.

Crossing	No. of female	Mean weight (g)	No. of harvest	No. of fry/ female	No. of fry/g body weight	No. of fry produced
Abbassa (F) x Zawia (M)	66	161.7	2	190	1.17	12 507
Maryout (F) x Abbassa (M)	66	187.0	2	158	0.84	10 424
Zawia (F) x Maryout (M)	66	268.5	2	159	0.85	10 494

1996 and 1997 showed that Maryout strain attained higher growth compared to Abbassa and Zawia strains (Tables 5 and 6).

At the end of the culture period, growth rate among crosses was significantly different (P=0.01). In 1996, the highest growth was shown by the crossing of Zawia x Maryout, followed by Maryout x Abbassa; Abbassa x Zawia was the lowest (Table 5). The same trend was observed in 1997 (Table 6).

SDS-PAGE and isozymes by using starch gel electrophoresis were carried out for the three strains and their crosses starting from the parents, first generations, first crossings, second generations and second crossings. The analysis showed variations among and between strains from three different locations.

Salinity tolerance of Nile tilapia

Differences related to performance traits exist among fish species as well as among strains within a species. Compared to most tilapia species, *O. mossambicus* is more salt-tolerant while *O. aureus* is more cold-tolerant. Within species, differences were detected among Egypt, Ivory Coast and Ghana strains of *O. niloticus*. Examples of such differences gave this research group reason to study the salinity tolerance for strains of *O. niloticus.*

A study by CLAR and the Genetics Department, Faculty of Agriculture, Ain Shams University, aims: (1) to determine the lethal salinity levels of some strains of *O. niloticus* and compare these with other tilapia species and (2) to develop salinity-tolerant *O. niloticus* through subsequent use of survivors in breeding program. The study is in progress, and new data on genetic parameters and heritability values for salinity tolerance will be estimated.

Genetic and physiological studies on fish from polluted locations

Seas, rivers and lakes are the eventual sinks for many of harmful or waste substances disposed of by humans. Aquatic life, including food fishes, is capable of absorbing and accumulating various chemicals especially heavy metals which cause adverse effects on the aquatic biota. These effects include deleterious changes which disrupt the metabolic activity at a biochemical level (Hinton et al. 1973).

Egypt's coastal lakes act as temporary reservoirs for drainage water and often are highly contaminated with anthropogenic materials. This is true particularly for Lake Manzala and Lake Maryout and to a lesser extent for Lake Edku (El-Rayis and Saad 1984).

Collaborative research between CLAR and the Department of Zoology, Faculty of Science, Zagazig University, is in progress to study the genotype of fish collected from different polluted areas, chromosomal aberrations, biochemical electrophoresis analysis for protein, and some isozymes and micronucleus test on freshwater fish.

Table 5. Comparison of growth performance for three strains of *O. niloticus* and their crosses after 105 days of culture in earthen ponds, 1996.

Strain	Abbassa (AB)	Zawia (ZW)	Maryout (MR)	AB (F) x ZW (M)	MR (F) x AB (M)	ZW (F) x MR (M)
Date of stocking	14 Jul 1996	14 Jul 1996	14 Jul 1996	1 Aug 1996	1 Aug 1996	1 Aug 1996
Growing period	105.00	105.00	105.00	105.00	105.00	105.00
Mean initial weight (g)	2.80	2.80	2.80	0.50	0.50	0.50
Mean final weight (g)	86.80	167.50	177.70	97.32	119.86	161.44
Survival rate (%)	93.00	90.00	98.00	91.00	90.00	95.20
Average daily weight gain (g/day)	0.80	1.57	1.60	0.92	1.12	1.53
Feed conversion ratio	1.13	1.78	2.30	1.51	1.67	1.80
Condition factor	2.22	2.51	2.73	2.26	2.33	2.46
Yield per feddan (kg)	339.00	633.20	731.40	345.25	424.30	614.67
Weight gain/fish (g)	84.10	167.70	168.90	96.82	117.63	160.94
Specific growth rate (%)	3.27	3.89	3.95	5.02	5.20	5.50

Table 6. Comparison of growth performance for three strains of *O. niloticus* and their crosses after 75 days of culture in earthen ponds, 1997.

Strain	Abbassa (AB)	Zawia (ZW)	Maryout (MR)	AB (F) x ZW (M)	MR (F) x AB (M)	ZW (F) x MR (M)
Date of stocking	10 Sept 1997	10 Sept 1997	10 Sept 1997	10 Sept 1997	10 Sept 1997	10 Sept 1997
Growing period	75.00	75.00	75.00	75.00	75.00	75.00
Mean initial weight (g)	1.40	1.20	1.80	1.40	1.20	0.30
Mean final weight (g)	32.81	41.87	46.60	36.03	33.00	39.75
Survival rate (%)	94.00	88.10	90.00	86.50	89.50	87.50
Average daily weight gain (g/day)	0.39	0.51	0.53	0.43	0.43	0.49
Feed conversion ratio	0.35	0.36	0.41	0.36	0.30	0.55
Condition factor	2.20	2.24	2.25	2.23	2.10	2.28
Yield per feddan (kg)	129.50	154.90	176.14	131.20	124.10	146.10
Weight gain/fish (g)	31.41	40.67	44.80	34.63	31.80	45.00
Specific growth rate (%)	4.21	4.73	4.33	4.21	4.54	6.51

The study is also undertaking hematological examination to determine the total erythrocyte count, hematocrit value, hemoglobin concentration and hematological index (mean corpuscle volume, mean corpuscle hemoglobin and mean corpuscle hemoglobin concentration). It also conducts biochemical analysis to determine the total protein, total lipid, and liver and kidney functions.

DNA fingerprinting

The Genetics Department, Ain Shams University, is doing genetic fingerprinting in *O. niloticus* and detecting some of its lines by isozyme, organ distribution and RAPD-PCR DNA markers. Three lines of *O. niloticus* collected from different locations - Lake Manzala, Lake Nasser (Foki line) and Sohag hatchery - were analyzed. Six isozyme systems (Adh, Acph, Est, Ldh, Mdh and To) were detected in 10 organs using starch gel electrophoresis technique. The data revealed that Esterase and Lactate dehydrogenase isozymes systems are more powerful in line-detecting organs, while the other systems are sufficient to differentiate line-detecting localities. The DNA markers using RAPD-PCR technique were carried out.

CLAR/INGA Genetics Project

This project is called "Genetic Enhancement of Egyptian Farmed Tilapia under Different

Environmental and Culture Conditions". Its research includes:

- evaluation of strains of *O. niloticus* to identify the ones that perform better under different environmental and climatic conditions (ponds, cages, ricefields);
- genetic characterization of different strains; and
- production of new breeds that are more suitable for Egyptian aquaculture conditions.

The data collected from evaluation work will be used to establish an effective selective breeding program.

For this research, four different stocks of *O. niloticus* were collected from Ismailia canal at the center of Delta area (around Abbassa area) with moderate temperature, Lake Manzala in the northern part of Delta, and Lake Nasser, south of Egypt, with high temperature climate and Maryout strain from Maryout hatchery, originally from north of Egypt with cold temperature climate. Reproductive performance of Ismailia, Manzala and Maryout stocks has been studied.

Winter growth evaluation of Nile tilapia strains

Temperature tolerance is a key factor in tilapia production in temperate zones and to some extent in subtropical zones where tilapia aquaculture is characterized by seasonal changes in water temperature (Lahav and Ra'anan 1997). During winter, water temperature may drop to levels that cause severe growth inhibitions and sometimes, mortality. A species or a hybrid with greater cold tolerance becomes more valuable and may significantly improve the profitability of the industry. Moreover, the relatively short growing season in temperate climates could be extended as species and/or strains of cold-tolerant tilapia are surviving and/or able to continue growing at temperatures few degrees less than their noncold tolerant counterparts. In a three-replicate experiment, three strains of *O. niloticus* were stocked in 0.1 ha earthen ponds starting 15 December 1998, as temperature was declining in order to compare the winter growth for the three strains. As the work progresses, new data on genetic parameters and heritability values for cold tolerance will be developed.

Genetic evaluation of African catfish

Taxonomists and systematists attempt to distinguish species and hypothesize lineage by conducting studies of genetically based differences and similarities among populations. Historically, genetic differentiation has usually been inferred from a comparison of morphological characters. There is, however, an increasing trend in taxonomy to supplement morphological, analogical, ethalogical, biochemical or karyotypic characters among populations as a means of detecting genetic divergence. The African catfish is widely distributed throughout Africa. It inhabits tropical swamps, lakes and rivers, some of which are subject to seasonal drying. In the northen and central part of Africa, it has been described as *Clarias lazera;* in the eastern part as *C. senegalensis;* in the western part as *C. mossambicus;* and in the southern part as *C. gariepinus*.

The research at CLAR aims to:

- evaluate different catfish strains collected from various environmental conditions;
- make a biochemical genetic analysis (SDS-PAGE and isozyme electrophoresis) and a RAPD-DNA marker analysis.

It plans to collect broodstocks of *Clarias* from three locations, Abbassa, Kafer El-Seikh and Nile River, and evaluate the origin of these strains.

Other Research Highlights

The following research activities are being undertaken at the National Institute of Oceanography and Fisheries, Alexandria:

- karyological analysis of two species of family Sparidae (*Sparus auratus* and *Lithognathus mormyrus)*;
- isolation of salinity-resistant gene from *Artemia salina*;
- genotype analysis of *Tilapia* spp. at Lake Manzala;
- determination of the effect of water pollution in Lake Manzala on RNA/DNA ratio in *Tilapia* spp. in Egypt;
- genetic differentiation of sarcoplasmic protein in family *Mugilidae* at different habitats;
- determination of genetic variability of *Mugil cephalus* in freshwater and marine habitats;
- determination of the effect of two herbicides, Saturn and Ronstar, on the cytological character of and on electrophoretic protein polymorphism of *C. lazera.*

At the General Authority for Fish Resources Development, genetic development within tilapia strains in Egypt was carried out through a selection

program in government hatcheries. *O. niloticus* (parent, first and second generations) was selected for body weight and total body length of each of the three lines (Saft–Khaled, Sohag and Foki lines).

At the Idfena Veterinary College, Alexandria University, research on tilapia includes commercial production of monosex tilapia and supermale production of *O. niloticus.*

Most research activities at the Genetic Engineering Research Institute, Monofaya University, Sadat City, deal with fingerprinting and gene transfer.

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A COMPARATIVE EVALUATION OF TWO TILAPIA STRAINS IN FIJI

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ABSTRACT

The reproductive, survival and growth performances of Nile tilapia *(Oreochromis niloticus)*, Fijian Chitralada strain and that developed from the Genetic Improvement of Farmed Tilapia (GIFT) program in the Philippines, were evaluated in two culture environments, integrated and nonintegrated.

Results showed that the Chitralada strain had more participating breeders and higher fry survival than the GIFT strain. The latter had higher fecundity but the brooders showed a high incidence of fry cannibalism. It also had a slightly better growth than that of Chitralada strain. Growth of the GIFT strain in the integrated system was better than in the nonintegrated one, while there was no significant difference in growth between the two systems for Chitralada strain.

Introduction

Tilapia genetics research started in 1993 in Fiji by the Fisheries Division, Ministry of Agriculture, Fisheries and Forests (MAFF) in collaboration with the Queensland University of Technology, Brisbane, Australia, and the Institute of Advanced Studies, University of Malaya, Kuala Lumpur, Malaysia, under a project entitled Genetic Identification and Stock Improvement of Tilapia in Malaysia and Fiji. Phase1 of this project was funded by the Australian Centre for International Agricultural Research (ACIAR) and was completed in December 1995.

During the first phase, the culture performance of four tilapias in Fiji, namely *Oreochromis mossambicus*, Israel and Chitralada strains of Nile tilapia (*O. niloticus*) and red tilapia hybrid, was assessed for three consecutive generations under two aquaculture production systems (integrated and nonintegrated ponds) to determine which of the strains was performing best. Results of the study showed that Chitralada strain was the best performing strain in Fiji.

This led to the second phase of the project on Genetic Improvement of Tilapia in Fiji and Redclaw

in Australia, funded also by ACIAR. The project commenced in January 1998 and aims in the first instance to compare the performance of the Chitralada strain with the GIFT strain, then undertake selection for three generations of best performing strain under Fijian conditions. Concurrently, microsatellite markers will also be developed through collaboration between Queensland University of Technology and the Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia. Individual performance in culture will be correlated with allelic variation to determine the potential for marker-assisted selection for further improvement of cultured strains.

Another objective is to provide training for research staff to enhance skills in experimental design techniques and statistical methodology and in tagging and related techniques for selection progams. The project also aims to develop extension procedures for farmers for the effective dissemination of genetic gain in improved breeds.

Future research will be towards repetition of the comparative breeding and growout trials of the two tilapia strains (GIFT vs. Chitralada).

Materials and Methods

Tilapia strains

The GIFT strain of *O. niloticus*, was imported from the Philippines in August 1997 and maintained at the Naduruloulou Fisheries Station. Fifth generation of Chitralada was imported from the National Inland Fisheries Institute, Thailand, in August 1988 and is currently being used by farmers throughout the country.

Evaluation of culture performance

Breeding of the two strains was carried out at Naduruloulou Aquaculture Station from 1 May to June 1998. In a breeding design structured to generate as many families per strain, 25 fine-mesh breeding hapas (1m x 1m x 1m) were each stocked with four females and two males, and were set up in a 2 500 m^2 pond. Brooders were fed with a grower mix feed twice daily until they spawned. Dead brooders resulting from damage from handling during stocking were replaced only during the first week.

Fry collection began soon after swim-up fry were observed. Spawned females were identified and transferred into separate holding ponds immediately after fry collection. For each strain, fry were collected on the same day and their number was estimated. They were stocked in nursery hapas at a density of approximately 200 fry per hapa. The fry were fed a fry mix feed comprising 50% fish meal and 50% rice bran three times a day. Collection was carried out every fourth day or whenever swim-up fry were observed. Fry ages were carefully noted, and upon transfer to larger mesh-size hapas, only fry spawned within 40 days of collection were pooled as a single batch. Early fry survival counts were made on days 20-24.

The fry were then nursed for 2 months until they reached fingerling size (10–11 g). The nursery hapas were changed every 14 days for the first month and thereafter transferred to bigger mesh size hapas (2 m x 2 m x 1.5 m) to allow for easy movement of water through the hapa.

Breeding efficiency (the proportion of females spawned), average fecundity (the total number of fry produced divided by the number of female spawners in the breeding cycle) and early fry survival (after 20 days) were calculated for each strain at the end of the breeding trial.

Growth performance trials were undertaken during September 1998 - January 1999. Trials were carried out in three replicate ponds integrated with ducks and in three nonintegrated ponds each approximately 600 m^2 in size and 1m in depth. Each integrated pond had a bamboo hut which housed 40 ducklings (2 - 4 weeks old) at the beginning of the growth cycle. Both types of ponds were limed (1 250 $kg \cdot ha^{-1}$ of hydrated lime) and manured with chicken manure (1 250 $kg \cdot ha^{-1}$) before stocking of fry. Using a communal pond concept while keeping the strains separate, 100 representatives from each strain were stocked into rearing hapas (2 m x 2 m x 1.5 m with mesh size 0.5 cm) in each of the six ponds in a randomized design. Initial weights were taken before stocking. A commercial tilapia pellet (29.9% crude protein) was used for feeding twice daily.

Sampling of individual weights and lengths was made on 30 fish per strain in each pond every 23 days (except for the first sampling which was 30 days). Sex was noted as soon as it could be

determined. Final fish weights and lengths were measured on all fish at the end of the fourth month (123 days). Rearing hapas were replaced at each sampling period to avoid fouling.

Water quality parameters were also measured during the growth trial and occasional algal blooms were flushed out by water exchange. Mean daily weight gain (MDWG), food conversion efficiency and sex ratios were calculated for each strain on completion of the growth trial.

Analysis of strain differences

Comparisons of means between and within strains, and between systems were carried out on Microsoft Excel using the t-test in descriptive statistics at p=0.05 (5% confidence interval).

Results and Discussion

Reproduction and survival

Fry of the Chitralada strain were sighted after 15 days while those of the GIFT strain were sighted after 23 days. Table 1 summarizes results of the breeding trial. The delay in breeding of the GIFT strain may have been due to temperature aside from the high mortality of brooders from handling and disturbances during replacements.

Table 1. Evaluation of reproduction and survival of Chitralada and GIFT strains of *O. niloticus*.

Breeding and fry production	GIFT strain	Chitralada strain
No. of females spawned /100	31	48
Average fecundity	211	194
Total no. of fry	6 532	11 760
Average weight of brooders (g)		
Male	800	700
Female	400	300
Survivors at 20 days	5 531	9 318
Average early survival (%)	84	79

Pond conditions

During the breeding trial, pond water pH in the morning ranged from 7.5 to 8.0 and in the afternoon from 8.3 to 8.4. Morning temperatures ranged from 24.4 to 26.9°C and afternoon ones, from 26.7 to 27.3°C. Dissolved oxygen readings ranged from 5.0 to 6.8 in the morning and from 6.9 to 8.0 in the afternoon.

During the four-month growth trial, water temperature and pH did not differ significantly between ponds. The pH morning readings ranged from 7.7 to 8.7, and temperatures from 24.7 to 30.6°C. Afternoon readings ranged from 8.5 to 9.6 for pH and 26.3 to 34.9°C for temperature (Tables 2 and 3). Of the three parameters (temperature, pH and dissolved oxygen), temperature was found to have a significant effect on growth and reproduction.

Growth trial

After 4 months, the average weight was higher for the GIFT strain, the difference being significant (P=0.05) only at the end of the trial (Fig. 1). The differences in growth may be attributed to the fact that majority of the GIFT fish had not reached maturation at the end of the growout period (4 months) as compared to the Chitralada ones, which

Table 2. Average temperatures (°C) during growth trial.

Month	Pond 1		Pond 2		Pond 3		Pond 4		Pond 5		Pond 6	
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Sep	24.7	26.4	24.9	26.5	24.9	26.4	24.8	26.4	24.7	26.2	24.8	26.3
Oct	27.6	29.6	27.5	29.2	27.7	29.9	27.7	29.4	28.0	29.7	27.5	29.6
Nov	29.7	32.3	29.6	32.4	29.8	31.9	29.5	32.0	29.7	32.0	29.5	31.9
Dec	30.4	34.9	30.0	34.8	30.6	34.9	30.0	34.8	30.0	35.0	30.0	34.9

Table 3. Average water pH during growth trial.

Month	Pond 1		Pond 2		Pond 3		Pond 4		Pond 5		Pond 6	
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Nov	7.7	9.0	8.1	9.5	8.2	9.6	8.1	9.3	7.9	9.3	8.0	9.3
Dec	7.8	8.5	8.3	9.3	8.5	9.2	8.7	9.6	8.4	9.2	7.7	8.9

Note: Meter was faulty during first two months.

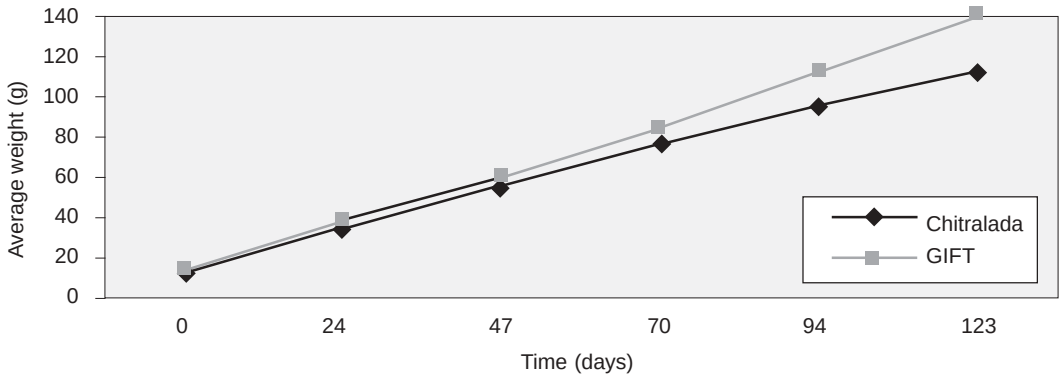

Fig. 1. Average weight of two strains of *O.niloticus* during growth trial.

were all mature and breeding at the time. In addition, GIFT strain had 60% males in the total sample compared to 48% males of Chitralada strain. Tables 4 and 5 show average weights for mixed sex at each sampling interval, and Table 6, the average weights for separate sexes.

It was evident from samples 1 to 4 that sexual dimorphism existed for both strains. Comparisons between strains at 94 days showed that on average, GIFT males had 17% and GIFT females had 11% growth advantage over Chitralada males and females, respectively. There was no significant difference found among females and among males of each strain.

However, comparing the sexes within strains showed a significant difference in GIFT strain in which males had 30% growth over females, and in Chitralada strain in which males had 25% growth over females (Table 7). Considering that individuals used in growth comparisons were selected randomly from different families, variable sex ratios may just be an effect of sampling. The skewed bias towards males observed in the GIFT strain may be caused by some degree of nonrandom sampling due to lower number of fry available for this strain.

Results of comparative production potential of GIFT and existing local strains of *O. niloticus* carried out in

Table 4. Average body weight (g) of GIFT strain of *O. niloticus* at different samplings.

Pond no.	No. of days					
	Initial 9 Apr 1998	S1 24 days	S2 47 days	S3 70 days	S4 94 days	final 123 days
P1 (NI)	14.50	43.10	71.10	87.20	124.00	135.00
P2 (NI)	13.30	42.80	46.60	71.10	87.90	153.00
P3 (I)	13.60	37.70	59.80	84.00	116.00	131.00
P4 (I)	15.40	33.10	58.30	93.20	125.00	155.00
P5 (I)	15.40	37.30	65.40	115.00	142.00	170.00
P6 (NI)	11.30	38.80	55.50	59.40	75.70	95.60
Overall	14.30 ± 00.38	38.80 ± 10.53	59.50 ± 03.43	84.00 ± 07.02	112 .00 ± 10.21	140.00 ± 10.60

Notes: P - pond; S - sampling number; NI -nonintegrated; I - integrated.

Table 5. Average body weight (g) of Chitralada strain of *O. niloticus* at different samplings.

Pond no.	No. of days					
	Initial 9 Apr 1998	S1 24 days	S2 47 days	S3 70 days	S4 94 days	final 123 days
P1 (NI)	14.50	38.40	73.50	94.10	121.00	126.00
P2 (NI)	12.70	33.80	38.20	52.10	77.00	109.00
P3 (I)	13.90	35.40	59.20	77.10	90.70	106.00
P4 (I)	14.10	35.40	51.50	64.80	80.90	111.00
P5 (I)	13.40	36.90	64.90	109.00	140.00	147.00
P6 (NI)	14.10	32.50	45.90	54.00	66.80	73.90
Overall	13.70 ± 00.26	35.40 ± 00.86	55.50 ± 05.27	76.30 ± 10.01	96.00 ± 11.60	112.00 ± 09.86

Notes: P - pond; S - sampling number; NI -nonintegrated; I - integrated.

Table 6. Average body weight (g) of two strains of *O. niloticus* by sex from three sampling intervals.

		Male	Female
Chitralada strain	S2 (47 days)	67.10 ± 04.10	46.6 ± 3.5
	S3 (70 days)	86.70 ± 11.20	60.9 ± 7.2
	S4 (94 days)	106.00 ± 13.10	80.0 ± 9.1
GIFT strain	S2	77.20 ± 03.50	49.0 ± 3.4
	S3	98.70 ± 08.20	72.6 ± 5.6
	S4	128.00 ± 11.20	90.0 ± 6.7

Table 7. Final sex ratio for two strains of *O. niloticus*.

Pond	Chitralada strain		GIFT strain	
	Female	Male	Female	Male
1	50	50	26	74
2	49	51	44	54
3	44	56	52	46
4	56	44	47	53
5	53	47	39	61
6	59	41	33	67
Total	311	289	241	355
%	52	48	40	60

Bangladesh also showed that GIFT strain had better growth than other strains after 3 months (GIFT average weight gain was 40% better than existing strains) (Mazid et al. 1996).

GIFT fish had higher MDWG and were better at converting feed into growth than Chitralada (Tables 8 and 9). MDWG during growout decreased and food conversion ratio increased after day 94 for both strains due to females breeding (eggs were observed in mouths of females) (Figs. 2 and 3). The significant difference in MDWG between two strains was also due to the higher number of males in the GIFT sample.

When comparing growth of the strains between two conditions (integrated and nonintegrated), there was no significant difference for Chitralada strain (Fig. 4) while for GIFT strain, there was a significant difference towards the end of the growout where the fish in the integrated system showed higher growth than in the nonintegrated system (Fig. 5).

The age batch designated in this study (within 40 days age difference) was found to be a reliable basis for comparing strains which did not result in problems of age or size differences. Palada-de Vera and Eknath (1993) found no significant effect of initial size differences on growth rate of *O. niloticus* strains across three batches collected at one-week intervals.

The genotype-environment interaction was not analyzed in this study. However, studies by Macaranas et al. (1997) at the same experimental site (Naduruloulou, Fiji), on two *O. niloticus* strains, Chitralada and Israel, showed no significant genotype-environment interactions within a range of environments.

Since this is the first experience with the GIFT fish, the project made some accomplishments with regard to breeding (fry cannibalism and handling of brooders) of this strain. Results of the first comparative

Table 8. MDWG (g) and food conversion rate (FCR) for Chitralada strain of *O. niloticus* at different samplings.

	Pond 1 : NI		Pond 2 : NI		Pond 3 : I		Pond 4 : I		Pond 5 : I		Pond 6 : NI		Overall	
	MDWG	FCR	MDWG	FCR	MDWG	FCR	MDWG	FCR	MDWG	FCR	MDWG	FCR	MDWG	FCR
S1	1.00	1.75	0.88	1.76	0.91	1.80	0.89	1.84	0.98	1.64	0.77	1.53	0.90	1.72
S2	1.53	0.58	0.19	3.52	1.03	0.68	0.70	0.59	1.22	0.58	0.58	0.64	0.88	1.10
S3	0.90	0.99	0.60	1.02	0.78	1.08	0.62	1.15	2.17	0.56	1.93	0.53	1.12	0.82
S4	1.10	0.80	1.04	0.59	0.57	1.48	0.63	1.26	1.05	1.18	0.53	1.23	0.82	1.09
Final	0.18	6.70	1.14	0.80	0.55	1.80	1.08	0.80	0.25	6.20	0.25	3.00	0.58	3.20

Notes: NI - nonintegrated; I - integrated.

Table 9. MDWG (g) and FCR for GIFT strain of *O. niloticus* at different samplings.

	Pond 1 : NI		Pond 2 : NI		Pond 3 : I		Pond 4 : I		Pond 5 : I		Pond 6 : NI		Overall	
	MDWG	FCR	MDWG	FCR	MDWG	FCR	MDWG	FCR	MDWG	FCR	MDWG	FCR	MDWG	FCR
S1	1.19	1.47	1.23	1.33	0.99	1.68	0.74	2.61	0.91	2.06	0.77	1.53	1.03	1.83
S2	1.22	0.64	0.17	2.20	0.96	0.62	1.10	0.80	1.22	0.58	0.73	1.48	0.90	1.05
S3	0.70	1.08	1.09	0.70	1.05	0.87	1.52	0.70	1.87	0.66	0.17	3.03	1.07	1.17
S4	1.50	0.74	0.68	1.07	1.33	0.69	1.33	0.71	1.40	0.87	0.68	0.90	1.15	0.83
Final	0.39	3.40	2.33	0.40	0.54	2.40	1.07	1.30	1.00	1.60	0.71	1.20	1.01	1.70

Notes: NI - nonintegrated; I - integrated.

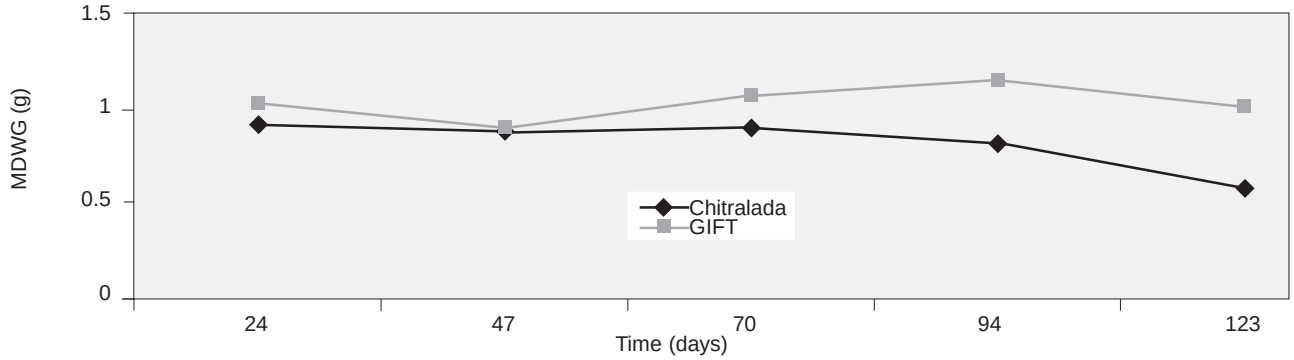

Fig. 2. MDWG (g) of two strains of *O. niloticus* during growout.

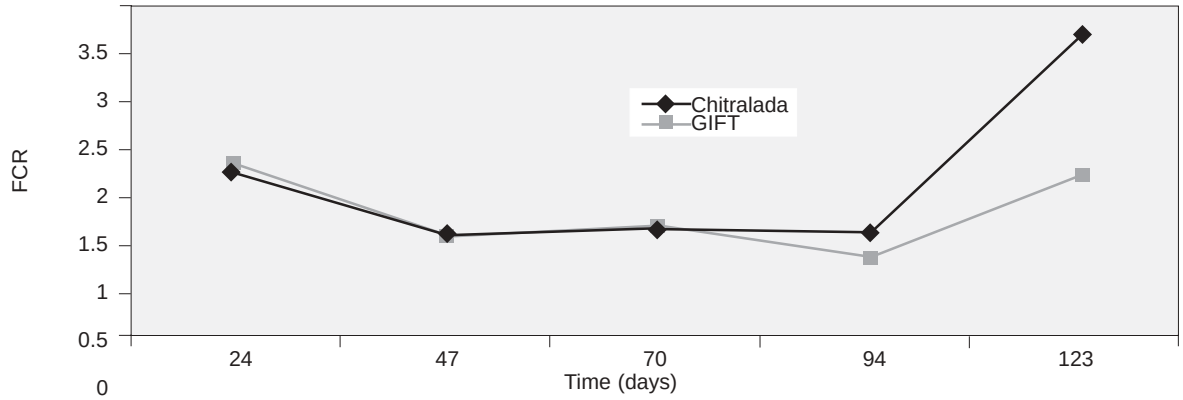

Fig. 3. Food conversion rate of two strains of *O. niloticus* during growth trial.

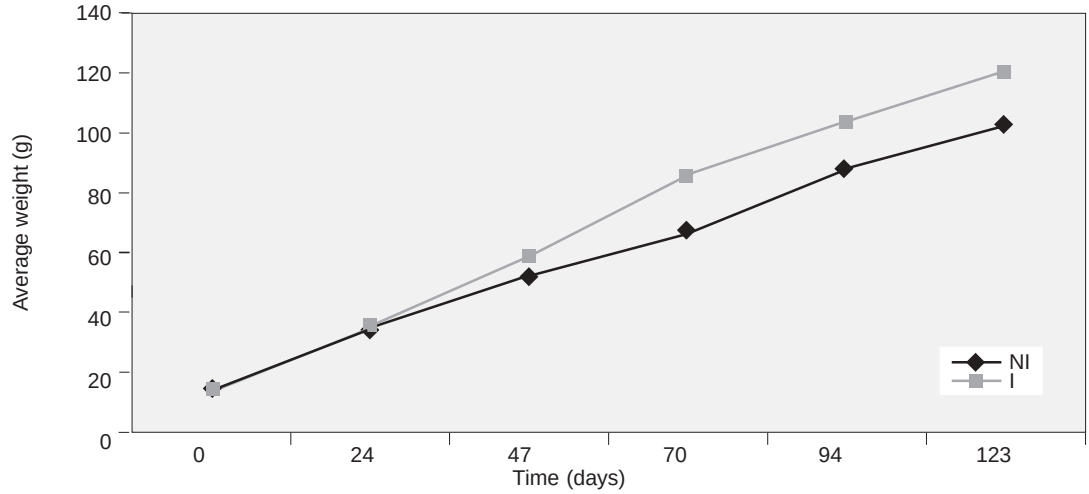

Fig. 4. Average weight of Chitralada strain of *O. niloticus* under different farming systems.

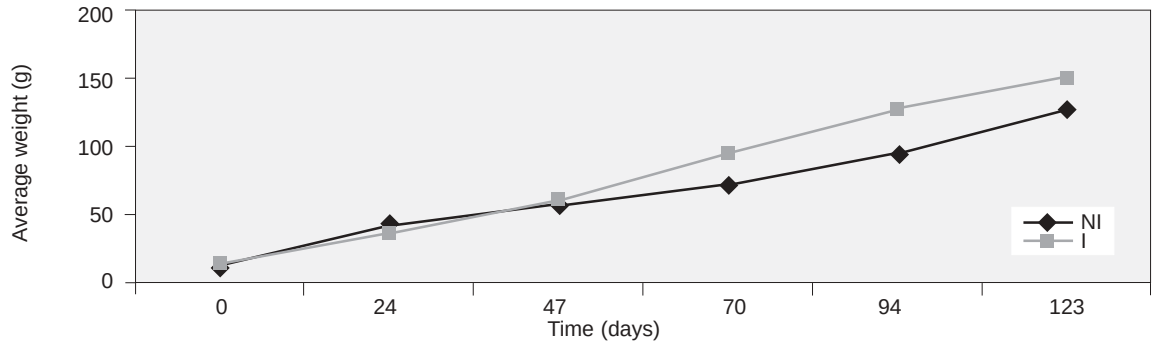

Fig. 5. Average weight of GIFT strain under different conditons.

breeding and growth trial indicated that the GIFT strain performed slightly better than the Chitralada strain. A more substantial statistical analysis of data from this trial is being carried out. To complete the evaluation of performance of the two strains, the trial was repeated in April-May 1999 for breeding and in June-September 1999 for growth.

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AQUACULTURE GENETICS RESEARCH IN INDIA : AN OVERVIEW

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ABSTRACT

Aquaculture genetics research in India is relatively recent. Initially, research was limited to production and evaluation of carp hybrids. Among the Indian carp species, as many as six interspecific and 13 intergeneric hybrids have been produced. Besides these, hybrid crosses were also made between Indian and Chinese grass carp, silver carp and common carp. The crosses between common carp and Indian carp resulted in sterile hybrids.

Methodologies for chromosomal engineering, leading to gynogenesis and polyploidy (triploidy and tetraploidy), were also developed for Indian and Chinese carps for application in aquaculture. Sex manipulation of fish, particularly in tilapia and common carp, were also carried out successfully.

A survey of some carp seed producing hatcheries in India indicated inbreeding. Selective breeding work was undertaken in Rohu (*Labeo rohita*) and Catla (*Catla catla*) to produce genetically improved seed and to develop proper breeding procedures for the hatchery managers in the country to avoid inbreeding in hatchery stocks.

An overview of these research efforts in genetic improvement is discussed in this paper.

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Introduction

Inland aquaculture in India is centered on carps, i.e., Indian major carps, catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), Chinese carps and common carp (*Cyprinus carpio*). Until the mid-1950s, fish culturists depended on wild collections of Indian carps seed from rivers. However, the success of hypophysation technique made spawning these fish under captivity possible. This has helped farmers in procuring seed from hatcheries.

However, in an attempt to produce more seed to meet the increasing demand, the seed producer (i.e., a qualified hatchery manager or a fish farmer) did not pay attention to the quality. Consequently, the carp seed produced from many hatcheries was reported to show signs of inbreeding depression, such as poor survival and growth, susceptibility to disease, deformities, etc.

Hence, it became essential to carry out research on genetic improvement of these carps through various approaches. In the 1960s and 1970s, fish genetic research was mainly restricted to cytogenetic studies and hybridization. Later, chromosome manipulation, developing breeding programs for important carp species and application of molecular techniques were emphasized.

Several national and central institutions and state agricultural and other universities undertake fish genetic research. Some of these are the Central Institute of Freshwater Aquaculture (CIFA), National Bureau of Fish Genetic Resources (NBFGR), Central Institute of Fisheries Education (CIFE), University of Agricultural Sciences (UAS), Bangalore (at Fisheries Research Station, Hesaraghatta, and College of Fisheries, Mangalore), Centre for Cellular and Molecular Biology (CCMB), Madhurai Kamraj University (MKU) and Bose Institute.

Genetics Research

Hybridization

In any genetic/stock improvement program, it is necessary to breed the candidate species artificially under controlled conditions. The success of induced breeding of Indian major carps paved the way for research on genetic improvement of carps. In the initial stages, simple interspecific and intergeneric hybridization was done to produce and evaluate the carps' useful traits for aquaculture. Hybridization is one of the methods used for combining desirable traits of selected species, with experiments demonstrating a high level of compatibility among Indian carps.

Altogether, six interspecific and 13 intergeneric hybrids were produced among the four species of Indian major carps belonging to three genera, i.e., *Catla*, *Labeo* and *Cirrhinus* (Reddy 1999). Over three decades of research on hybridization showed that these major carps with distinct morphological characters are highly compatible and able to produce viable and fertile hybrid progenies. Mature interspecific and intergeneric hybrids could be induced to produce F_2 progeny or backcross and triplecross hybrids.

The growth exhibited by most of these hybrids was intermediate, i.e., better than the slow-growing parent as in the case of interspecific hybrid between *L. rohita* and *L. calbasu* or intergeneric hybrids between *L. rohita* and *C. catla* or *C. catla* and *C. mrigala*, etc.

Hybridization between Indian major carps (*C. catla* and *L. rohita*) and medium carp *L. fimbriatus* also had a similar trend in growth with hybrids showing superiority over slow-growing parents (Basavaraju et al. 1989, 1990 and 1995).

With regard to hybridization between Indian major carps and Chinese grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*), no compatibility was observed. Almost all of these died either during the embryonic development or soon after hatching. Only a few hybrids between *C. catla* and *H. molitrix* survived.

Relatively better survival of the hybrid progeny was observed in the crosses between female common carp (*C. carpio*) and males of *C. catla, L. rohita* and *C. mrigala* compared with the reciprocal hybrid crosses particularly between female *L. rohita* and male *C. carpio* (Kowtal and Gupta 1985; John and Reddy 1987; Khan et al. 1989).

It is necessary to prevent indiscriminate hybridization. These carps are highly compatible to interbreed. Also, since almost all interspecific and intergeneric hybrids were found fertile, one should be more cautious of their crossbreeding as this may cause serious damage to the original gene pool through genetic introgression (Padhi and Mandal 1994).

Chromosome manipulation

Chromosome manipulation as a tool to improve genetic status of fish was initiated at CIFA and in other institutes.

Meiotic gynogenesis was successfully induced in Indian major carps for the first time in *L. rohita, C. catla* (John et al. 1984) and *C. mrigala* (John et al. 1988). Efficacy of shock, i.e., thermal and pressure, and duration of treatment seem to vary from species to species. Effective intensity lies at some point around the sublethal level. Thus, in all species of Indian carps, cold shock below 10°C and heat shock above 42°C proved to be lethal (John et al. 1984; Reddy et al. 1993).

The first successful induction of mitotic gynogenesis in *L. rohita* was reported by Reddy et al. (1993). The use of milt from heterologous species preferably having low compatibility to hybridize may be more desirable for easy elimination of paternal genetic input with irradiation.

Induction of androgenesis in Indian major carps was not very successful as egg consists of huge mass of cytoplasm and yolk that prevent the proper exposure of the chromosomes to UV rays.Some preliminary attempts have been made to induce artificial induction of triploidy and tetraploidy in Indian major carps with varying degrees of success (Reddy et al. 1987; 1990). The genetics division of CIFA succeeded in inducing triploidy in *L. rohita* and tetraploidy in *L. rohita* and *C. catla*. Common carp triploid showed 60-100% more growth than its diploid siblings (Reddy et al. 1998). Triploid *C. idella* can be utilized in openwater reservoirs to control aquatic weeds without propagating itself as it is supposed to be sterile.

Triploidy was successfully induced in *C. carpio* through heat shock treatment to produce sterile individuals as an approach to control unwanted reproduction in grow-out ponds. Heat shock at 40°C, 1-3 min after fertilization for 1.5 min resulted in 100% triploidy. The triploid *C. carpio* was found to grow as fast as diploid and yield 15-18% extra edible meat because of low GSI in case of triploids (Basavaraju et al. 1998).

An attempt to induce sterility in freshwater catfish *Heteropneustis fossillis* through heat shock was partially successful. Heat shock for 3 min at 40°C, 4 min after fertilization yielded 46% triploids.

Current research in chromosomal engineering in Indian major carps aims to produce allotriploids of carp hybrids (*C. catla* x *L. rohita* and *L. calbasu* x *L. rohita)* and to evaluate them for resistance against parasitic infection. In *C. carpio*, the objective of chromosome manipulation is to produce sterile fish to optimize production in aquaculture.

Sex manipulation

Research on manipulation of sex (production of sterile and monosex population) as a tool for increasing production has been in progress in many institutes in India. Administration of 17-α methyltestosterone (MT) through diet to eight-day old fry for a period of 45 days induced complete sterility in *C. carpio*. Compared with normal fish, sterile fish grew faster (21%) and had better resistance against bacteria (Mohire et al. 1999).

In progress at UAS, Bangalore, are studies on the production of monosex population of *C. carpio* through hormone sex reversal and progeny testing as an alternative approach to overcome sexual maturation and unwanted reproduction. UAS, in collaboration with the International Center for Living Aquatic Resources Management (ICLARM), is carrying out research to evaluate performance of crossbreeds of Indian major carp and to assess inbreeding of *C. catla* from different hatcheries of Karnataka state and the usefulness of sterile triploid and monosex *C. carpio* in aquaculture.

Selective breeding

CIFA, in collaboration with the Institute of Aquaculture Research of Norway (AKVAFORSK), undertook genetic improvement of *L. rohita* through selective breeding. The project started in 1992, and the first phase was completed in 1997. The second phase is in progress. *L. rohita* was chosen as the model fish, not only because of consumer preference, but also because of its poor growth in many polyculture systems. Besides, *L. rohita* is also prone to disease, particularly parasitic infection.

The founder population of *L. rohita* was collected as fry/fingerlings from five different rivers, i.e., Ganga, Gomati, Yamuna, Sutlej and Brahmaputra, in addition to CIFA hatchery stocks. The stocks were marked by M-prucian blue dye marking/fin clipping for communal rearing in the same pond to raise them to broodfish.

Full-sib families were produced as maternal/paternal half-sibs. Full-sib families were reared separately in nursery ponds until they reached 10-15 g size. Individual tagging was done by using passive integrated transponder (PIT) tag and stocked in communal ponds for rearing in monoculture or polyculture with *C. catla* and *C. mrigala*.

After nearly one year of rearing, estimation of breeding value was done following combined selection. Individuals were ranked according to their breeding value and the top 300-500 individuals were selected as broodfish for the next generation. Individuals with breeding value around the mean value were taken as control groups.

Full-sib progeny groups were produced from selected group of broodfishes after avoiding full-sib/half-sib mating. Mass breeding was conducted for production of control group progeny from control broodfishes. After one year of rearing, realized response was calculated taking growth as the trait of selection (Reddy et al. 1998).

Large differences in growth between full-sib families within stocks were observed in both monoculture and polyculture systems, which prove that the founder stocks of *L. rohita* have large genetic variation and the selective breeding program did not start from a closed population (Table 1).

Growth and survival of wild (riverine) stocks were equal or better than that of the farmed (local) stocks, indicating that the present procedure followed by hatcheries for seed production is improper (Reddy et al. in press).

Ranking of full-sib groups is highly consistent for growth in monoculture and polyculture systems, showing that different breeding plans are not required for different production systems.

Diallele crosses of four riverine and farmed stocks were conducted. Negative heterosis effect was observed in growth of *L. rohita*, indicating that pure breeding might be more suitable for selection (Gjerde et al. 1999).

Realized selection responses were calculated after each generation of selection for first and second line base populations (Fig. 1). Since genotype interacts sensitively with environment, these findings have to be tested under different agroclimatic conditions in the country. Field experiments to this effect were started in Ludhiana (Punjab), Bangalore (Karnataka), Vijaywada (Andhra Pradesh) and Kausalyaganga state fish farm (Orissa).

Genetically improved *L. rohita* was supplied to some carp hatcheries in India to replace their broodstock. Studies for formulation of effective feed for improved *L. rohita* are in progress.

In 1995, a research program to assess the genetic status of Indian major carps with reference to *C. catla* and to start a sustainable breeding program was initiated at the Fisheries Research Station, UAS, in collaboration with the Fish Genetic Program of the University of Wales, UK. A survey on broodstock management and breeding practices by major carp hatcheries revealed that these hatcheries function as isolated units. The survey also indicated that the stocks are likely to be inbred (Table 2) and that their performance will possibly decline if corrective measures are not taken. Germplasm from four wild stocks were collected and will be evaluated. Progress has been slow due to late maturity (3+ years) and difficulty in getting simultaneously ripe breeders of all stocks.

Genetic characterization

Identification and genetic characterization of wild and hatchery populations are important since broodstock with good genetic background is necessary for a successful breeding program. The knowledge from such investigations can be used in optimizing and sustaining yield, stock management and conservation of genetic diversity. The work being undertaken in the biotechnology division of CIFA involves molecular

Table 1. Average heterosis for body weight and survival of *L. rohita*.

	Body weight (g)		Survival (%)	
	Monoculture	Polyculture	Monoculture	Polyculture
Pure stocks	445.3	446.5	90.0	68.8
Crosses	437.7	414.7	90.0	74.7
Heterosis	-7.7 + 10.3	-31.8 + 13.5	0.00 + 3.1	+5.9 + 4.3

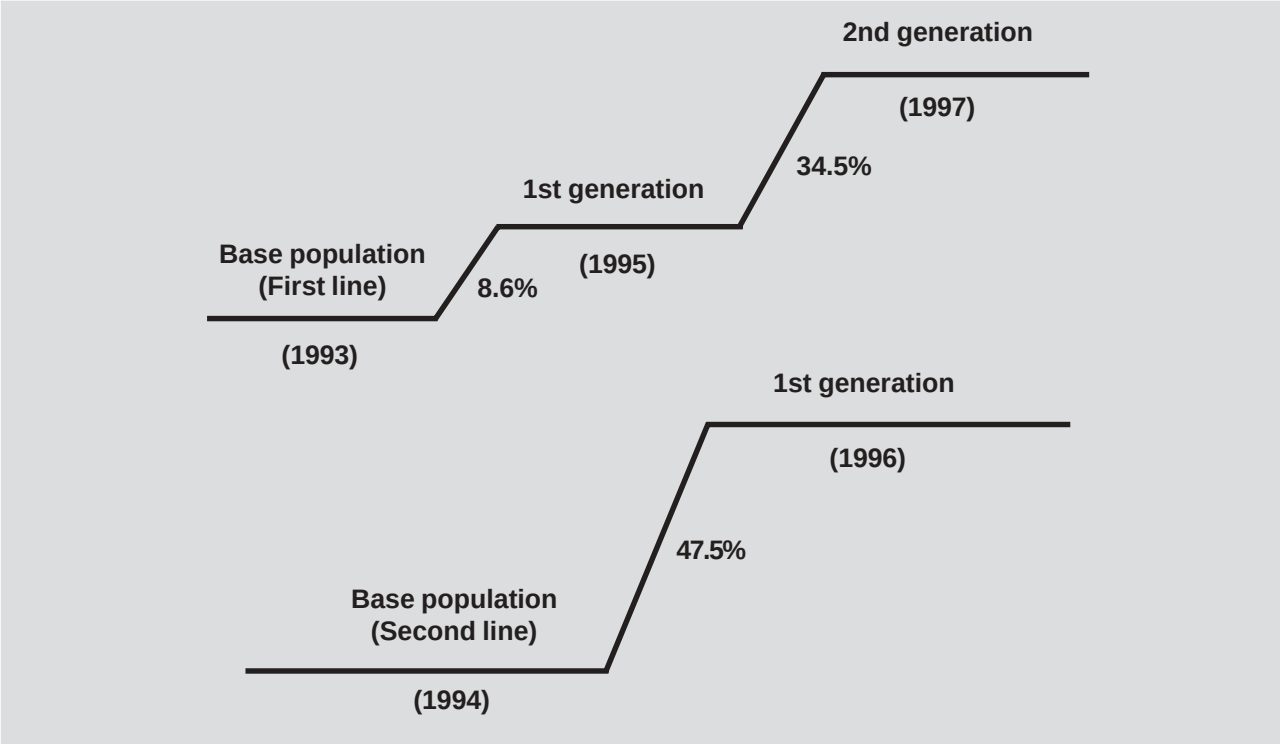

Fig. 1. Realized selection response of *L. rohita* (Das Mahapatra et al. 2000).

genetic characterization of Indian major carps using different types of DNA markers.

Gene mapping

Gene mapping is done for localization and functional characterization of economically important trait genes that will improve the breeding program through marker-assisted selection (MAS). The work involves isolation and development of highly polymorphic type-II DNA markers and, subsequently, development of a genetic linkage map for Indian major carps with particular reference to *L. rohita*. The long-term objective is the identification of trait-associated genes such as disease resistance and body growth.

At present, CIFA, in collaboration with ICLARM, is undertaking studies for the utilization of DNA fingerprinting in *L. rohita* breeding program to: (1) determine DNA profiles of selected, unselected and control stocks of *L. rohita* being investigated under the selective breeding program; (2) correlate performance with DNA markers within the sampled groups for future use in MAS; and (3) determine genetic tags for breeding programs.

Attempts are being made to identify RAPD markers, if any, that could differentiate between growth-selected and control animals. Simultaneously, probe-based DNA fingerprinting was also initiated on selected, unselected parent and progeny DNA restriction enzymes.

Table 2. Estimated effective population sizes and rates of inbreeding for major stocks of *C. catla* in Karnataka state fish hatcheries.

Hatchery	Species	N_e	DF (% per annum)
Tunga Bhadra Dam	catla	55.5 (10.5)[a]	0.9 (04.7)
	rohu	23.5 (12.3)	2.1 (04.0)
	mrigal	18.4 (13.5)	2.7 (03.7)
Bhadra Reservoir Project	catla	18.4 (08.5)	3.5 (05.9)
	rohu	14.2 (06.9)	3.5 (07.2)
Kabini Reservoir Project	catla	11.4 (04.7)	4.4 (10.6)
Shivapura Fish Farm	Insufficient data		
Vanivilas Sagar Fish Farm	Insufficient data		
Munirabad	Insufficient data		

The Department of Biochemistry and the Fisheries Research Station, UAS, have jointly taken up research on population genetics of peninsular carp *L. fimbriatus* using genetic markers. This study, using RAPD marker, indicated 37% polymorphism in different populations while studies with 21 enzyme systems did not show polymorphism. The use of mt-DNA PCR-RFLP as an alternative marker is under study.

Biochemical genetics of two reciprocal hybrids between *L. fimbriatus* and *C. catla* and their parents was studied electrophoretically using several protein and enzyme markers. The myogen and serum protein pattern showed high degree of homology among the parents and absolute homology among the reciprocal hybrids. The ontogenetic expression of LDH showed some degree of gene expression in the early development studies (Basavaraju and Keshavappa et al. 1996).

Conservation of Endangered Species

To replenish the declining population of mahseer (*Tor putitora*), a seed ranching program was initiated at Garampani in Kosi stream in Uttar Pradesh hills jointly by NBFGR, Lucknow; National Research Center (NRC) on Coldwater Fisheries, G.P. Pant University and State Fisheries Department.

Studies on short-term preservation of spermatozoa of Deccan mahseer (*T. khudree*) were also done. The trials were conducted using plastic vial/polythene bags at ambient temperature (AT) and 4°C, with or without oxygen. In general, the spermatozoa of mahseer reared at 18-24°C were motile for a longer period (132 hours). Spermatozoa stored at 4°C showed better motility than those maintained at AT (Basavaraja et al. 1999).

Genetic markers

Screening of *L. rohita* founder stocks used for genetic improvement undertaken by CIFA in collaboration with NBFGR indicated the presence of adequate genetic variability and absence of genetic contamination.

Screening of two populations of *L. rohita,* from Ganga and Gomati Rivers with isozymes and isoelectric focusing of eye lens proteins revealed polymorphism in XDH, PGM-1, PGM-2, EST-1G6PDH and ACP out of 20 isoenzymes studied at NBFGR.

With the use of a cost-effective package of genetic markers to quantify genetic contamination of hatchery stocks of Indian major carps, significantly higher levels of introgressions than with phenotypic markers were revealed. In Indian major carps, two mt-DNA genes were successfully amplified with polymerase chain reaction and amplified reaction fragment pattern for 2.5 kb was obtained for five restriction enzymes. Polymorphism was indicated in the pattern obtained with Eco R1 (NBFGR).

Genebanking

A compendium entitled "Fish biodiversity of India" containing information on systematics, habitat and distribution of 2118 finfishes has been made by NBFGR. The bureau has also achieved some success in using cryopreserved sperm to transfer germplasm for crossbreeding program between distant and discrete populations. The farmed *C. carpio* at Bilaspur (Himachal Pradesh) was crossed with frozen sperms of wild stocks of Ooty (Tamil Nadu) and Rewalsar. Rewalsar stock was found to be superior, and Himachal Pradesh State Fisheries was advised to replace existing farmed stocks of *C. carpio*, which was found to be contaminated with goldfish genome. A similar program is in progress for rainbow trout at Ooty, Tamil Nadu.

The NBFGR genebank has sperms of eleven species: *C. catla, L. rohita, C. mrigal, C. carpio, Salmo gairdneri, Salmo trutta, T. putitora, T. khudree, Tenualosa ilisha, Labeo dussumieri and Horabagus brachysoma.*. The improvement of the technique over time resulted in hatching with a range of 65%-100% with majority between 80%-90%. Hatchlings have been produced from five-year old frozen sperms of *L. rohita*.

Crossbreeding of Nilgiri's rainbow trout with faster-growing stock from Himachal Pradesh to achieve better growth was successfully undertaken. The fingerlings produced from crossbreeding of Tamil Nadu rainbow trout with Himachal Pradesh stocks using cryopreserved milt have exhibited higher growth. These results showed that cryopreserved milt could be utilized to transfer genome.

Sperm cryopreservation protocols were developed for the first time for the above species by NBFGR.Viable larvae were produced from cryopreserved milt raising the possibility of stock enhancement in depleted areas.

Other institutes like CCMB and the National Institute of Immunology and universities such as MKU and Jawaharlal Nehru University (JNU) are developing transgenic fish technology, including construction of

genomic and C-DNA libraries of Indian carps *C. catla*, *L. rohita* and Indian catfish *H. fossilis* and *Clarias batrachus*, and isolation of growth hormone gene from these libraries.

At CCMB, construction of c-DNA libraries from pituitary glands of *C. catla* and *L. rohita* and sequencing of c-DNA clones of *C. catla* were carried out. At JNU, construction of genomic DNA libraries of *C. catla* and *L. rohita*, cloning and characterization of genomic clones and encoding interferon gamma gene(s) in these species are being undertaken. MKU is also undertaking c-DNA cloning of growth hormone (GH) gene of *H. fossilis* and *L. rohita*; screening and isolation of full-length c-DNA of growth hormone of *H. fossilis* and *L. rohita*; and mapping and sequencing of full length c-DNA of GH gene of these two species.

Future Genetics Research

Following are key areas for genetics research in the future:

- Development of breeding programs for important aquaculture species;
- Extra traits for selection in *L. rohita* breeding program, such as disease resistance and quality of flesh;
- Determination of suitable age and size of broodfishes for production of quality seed and development of suitable plan for genetic improvement of carps for hatchery managers;
- Application of molecular markers and genetic techniques for breeding programs;
- Continued research on genetic engineering; and
- Testing and dissemination of research outputs to end-users.

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AQUACULTURE GENETICS RESEARCH IN INDONESIA

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ABSTRACT

This paper presents genetics research conducted by the institutional members of the Indonesian Network of Fish Genetics Research and Development (INFIGRAD) during 1998. The research included: (1) identification and genetic characterization of freshwater and brackishwater fish and (2) genetic improvement through selection and hybridization. This paper also reports on the contribution of the GIFT strain Nile tilapia (*Oreochromis niloticus*) to the development of floating net culture in reservoirs and on the research collaborations with the International Network on Genetics in Aquaculture/ International Center for Living Aquatic Resources Management on common carp and with the L'Institut Francais de Recherche Scientifique pour le Developpement en Cooperation on genetic characterization of catfish.

Introduction

Fresh and brackishwater aquaculture contributed 733 000 t (16.5%) to the total fish production of about 4.452 million t in Indonesia (DCFA 1998). Of this, freshwater aquaculture production was about 328 800 t, of which common carp (*Cyprinus carpio*) was 54.3% (Table 1). Nile tilapia (*Oreochromis niloticus*), which was first introduced in Indonesia in 1969 from Taiwan has been widely accepted by fish farmers. Common carp and Nile tilapia (recently the GIFT strain) are cultivated intensively, especially in floating net cages in reservoirs.

Common carp culture started in West Java in the middle of 19th century (Ardiwinata 1971) and subsequently, was taken up in other parts of Java, Sumatra and Sulawesi islands since early 20th century. There are at least ten stocks of common carps known in Indonesia: Punten, Sinyonya, Majalaya, Domas, Merah, Kancra-domas, Kaca (mirror carp), Kumpai, Taiwan carp and Koi (Japanese fancy carp) (Taniguchi et al. 1992). Recent collections include Rajadanu, Wildan Cianjur and Sutisna Kuningan (Nugroho and Wahyudi 1991).

In recent years, the genetic improvement of common carp and Nile tilapia has been considered important in aquaculture operations. Research on genetic characterization of both fresh and marine fishes is also gaining importance and has been part of the government's conservation efforts.

Table 1. Production (t) of important freshwater culture species in Indonesia in 1996 (DGF 1997).

Species	Type of culture			Production	
	Pond	Ricefield	Cages	t	%
Common carp *(C. carpio)*	069.091	074.782	34.489	178.362	054.3
Java barb *(Puntius gonionotus)*	019.753	013.212	00.221	033.186	010.1
Nilem carp *(Osteochilus hasselti)*	017.226	000.381	00.969	018.576	005.6
Mozambique tilapia *(O. mossambicus)*	016.174	003.738	01.111	021.023	006.4
Nile tilapia *(O. niloticus)*	017.665	002.062	05.941	025.668	007.8
Other species	043.009	007.039	01.900	051.948	015.8
Total production	182.918	101.214	44.631	328.763	100.0

Research activities conducted in 1997/1998, included: (1) identification and genetic characterization of wild, freshwater and brackishwater cultivated species, such as common carp, catfish (*Pangasius* sp. and *Clarias* sp.), milkfish (*Chanos chanos*), groupers, eel and shrimp; (2) genetic improvement of *C. carpio*, *O. niloticus* and *Macrobrachium rosenbergii*; and (3) hybridization of *C. carpio* and *O. niloticus*. Research on production of gynogenetic common carp for parent stocks of hybrids was undertaken earlier. Although brackishwater species played an important role in the development of the aquaculture industry, the genetic research of even the most important species is just in the early stages, with characterization of stocks and search for better strain from the wild. The other research includes the development of technology for mass production of seed, including gonad maturation of broodfish, spawning, hatching, and larval and fry rearing.

Since intensive aquaculture development needs a large supply of high-quality seed of genetically improved fish, research on genetic improvement is receiving high priority. The introduction of the GIFT strain *O. niloticus* from the Philippines, in 1994 contributed to the development of intensive culture in floating net cages and of the seed production industry in West Java, as the aquaculture center in the country.

Information provided in this paper was collected from:

- institutions under the Agency of Agricultural Research and Development:
  - Research Institute for Freshwater Fisheries (RIFF)
  - Gondol Research Station for Coastal Fisheries (GRSCF)
- universities
  - Department of Fisheries, University of Brawidjaja (UNBRA)
  - Faculty of Fisheries, Bogor Agricultural University (BAU)
- Agency for Assessment and Application of Technology (AAAT)
- institutions under the Directorate General of Fisheries, e.g., Freshwater Aquaculture Development Center (FADC).

These institutions are members of the Indonesian Network of Fish Genetics Research and Development (INFIGRAD). Other members of the network include the: Faculty of Fisheries, University of Riau; Department of Fisheries (DOF), University of Bung Hata; Faculty of Fisheries, University of Pajajaran; DOF, University of Gajah Mada; Faculty of Fisheries, University of Lambung Mangkurat; and Faculty of Fisheries, University of Hasanuddin. Table 2 lists the different aspects of genetics research being carried out by the institutions. Also, there is an ongoing research collaboration between the Central Research Institute for Fisheries (CRIFI)/RIFF and the International Network on Genetics in Aquaculture/ International Center for Living Aquatic Resources Management (INGA/ICLARM) called Genetic Improvement of Common Carp, and between CRIFI/ RIFF and the L'Institut Francais de Recherche Scientifique pour le Developpement en Cooperation, called Characterization, Utilization and Maintenance of Biological Diversity for the Diversification and Sustainability of Catfish Culture in Southeast Asia.

Genetics Research

Research was conducted by 11 institutions (including research collaborations) on five freshwater and five brackishwater fish species (Table 2).

Table 2. Genetics research on freshwater and brackishwater fish species and the institutions involved in Indonesia.

Species	Characterization	Gynogenesis	Selection	Hybridization	Institutions involved
Freshwater					
C. carpio					
	RAPD				AAAT
		Punten strain			UNBRA and Punten Hatchery
		Majalaya, Sinyonya			FADC
		Majalaya, Sinyonya, mirror carp			BAU
			Combined multitrait		CRIFI/RIFF and INGA/ ICLARM
Clarias spp. (1)	Allozyme				ORSTOM and RIFF
C. batrachus (2)	Isozyme electrophoresis				RIFF
Pangasius spp.	Allozyme				ORSTOM and RIFF
O. niloticus			Heritability, family	Diallele crossing	FADC
M. rosenbergii			Family		RIFF
Brackishwater					
C. chanos	Isozyme electrophoresis				GRSCF
Epinephelus spp.	Isozyme electrophoresis				GRSCF
Chromileptes altivelis	Isozyme electrophoresis				GRSCF
Anguilla bicolor	RAPD				AAAT
Penaeus monodon	Isozyme electrophoresis				GRSCF

Carps

*Documentation of the genetic resources of carps (*Cyprinus carpio, Barbodes gonionotus, Osteochilus hasselti, Leptobarbus hoeveni *and* Neolissochilus thienemanni*) in Indonesia*

This activity was a collaboration between CRIFI/RIFF and INGA/ICLARM. Literature searches were made from 12 faculties or divisions of fisheries of universities in West, Central and East Java and West Sumatra, and from related fisheries institutions, such as the Institute for Evaluation and Application of Technology, Provincial Fishery Extension Services, Central Hatchery and RIFF. Questionnaires were distributed to the Provincial and Regional Fishery Extension Services and Central Hatcheries in West and Central Java. Common carp genetic resources, which focused on strains of Rajadanu, Wildan Cianjur, Sutisna Kuningan and Majalaya, were documented. Information was collected on the biology of species; status of genetic quality evaluation and utilization of genotypically different common carp.

Establishment of synthetic base population of common carp from four strains

This study is being carried out by CRIFI/RIFF and INGA/ICLARM. At the first stage, complete diallele crossing of three strains (Rajadanu, Wildan Cianjur and Majalaya) and one cross of Sutisna Kuningan was made, with a total of 10 crossings. Each strain consisted of five females and five males. Crossing was accomplished by artificial fertilization, where eggs of each female were fertilized with sperm of five males. Simultaneous ovulation occurred after two injections with Ovaprim (combination of GnRH and domperidone). Fertilized eggs were distributed to *kakabans* (*Arenga* fiber egg collectors) set in hapas. The eggs of each female were hatched in five hapas.Rearing of eight-day old larvae was carried out in fine-mesh (1-2 mm) net cages set in two ponds, in which 50 net cages of 2 x 1.2 x 1 m in dimension were set in each pond. The stocking density was 1 300 larvae per cage. In one month, the larvae grew to fry with individual weight of about 1 g. The next step was rearing of fry to fingerling in net cages with

larger mesh. The base population will be established through 150 tagged fingerlings per full-sib family. This will be conducted in the second year.

Data collected from each breeder were fecundity, egg diameter, hatching rate, larval size, fry size and survival rate.

Gynogenesis of Punten common carp

Punten common carp is a well-known strain in Indonesia. Its phenotype characteristics include big belly and relatively wide body. The original Punten strain was the result of selection done by Goossens in 1928-1930 in Punten Hatchery, West Java (Ardiwinata 1971). This strain is not popular with fish farmers and competes with the widely spread Majalaya strain. In view of this, the Punten strain has become rare and is difficult to find. Punten Hatchery, in collaboration with UNBRA in East Java, has tried to conserve and make gynogenesis of the existing strain. This effort produced gynogenetic Punten with different color variation, red, green and blue, with the ratio of standard length to body depth 1:2.4; 1:2.5-2.7; and 1:2.6-2.9, respectively (Hasanudin 1998).

Gynogenesis of Majalaya and Sinyonya strains of common carp and their diallele crossing

The Majalaya strain is a big belly type one, with wide body depth and greyish-green scales. The Sinyonya strain is elongated and yellow. The eyes of the young fish are normal, while those of the old fish are covered by the eyelid membranes. The second generation of mitotic-gynogenetic diploid was produced for both strains. This diploid has a larger degree of phenotypic variation than the normal fish (Sumantadinata et al. 1990).

Diallele crossing was made to obtain better results. Progeny produced by crossing male Majalaya and female Sinyonya showed better growth and survival rates than the reciprocal and pure lines. The colors of the progeny were 70% green; yellow, 20%; and in between both colors, 10% (Hadadi et al. 1998).

Qualitative and quantitative characters of three C. carpio *strains*

The second generation of gynogenetic diploid common carp strains of Majalaya, Sinyonya and mirror carp were produced in 1992. Some fish of these strains were sex-reversed by oral administration of 17α-methyltestosterone. Females and the sex-reversed males were matured in early 1994. The fish were grown in earthen pond and in running water system with intensive feeding. Characteristics of the young fish to market size were observed and analyzed .

Slight differences among strains were found in the egg size before and after ovulation and in their further development. Fecundity of Sinyonya was higher than that of the mirror and Majalaya strains, but their fertilization and hatching rate were relatively similar. The growth rate of mirror and Sinyonya strains were similar under still or running water conditions, but they were better than that of Majalaya strain. The survival rate of mirror carp was the lowest. As regards body composition, the Sinyonya strain had the highest percentage of fillet; Majalaya strain, biggest viscera; and mirror carp, fillet remnant. The total quantity of muscular spines of the Sinyonya carp was the lowest. Proximate analysis of flesh showed it had the highest protein content and the lowest lipid content (Arfah and Sumantadinata 1998).

Application of RAPD-PCR method for analyses of DNA polymorphism of C. carpio

Six strains of *C. carpio* (Majalaya, Sinyonya, Punten, Domas, mirror and Merah [red]) were collected from Wanayasa Hatchery, fish farmer ponds and floating net cages. Blood samples were collected for extraction of total DNA, visualization of DNA by electrophoretic gel agaros and ethidium bromide staining, test and assessment of DNA quality, and reaction of PCR by RAPD method. The results showed no difference in typical band pattern for the four strains, except Majalaya and Sinyonya. For these two strains, DNA polymorphism can be detected with the application of RAPD-PCR (Faizal et al. 1998).

Catfish

Identification and characterization of Clarias *and* Pangasius *sp.*

Collaborative research involving six institutions has been carried out, since November 1997. The institutions were ORSTOM and Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (France); Katholieke Universiteit Leuven (Belgium); Cantho University (Vietnam) and CRIFI/RIFF (Indonesia). The research project was entitled “Characterization, Utilization and Maintenance of Biological Diversity for the

Table 3. Heterozygosity of each locus for each population of *C. batrachus*.

Locus	Allele	Locality					
		Lamongan	Depok	Parung	Palembang	Plaju	Tegineneng
Aat	100	0.925	0.925	0.925	0.800	0.925	0.925
	80	0.000	0.000	0.000	0.125	0.100	0.000
	60	0.075	0.075	0.075	0.075	0.100	0.075
H^1		0.139	0.139	0.139	0.252	0.145	0.139
Mpi	200	0.950	0.950	0.950	0.950	0.950	0.950
	110	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.050	0.050	0.050	0.050	0.050	0.050
h^2	40	0.095	0.095	0.095	0.095	0.095	0.095
		0.795	0.795	0.795	0.795	0.795	0.795
Gpi	120	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.025	0.025	0.025	0.025	0.025	0.025
H	50	0.048	0.048	0.048	0.048	0.048	0.048
		0.950	0.950	0.950	0.950	0.950	0.950
H		0.038	0.038	0.038	0.057	0.059	0.061

Notes: H - total heterozygosity; h - heterozygosity per locus.

Diversification and Sustainability of Catfish Culture in Southeast Asia".

The research work consisted of two parts. The first part aimed at identification and characterization of species and populations of the genera *Pangasius* and *Clarias* of actual or potential interest for aquaculture. Three complementary approaches were used: morphological analysis, estimation of genetic variation (protein electrophoresis, mitochondrial and microsatellite DNA analysis), and characterization of gill parasites communities (Monogenea). The results showed the: (1) phylogenetic relationships among Pangasiid catfish species (Siluroidei, Pangasidae) using allozyme data; (2) phylogenetic relationships among Clariid catfish species (Siluroidei, Clariidae) using allozyme and mitochondrial data; and (3) diversity of gill parasites of some catfish host species in Southeast Asia.

The second part of the research dealt with diversification and optimization of aquaculture production. The results showed: (1) aquaculture potential and artificial propagation in Pangasiids and (2) optimization of culture practices of *Pangasius hypopthalmus.*

Intraspecific characterization of Clarias batrachus *strains from Java and Sumatra*

The aim of this study was to identify the enzymatic characters of *C. batrachus* populations on the islands of Java (Depok, Parung and Lamongan) and Sumatra (Palembang, Plaju and Tegineneng). Eight enzymes were used : Adh, Gpi, Idh, Mpi, Sdh, Aat, Fbp and Ipo. The samples analyzed were taken from liver tissues. Twelve loci were found: Aat-1, Aa-2, Adh-1, Fbp-1, Gpi-1, Gpi-2, Idh-1, Ipo-1, Ipo-2, Mpi-1, Mpi-2 and Sdh-1, which showed heterozygosity (Gpi, Mpi, Aat and Ipo) and homozygosity (Idh, Adh, Sdh and Fbp). The total heterozygosity of the Javanese local catfish showed lower value (H= 0.038) than that of Sumatra's (H= 0.057 to 0.061) (Table 3) (Hadie et al. 1998).

Results indicated the possibility of less random mating due to a decrease in population as a result of intensive capture or introduction of other species, i.e., *C. gariepinus*. The introduction has been dominated by the local catfish, as it is easy to culture, has higher fecundity and faster growth, and is more resistant to diseases. At present, *C. batrachus* has become a threatened species. In Sumatra, where there is smaller population and vast area of open waters (rivers and swamps), there is a larger population of catfish.

Nile tilapia

Heritability of growth rate of O. niloticus

Three strains of *O. niloticus,* GIFT, Chitralada and Nile tilapia 69 (introduced in Indonesia in 1969 from Taiwan), each consisting of 25 families, were studied. GIFT showed higher heritability than both Chitralada and Nile tilapia 69. The values after a two-month rearing period were 0.74, 0.66 and 0.17, and after

nine months, 0.45, 0.37 and 0.05, respectively (Widiyati et al. 1998).

Evaluation of diallele crossed tilapias

Diallele crossing of tilapias was carried out for the following strains: (1) Chitralada (black) strain of *O. niloticus* and Philippine red tilapia; (2) GIFT strain of *O. niloticus* (black) and red tilapia (NIFI – National Inland Fisheries Institute, Thailand); (3) GIFT strain and Philippine red tilapia; (4) Nile tilapia 69 (black) and NIFI strain; (5) Nile tilapia 69 and Philippine red tilapia; (6) Chitralada strain and GIFT strain; (7) Chitralada strain and Nile tilapia 69; and (8) GIFT strain and Nile tilapia 69.

The growth rate and color composition were observed for 16 weeks. Crossing male GIFT strain with other strains showed better growth rate (117-123 g) than either crossing male Chitralada strain with other strains (89-100 g) or pure GIFT strain (average weight, 98 g). The highest percentage of red tilapia (46%) was obtained from crossing male GIFT strain and female Philippine red tilapia, followed by yellow (42%) and black (11%) types (Hadadi et al. 1998).

Milkfish

Genetic variation of *Chanos chanos* samples (natural and hatcheries F_1 and F_2) and performance of F_1 and F_2 were examined. Among 15 enzymes, 29 loci were detected. Eleven were polymorphic, namely, Adh, Aat-1, Est-2, Gpd, Gpi-1, Gpi-2, Idh-1, Ldh-1, Mdh-1, 6-Pgd, Pgm-2. Reduction in genetic variability was found in the hatchery stock (F_2). The reductions were 50% in the number of polymorphic loci, 22.36% in the number of allele per locus and 37.78% in the heterozygosity. The actual number of broodstock contributing in F_2 was 14 out of 50 fish individuals in the spawning tanks. GPI locus among the mostly polymorphic loci could be used as a marker in genetic improvement program. In order to avoid reduction in genetic variability in hatchery stocks, it is proposed to increase the number of broodstock for mass production of milkfish fry (Sugama and Prijono 1998).

Grouper

The study of genetics and characteristics of groupers *Epinephelus* spp. and *Chromileptes altivelis* was conducted by the Research Station of Research Institute for Coastal Aquaculture in Gondol, Bali. Four species of groupers (*E. fuscogutatus*, *E. coioides*, *E. microdon* and *E. bonthoides*) and one species of *C. altivelis* were analyzed by isozyme electrophoresis for 13 enzymes, such as Adh, Aat, Est, Gpi, Idh, Ldh, Mdh, Me, 6-Pgd, Pgm, Sdh, Sod and Sp. Using TC-8 and CAPM-6 buffer systems combined with skeletal muscle and liver tissues, six polymorphic loci were detected in *E. coioides* and *C. altivelis*, four in *E. fuscogutatus* and *E microdon* and two in *E. bonthoides*. Genetic variability was highest in *E. coioides*, 1.78 allele per locus and 0.89 heterozygosity. The shortest genetic distance (D) was found between *E. coioides* and *E. bonthoides* with D value of 0.0832. D values between *C. altivelis* and other species were significant (D±0.8). The results also suggest that Adh locus is a reliable marker for species identification (Sugama and Trijoko 1998).

Eel

RAPD-DNA method on *Anguilla bicolor* elver was applied. Elvers were collected from Cimandiri River (1-2 km from the river mouth) on south coast of West Java. The samples of tissue were extracted for the total DNA assessment of the concentration of DNA, test of the quality of DNA with restriction enzyme, RAPD-PCR. The results showed that RAPD-PCR method can be applied to distinguish different populations of elvers (Amarullah et al. 1998). The study was conducted by AAAT.

Giant freshwater prawn

A study was conducted to evaluate response to selection on dress-out characteristics of the synthetic population of *Macrobrachium rosenbergii*. The character evaluated was the percentage proportion of carapace to the standard body length. Base population was established with three collections from Kalipucang, Tanjung Air (both located in West Java) and Musi (South Sumatra) through half-sib mating. Larval and postlarval rearings were conducted in concrete tanks using clear water system with temperature of 28-31°C. Growout was done in earthen ponds of 400 m^2. The F_1 synthetic population of five months old showed average body weight of 23.56 ± 10.37; maximum and minimum body weight of 53.30 and 6.20 g, respectively; coefficient of variation, 44.21%; heritability, 0.56% (standard error 0.07); average dress-out 56.63±3.39%; coefficient of variation, 6.0% (Emmawati et al. 1998). The average dressing was higher compared to the average of the character at farmer level (45%) (Hadie et al. 1991). The selection indicated the possibility of improving dress-out

percentage in *M. rosenbergii*. Response of selection, selection differential and intensity, and predicted average dress-out of F_2 are presented in Table 4.

Shrimp

The association between heterozygosity at two loci of isocitrate dehydrogenase (Idh) and glucose phosphate isomerase (Gpi) and the development rate in *P. monodon* were examined. The purpose of this study, which was conducted by GRSCF, was to test the prediction that heterozygous prawn develops faster than the homozygous one. There were significant differences in fecundity, hatching rate and vitality between heterozygous and homozygous individuals. Larvae derived from heterozygous broodstock developed faster than those from homozygous one. The results suggest that heterozygous individuals are superior to homozygous ones (Sugama et al.1998).

The study of genetic variation and population structure of *P. monodon* was another collaborative research among GRSCF, Faculty of Agriculture, Kochi University, and Faculty of Fisheries, BAU. Five populations of *P. monodon* in the coastal waters of Indonesia (Aceh, Madura, Bali, West Nusa Tenggara and South Sulawesi) were sampled electrophoretically for evaluation of genetic variation at 21 loci. Six loci (Est-2, ±- Gpd, Gpi, Idh, Ldh-1 and Mdh-1) were polymorphic in at least one of the samples. All polymorphic loci at all localities were in Hardy-Weinberg equilibrium. The genetic variability of the species, as indicated by number of alleles per locus (Na) and heterozygosity (H), was low, with average (Na=1.428), ranging from 1.333 to 1.523 and H=0.034, ranging from 0.023 to 0.047. The mean genetic distance between population pairs was 0.00045, ranging from 0.00004 to 0.00112. Clustering samples according to their paired genetic distances showed that the population of *P. monodon* consisted of three geographical groups. However, the absolute amount of genetic variation among populations sampled appeared to be very low (Sugama et al. 1998).

Table 4. Response, selection differential, intensity, and dress-out of *M. rosenbergii.*

Parameter	Value
Parental response, F_1 (%)	07.69
Selection differential	13.74
Selection intensity	04.05
Average dress-out (%)	56.63
Prediction of average dress-out (%)	64.32

O. niloticus (GIFT strain) culture in Indonesia

The GIFT strain *O. niloticus* was introduced in Indonesia in 1994 as part of the INGA program. The fry were studied in three locations, RIFF at Bogor, FADC at Sukabumi and Cangkringan Hatchery at Yogyakarta. Studies on growth rate, conducted in three locations in West Java, showed that GIFT performs better compared to *O. niloticus* strains already existing in Indonesia.

The economic crisis during 1998 had a strong impact on intensive fish culture, primarily because the high price of pelleted feed, which increased by 70% (from Rp 1 400 to Rp 2 400 or from US$0.15 to US$0.30). However, the number of pellet feed industries also increased; trademarks increased from four to 12, with different qualities. The quality of some feeds is poor resulting in low feed conversion. Efforts were made for the intensive fish culture to survive and become profitable through the use of fish species that are easy to handle and have fast growth rate, good marketability, and efficient feed conversion. GIFT strain has met these requirements particularly with regard to rearing in floating net cages in Saguling, Cirata and Jatiluhur reservoirs in the province of West Java.

On the other hand, the use of *C. carpio*, the lead species cultured in cages, has decreased dramatically; at present, it is estimated to be less than 10%. A total of 25 560 cages (7 x 7 x 3 m^3) need a large supply of GIFT fry. The high demand is due to the shift from *C. carpio* carp fry to GIFT production, and the establishment of large-scale GIFT hatcheries by new investors.

In single-bottom floating net cages, only GIFT fish or *C. carpio* are stocked, while in double-bottom cages, *C. carpio* are stocked in the inner cage and GIFT fish in the outer cage. Feeds are applied only to *C. carpio*, while GIFT fish are fed food droppings from the inner layer, in addition to periphyton growing on net walls. The rearing period varies from 60 to 110 days. In Jatiluhur reservoir, the stocking densities per cage in single-bottom net cages varied from 60 to 130 kg of *C. carpio* or 100 to 150 kg of *O. niloticus*. The production was from 875 to 1 940 kg of *C. carpio* and from 626 to 1 200 kg of *O. niloticus* per cage. In double-bottom cages, stocking densities of *C. carpio* were 50-60 kg in inner cages and 18-50 kg of *O. niloticus* in lower cages. The production was 1 080-1 326 kg and

160-313 kg, respectively (Krismono 1998). The fish are usually harvested at the individual size of 250-300 g with the price of GIFT strain at the producer level of Rp 3 500 (US$0.44) and for individual fish size over 500g the price was Rp 4 500 (US$0.56). Production of *C. carpio* and *O. niloticus* in floating net cages in Cirata reservoirs is shown in Table 5.

Brackishwater ponds, with a maximum salinity of 15 ppt, is another source for culture of GIFT strain. In Karawang area (West Java), such culture has been carried out since 1997. There are 130 units of ponds of 4 000 m^2 each that can produce 1.7-2.0 t/pond in 110-day rearing period with a stocking density of 10 000 fingerlings (3-5 cm). Fish are harvested at the average individual size of 200 g, and the survival rate is about 80-85%. A trial in ponds of 3 000 m^2 each, with a stocking density of 75 000 fry of 1 g /fish produced fingerlings of 15-20 g/fish with a survival rate of 80% in 110-day period. To maintain the genetic quality of GIFT strain, broodstock management should be done by seed producers, with assistance from authorities and the scientific community.

Table 5. Production of *C. carpio* and *O. niloticus* in floating net cages in Cirata reservoirs.[1]

	Number of cages (unit)		Production (t)		
	Available	Operating	*C. carpio*	*O. niloticus*	Total
1996	10 950	8 700	15 896	9 218[2]	25 114
1997	25 558	15 289	36 532	12 639[3]	49 171
1998	17 477	10 485	9 349	5 916[4]	15 265

[1]Data collected from Fish Culture Station of Saguling-Cirata reservoirs.
[2]Consisted of Nile tilapia 69.
[3]Mostly Nile tilapia 69.
[4]Mostly GIFT strain.

Future Research

Following are various future research collaborations on genetic research in Indonesia:

- CRIFI/RIFF and INGA/ICLARM - genetic improvement of carp in Asia: establishment of base population of the second ten crossings among strains of Rajadanu, Wildan Cianjur, Majalaya and Sutisna Kuningan. During 2000/2001, the project will continue to undertake estimation of the breeding value of 100 half-sib and 200 full-sib families from the best cross of the first generation.
- CRIFI/RIFF and ORSTOM - characterization, utilization and maintenance of biological diversity for the diversification and sustainability of catfish culture in Southeast Asia. For genetic characterization of Clariidae and Pangasidae, more samples will be collected from rivers in Java, Sumatra and Kalimantan, and analyzed thorugh protein electrophoresis and mtDNA.
- INFIGRAD (Fig. 1):
  - enzymatic characterization of *C. carpio* – to be conducted by RIFF for four strains of *C. carpio*, i.e., Majalaya, Rajadanu, Sutisna Kuningan and Wildan Cianjur;
  - DNA polymorphism analysis for carp species – to be carried out by AAAT;
  - hybridization of *C. carpio* strains – to be done

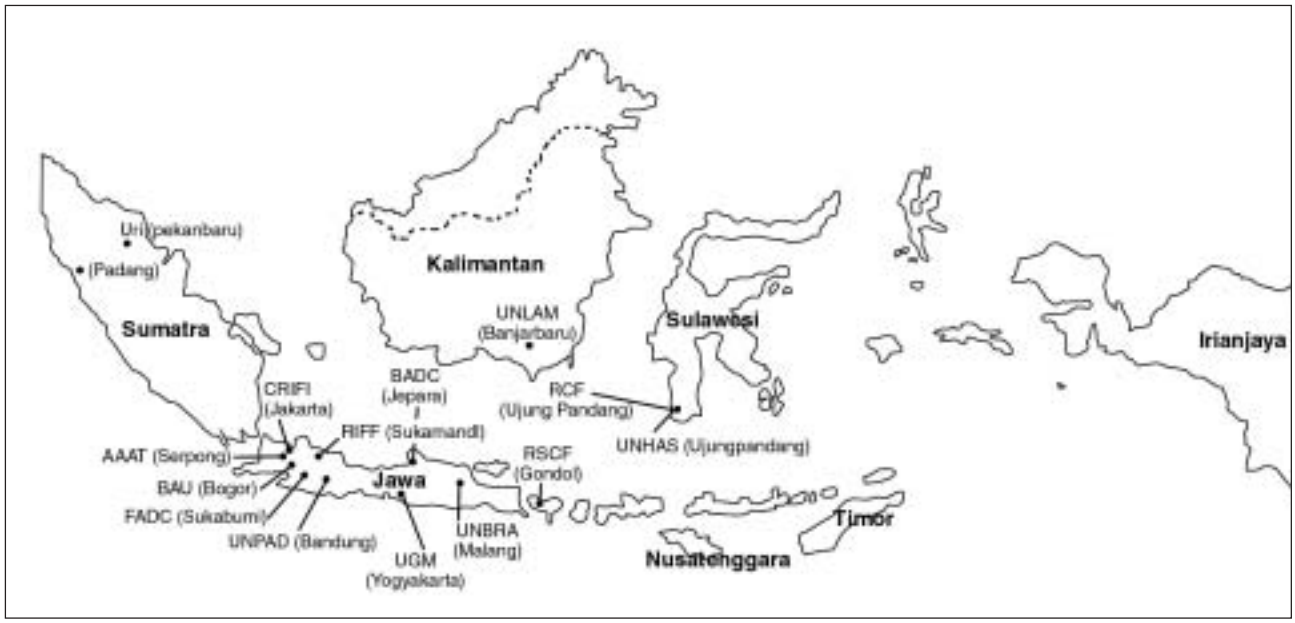

Fig. 1. Distribution of members of Indonesia Network of Fish Genetic Reseach and Development (INFIGRAD)

by FADC at Sukabumi;

- evaluation of heterosis of *Pangasius* hybrids (*P. jambal* and *P. nasutus*) – to be conducted by RIFF at Sukamandi;
- family selection of the sixth generation of GIFT strain of *O. niloticus* – to be conducted by RIFF, Sukamandi;
- study on the application of RNA:DNA ratio to evaluate growth of tilapia – to be done by AAAT, Serpong;
- hybridization of tilapia – to be done by FADC, Sukabumi;
- morphological and DNA characterization of *P. monodon* - to be carried out by GRSCF and Faculty of Fisheries, BAU; and
- studies on allozyme heterozygosity and growth rate of *C. chanos* fry – to be done by GRSCF.

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AQUACULTURE GENETICS RESEARCH IN MALAWI

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ABSTRACT

Aquaculture genetics research is carried out at the National Aquaculture Centre at Domasi, Malawi, by staff of the University of Malawi (UM) and the Department of Fisheries. The main species cultured are from genera *Tilapia* and *Oreochromis*. The genus *Oreochromis* was domesticated without proper identification of the various species especially in the subgenus *Nyasalapia* where three species (*O. karongae, O. squamipinnis,* and *O. lidole*) are difficult to identify before they reach breeding size. It is, however, observed that local fishers can identify these species using morphological characters. There have been no attempts to carry out deliberate domestication selection in the small-scale farms with the prevailing practices probably leading to deterioration of stock performance and decline in genetic diversity. Studies showed that wild populations grow faster than domesticated populations, and results of mass selection on collimated individuals suggest that selected individuals grow faster than unselected individuals. Studies of population genetics of domesticated and wild tilapias are underway at UM.

Introduction

The aquaculture industry in Malawi is characterized by small-scale farmers, and tilapia is the major fish species cultured. The Government has put a ban on importation of exotic species, which have been domesticated and selected, to fairly greater extent than the indigenous species. The ban is intended to protect the genetic integrity of fish populations in the natural water bodies.

Although aquaculture has developed to considerable extent in Malawi, compared to other African countries, it is still faced with the problem of culturing species with low growth rate. This, among other factors, is due to poor domestication protocols in the government and private farms. There is little regard for quality of germplasm being cultured. Small-scale farmers and government stations do not practice deliberate and effective genetic selection. They reserve a portion of fingerlings from grow-out ponds at the time of harvest for the next grow-out cycle. Although no criteria are known to be used in selecting the fingerlings, strong indirect or domestication selection can nevertheless occur, e.g., if the fingerlings come from very few parents or from particularly early or late maturing parental stock. Not much is known at present if such a system depletes genetic variation or

not, but DNA analysis of farm populations indicates that depletion is actually rather rapid.

The species cultured have not been properly identified or characterized. As a result, information has not been reproducible, consistent or easily transferable among programs. The domestication efforts of the *Nyasalapia* species have been carried out without sorting out the confusion surrounding the identity of the various species of *Oreochromis* (*Nyasalapia*) sp. Lack of documentation of the available genetic material is due not merely to lack of facilities and trained personnel to identify the species, but also to lack of knowledge of the structure of the gene pool of species relevant to aquaculture.

The increasing pressure from human population growth and activities associated with the lakes on aquatic biodiversity in Malawi is of particular concern. Cyprinids, like *Opsaridium microleps* and *Labeo mesops*, have disappeared from their traditional natural habitats where they used to occur in abundance. *O.* (*Nyasalapia*) sp. catches have declined considerably in the wild. The country is currently faced with the challenge of conserving the aquatic biodiversity in the lakes. *Ex situ* conservation is not economically feasible in such a country where financial resources are limited; the economic feasibility of *in situ* conservation offers achievable promise as an alternative means of preserving the otherwise threatened biodiversity. Several reservoirs exist in the country, but most of them are underutilized for fishery production.

Tilapia Genetic Resources in Malawi

In Malawi, the genus *Tilapia* is represented by *T. rendalli* while the genus *Oreochromis* is divided into two subgenera: *O. Oreochromis* and *O. Nyasalapia*. *Oreochromis* is composed of *O. shiranus* sp. (subdivided into *O. shiranus shiranus* and *O. shiranus chilwae*), *O. mossambicus* and *O. placidus*. The *O.* (*Nyasalapia*) species flock is composed of *O. Ny. karongae*, *O. Ny. lidole*, *O. Ny. squamipinnis* and *O. Ny. saka*. The taxonomy of *O. Nyasalapia* sp. is based almost entirely on morphometric data, some of which are extremely preliminary. Contrary to the species division listed above, multivariate morphometric analysis carried out recently suggests that there are only three species of *O. (Nyasalapia)*, i.e., *O. karongae*, *O. lidole* and *O. squamipinnis*. *O. saka* is classified as a junior synonym of *O. karongae* (Turner and Robinson 1990). Difficulties in identifying the *O. Nyasalapia* species have deterred proper broodstock handling procedures and development of improved strains for aquaculture.

Affinities

The *O. Nyasalapia* species flock appears morphologically to be the derivatives of *S. galilaeus* group (Fryer and Iles 1972) and they have affinities with *O. machrochir* and *O. rukwaensis*. Thys van Audernaerde (1968, cited in Sodsuk et al. 1995) proposed a separate subgenus *Nyasalapia* as part of the genus *Tilapia* which would be only restricted to the Lake Malawi species and subgenus *Lowuwiala* to include all other tasseled male species, for instance *O. (Ny.) machrochir*.

O. shiranus sp. is divided into two subspecies based on morphological and meristic characteristics (Trewavas 1983), *O. sh. chilwae* and *O. sh. shiranus*. The subspecies are from a common ancestor *O. shiranus* which existed in Rovuma River into which the ancient Lakes Chilwa-Chiuta drained to the Indian Ocean via Lujenda River. The most nearly related species is *O. rovumae*, and together with *O. mossambicus* and *O. placidus*, they belong to the *mossambicus* group of tilapias (Fryer and Iles 1972).

Zoogeographical distribution

The *O. Nyasalapia* sp. is endemic to Lakes Malawi and Malombe only. The fishes are distributed throughout Lake Malawi from north to south. Their population in the lake is reported to be declining. *O. shiranus* and *T. rendalli* are the most widely distributed tilapias in the country. They are found in Lake Malawi and Malombe, lagoons and rivers. *O. mossambicus* and *O. placidus* are only found in the Lower Shire (Table 1).

In aquaculture, the species have been moved without regard to their natural geographical distribution. The *O. Nyasalapia* subgenus has been stocked in reservoirs and fishponds outside their natural regions of distribution.

Indigenous knowledge on characterization of tilapias

It has been observed that fishers at the lakes and along the Shire River have their own methods of distinguishing among fish species based on morphometric characters and fish behavior. This knowledge has been passed on from several

Table 1. Distribution of tilapia in the water bodies of Malawi.

Water bodies	Species
Lake Malawi, Lake Malombe and Upper Shire	*O. shiranus shiranus* *O. Nyasalapia* species flock *T. rendalli*
Middle Shire	*O. shiranus shiranus* *T. rendalli*
Lower Shire	*O. mossambicus* *O. placidus* *T. rendalli*
Reservoirs	*O. shiranus hybrids* and pure strains *O. Nyasalapia* sp. *O. mossambicus/O. placidus* hybrids
Rivers and lagoons	*O. shiranus* sp. *T. rendalli*
Aquaculture	*O. Nyasalapia* sp.(distributed in all regions of the country but only recently domesticated)
	O. shiranus shiranus (common in all regions) *O. shiranus chilwae* (common in southern and central regions) *O. mossambicus* (common at Kasinthula Fish Farm, the species has hybridized with *O. shiranus* in Zomba district)
	O. placidus (rare, mainly hybridized with *O. mossambicus)* *T. rendalli* (common in all regions)

generations but has not been well documented. A nationwide survey of indigenous knowledge of the fishing communities on the taxonomy and behavior of tilapias has been completed and data are being analyzed.

Also, a survey of local names of tilapia species was carried out throughout the country. The main interest was in determining the local classification system for species in the *O. Nyasalapia* subgenus which are difficult to identify before breeding stages. These species are as follows:

- *O. squamipinnis* - deep body and sharp bend on the forehead; breeding males have characteristic vertical blue band on the forehead;
- *O. karongae* - deep body with depression on the forehead; males do not have vertical bands on the forehead as in *O. squamipinnis*; and
- *O. lidole* - big head and small body; generally slender in appearance.

Quantitative Genetics

Comparison of growth between wild and domesticated populations

Wild broodstock populations of *O. shiranus chilwa* were collected from Lakes Chilwa and Chiuta for domestication. F_1 progenies were compared to populations that have been at the National Aquaculture Centre (NAC) for several years. The experiment was carried out between August 1997 and April 1998. The results obtained in 1998 indicated that wild population grew faster than domesticated one, 16.6 g and 13.3 g, respectively (Table 2).

Mass selection on collimated populations

Broodstock of *O. shiranus* were collected from domesticated populations at the NAC. These were stocked in three ponds where they bred naturally. Fingerlings were collected and stocked randomly into 16 200 m^2 ponds at stocking rate of 5 fish m^{-2}. After one month, standard length (SL) measurements

Table 2. Growth comparison between wild and domesticated populations of *O. shiranus*.

Month	Mean weight (g)	
	Wild fish	Domesticated fish
Aug	05.3 ± 0.00	03.8 ± 0.00
Oct	06.2 ± 0.00	04.9 ± 0.01
Nov	10.0 ± 0.06	06.9 ± 0.03
Jan	12.2 ± 0.03	09.7 ± 0.06
Mar	16.5 ± 0.03	12.3 ± 0.03
Apr	21.9 ± 0.03	17.4 ± 0.05

were taken from fish in eight of the 16 ponds. All length measurements were converted to weight (W) using the formula, $W = 2.46x10^{-2}SL^{2.79}$. Individuals of the weight range 7.3-14.1 g were selected and restocked at stocking rate of 800 fish per pond. The fish were grown for another 3 months before SL measurements were taken. The average length was 17.2 g and range was 11.4-30.4 g. Fish of the range 20.7-30.4 g were selected for grow-out for 6 months, August 1998-January 1999. The mean size of selected population was 24.7 g and selection differential was 7.2 g. Stocking rate was 300 fish per pond. The rest of the fish were discarded. Length measurements were taken monthly.

The second group formed a control population which was stocked in eight ponds at 5 fish m^{-2} and then randomly trimmed to 800 fish per pond after a month. Two months later, the stocking density was reduced to 300 fish per pond and grown for 6 months, from August 1998 to January 1999, to compare the growth between selected and unselected populations. The stocking size range in this control population was 4.9-37.1 g and mean was 23.1 g.

In both populations, fish were fed 10% body weight with maize bran; chicken manure purchased from nearby chicken farms was applied to the ponds to promote primary productivity.

Selected populations grew faster than unselected ones (Table 3). Overall, the population had very low growth rate, probably supporting the fact that domesticated tilapias in Malawi have poor growth rate than their wild counterparts.

Population genetics

Studies of genetic characterization and population structure of *O. shiranus* sp. have been carried out at UM in collaboration with Dalhousie University of Canada with funding from the International Development Research Centre. In these studies, microsatellite DNA markers were used to determine the level of genetic diversity in wild and domesticated populations. Considerable decline in genetic diversity was reported in populations which were domesticated for longer period (Ambali 1996; Ambali et al. 1999).

Table 3. Growth comparison between selected and unselected populations of *O. shiranus*.

Month	Mean weight (g)	
	Unselected fish	Selected fish
Aug	23.12 ± 0.04	24.69 ± 0.02
Nov	23.46 ± 0.00	34.06 ± 0.03
Dec	25.77 ± 0.00	37.68 ± 0.04
Jan	29.00 ± 0.01	39.02 ± 0.04
Feb	29.40 ± 0.01	39.02 ± 0.03
Mar	31.02 ± 0.00	42.04 ± 0.00

A DNA analysis laboratory has been established at Chancellor College, UM, with funding from the Japanese International Cooperation Agency. Activities in the laboratory include characterization of indigenous tilapias and cichlid species with potential for aquaculture.

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GENETICS FOR IMPROVEMENT OF FISH IN MALAYSIA

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ABSTRACT

The application of genetics in aquaculture in Malaysia and the realization of the need for conservation and development of genetic resources are very recent. Many of the ongoing projects on these have yet to obtain results that are therefore still unpublished. This paper discusses briefly some relevant work on documentation of genetic resources, evaluation of resources, quantitative genetics and biotechnological developments. Research primarily carried out by the University of Malaya (UM) is presented in this paper. Research carried out by other projects is either unpublished or unavailable at the time of writing this paper.

Introduction

In the National Agriculture Policy (January 1999) announced by the Minister of Agriculture, Government of Malaysia, the need for sustainable development of fisheries industry was well emphasized.

The demand for fish increases with a growing population. According to Mazlan (1997), Malaysia, with an average per capita fish consumption of 35 kg, requires approximately 796 100 t of fish by the end of this century. To meet this demand, integrated planning for land resource management, protection of aquatic environment and development of an efficient fish industry are vital steps.

The development of a modern industry, as envisaged by the government, requires the establishment of sound breeding programs for important food species and this is usually accompanied by an expansion of programs for genetics research. This is the focus of this paper. Ornamental fish breeding is equally important though in the developing market, both within and outside the country.

Conservation and Conservation Genetics

Genetic diversity of freshwater fish has been documented. The dominant commercial species are carps, catfish and tilapia. Carps and catfish have also the most diverse species. There are nearly 300 fish species in Malaysia, the conservation status of which are not exactly known. The IUCN Red Data Book lists only two species (*Scleropages formosus* and *Probarbus jullieni*) from Malaysia as endangered. However, there are at least seven other species which may be categorized as rare or endangered (Table 1). Others

(e.g., Asian Wetland Bureau, Kuala Lumpur) believe that at least 17 species are rare. ICLARM also developed a global database, which has information on Malaysian species, but the exact status of genetic resources is insufficiently known and can only be ascertained through research on documentation and evaluation. For the rare species, methods of conservation need to be developed, which include stock identification, monitoring population size and live conservation or cryopreservation.

While the Department of Fisheries (DOF) has extensive programs for turtle conservation in the East Coast of Malaysia (Ibrahim 1998, pers. comm.), efforts have been initiated in cryopreservation of gametes and embryos for a few endemic species and marine finfish and in in-vitro culture of *Oreochromis* spp. (pers. comm.: Ali 1999, Khadijah 1999 and Norhanizan 1999).

A collaborative research on genetic identification of various strains of *Oreochromis* spp., carried out by Queensland University of Technology, University of Malaya and Universiti Putra Malaysia (UPM), will pave the way for future conservation genetics work in Malaysia.

Electrophoresis of 32 protein loci, 18 of which are polymorphic, in seven tilapia strains (*O. mossambicus*; *O. niloticus*, Chitralada strain; *O. niloticus*, Philippines strain; *O. niloticus*, Israel strain; *O. niloticus*, local strain; *O. aureus*, Singapore strain; and a Taiwanese hybrid strain) showed very close genetic relationship among the strains (Selvaraj et al. 1994).

Genetic similarity or distance index may be useful in conservation work. Only one of a set of strains may be conserved if they are genetically similar in terms of performance. Laboratories at UPM are now engaged in similar studies using molecular markers, instead of protein markers (pers. comm.: Selvaraj 1999 and Tan 1999). Preliminary results are presented below, under "Molecular genetics" portion.

Table 1. Some rare (R) or endangered and extinct (E) species in Malaysia.

Species	Status
Balantiocheilos melanopterus	R
Dischorondontus halei	R
Luciosoma trinema	R
Parachela maculicauda	R
Probarbus jullieni	E
Phallostethus dunckeri	E
Scleropages formosus	E
Helicophagus wandersii	E
Ceratoglanis scleronema	E

Genetics and Breeding

Genetics research and its application in Malaysia are still conspicuously inadequate. A survey of government and private breeding farms and hatcheries showed that most seed stocks for finfish production are imported (Chew and Mukherjee 1988).

Currently, species produced locally in hatcheries include shrimp, tilapia, Javanese carp, bighead carp, common carp, local catfish and seabass. For culture of cockles, mussels, grouper and snapper, seeds are always collected from the wild. Also, imports of exotic carps, especially grass carp, red tilapia, catfish, seabass and red snapper seedlings continue. Only seabass hatchlings and tiger prawn fry are exported (DOF 1996).

Research

Hatcheries have almost no research and documentation is scanty. Whatever little research has been done in genetics and breeding during the 1980s and 1990s is presented below.

Electrophoretic markers

There have been various studies on electrophoretic markers. Genetic relationships have been studied on *Oreochromis* spp. (Tan et al. 1989; Selvaraj et al. 1994); *Trichogaster pectoralis* (Tan et al. 1980); and *Macrobrachium rosenbergii* (Patimah, pers. comm.). Temporal variation (Yao 1980; Hasnah 1982; Surinderpal 1984; and Chew 1986), genetic control of isozymes (Tan et al. 1980; Patimah et al. 1989) and discriminating loci between *Penaeus monodon* and *P. merguiensis* (Daud et al. 1995) have been studied.

Karyotyping

There were several contributions in the creation of a database of karyograms of locally available fish (Bongso et al. 1980; Jamuna 1989; Das 1990). Work by Das (1990) illustrates the qualitative and quantitative variations between carp and tilapia species with respect to macrochromosomes. Jamuna's (1980) work relates the differences among ornamental species (guppy and koi).

Chromosome engineering

Induction of polyploidy in walking catfish, *Clarias batrachus*, by controlling water temperature was successful (Siraj et al. 1992). Growth performances and gonadal development of diploid and triploid were compared with a 30% protein feed. While the growth pattern was not significantly different ($P>0.05$), gonads of male and female triploids were poorly developed compared to those of diploid.

Siraj et al. (1993) achieved 67% gynogenetic fry in Lampan Jawa *Barbodes gonionotus*, using ultraviolet (UV)-irradiated sperm of *P. schwanenfeldii* followed by cold shock at 2oC.

Triploidy induction in the hybrid catfish (female *Clarias gariepinus* x male *C. macrocephalus*) has been successful using cold shock (Mohidin 1995). Production of gynogenetic female of *C. macrocephalus* with UV radiation and subsequently 92% sex reversal of gynogenetic females treated with oral administration of several dose levels of synthetic androgen (10, 50 and 100 mg/kg of feed) were attempted by Mohidin (1995). The presence of hermaphrodites, as expressed by both testicular and ovarian tissues, proved that the treatments did not result in total sex reversal.

Genetic evaluation

Genetic evaluation of six random-mating, parental tilapia strains in Malaysia (*O. niloticus*, Thai strain; *O. aureus*, Singapore strain; *O. niloticus*, Israel strain; *O. mossambicus*, Thai strain; and *O. niloticus*, local strain) for three generations in integrated (with ducks) and nonintegrated ponds showed consistent superiority of *O. niloticus*, Thai strain, in terms of body weight at different ages and carcass composition at 24 weeks. Performance in integrated ponds was better than that in nonintegrated ones (ACIAR 1997).

Hybridization

During the 1950s and 1960s, the Fisheries Research Institute at Batu Berendam, was involved in crossbreeding of various tilapia species. Performance of crossbreds, in general, was found to be better than their corresponding parental strains. Hickling's (1960) study first showed the possibility of all male hybrids by interspecific hybridization. The variation in sex ratio of progeny in different interspecific crosses from the usual 1:1 ratio was later explained by suggesting that *S. mossambica* (*O. mossambicus*) has a homogametic female and a heterogametic male while the reverse is true in the case of *S. honorum* (Chen 1969). However, this could not totally explain the results of subsequent crosses.

Intraspecific and interspecific reciprocal crosses involving *O. aureus* and *O. niloticus* suggested significant increase in growth and fecundity of hybrids compared to those of the parental species. Significant reciprocal difference (*O. niloticus* males x *O. aureus* females vs. *O. aureus* males x *O. niloticus* females) in terms of the above two characters and the departure from 1:1 sex ratio suggested that the *O. aureus* strain may have homogametic males (Mukherjee and Geeta 1992). Results of the chromosome study of Das (1990) partially confirmed this.

Phenotypic selection of families of *O. niloticus* Philippine strain, and of *O. aureus* that produce more males on crossing, and subsequent mating between these families produced 67% male progeny and 33% female progeny. Similar selection for another generation did not improve further the sex ratio in favor of males (Table 2). Therefore, it was not possible to produce all male progeny which was originally planned in a three-year experiment.

Geeta (1995), using four populations from University of Malaya (two populations of *O. niloticus* and one each of *O. aureus* and *O. mossambicus*), performed a reciprocal diallele cross to estimate general combining ability (GCA), specific combining ability (SCA) and reciprocal effects for such traits as growth, fecundity, hatchability, fry survival, fry growth and various carcass characteristics. The following conclusions were drawn from her study:

1. Body weight at different ages had significant GCA which had greater influence than SCA.
2. Survival rate showed significant reciprocal effects, mainly when *O. niloticus* females were used in the reciprocal crosses.

The above study also showed V_A (additive genetic variance) for body weight was much higher than V_D (dominance genetic variance) and therefore intrapopulation selection may be effective in tilapia populations.

In another experiment with tilapia, two pure strains of *O. niloticus* obtained directly from a breeding farm in Taiwan were crossed (Khadijah 1999, pers. comm.). Mean birth weight and standard length at 24 weeks of

two pure strains, their F_1 and F_2 hybrids were recorded. Taiwan B was significantly heavier and longer than Taiwan A in three subsequent generations of testing, when the strains were not subjected to any selection pressure. Mean of F_1 hybrids was intermediate between the mean of the putative parents, but intermediacy was not exact. To measure this, hybrid index over two generations was estimated (Table 2) using the formula:

$$V_h = 1/g \sum_{i=1}^{2} (h_i - M_{li})/(U_{2I} - U_{li}),$$

where g is no. of generations; h_i is mean of the reciprocal F_1 ; M_{1i} is mean of two parent groups; U_{li} is mean of the inferior parent group; and U_{2I} is mean of the superior parent group.

Hybrid index of body weight was much higher than similar index for total length and standard length.

Genotype x diet interaction has also been observed for almost all morphometric traits in experiments conducted at UM (Eriyusni et al. 1998), with two *O. niloticus* and one *O. mossambicus* strains and two diets (commercial and UM diets prepared from mainly local ingredients).

Apart from tilapia hybridization, crossing between species is being carried on *Clarias* spp. The local *Clarias batrachus*, although has low growth and is difficult to culture, is tasty. This species has been crossed with exotic African catfish *C. gariepinus* and with *C. macrocephalus*. Hybrids of these two crosses have higher survival rate than that of their parent species (Ong 1992b).

Giant freshwater prawn-breeders from hatchery versus wild stocks

A study was undertaken on giant freshwater prawn (*Macrobrachium rosenbergii*) to compare the performance of wild stock and pond breeders (Zainoddin 1995). This study, involving four generations, showed that growth and survival rates of stocks derived from pond breeders during larval stage were better than those from wild stocks. On the other hand, growth performance of the stocks from wild breeders during postlarval stage was better than those derived from pond breeders.

Table 2. Hybrid index of some traits in crosses of tilapia strains from Taiwan after 24 months.

Trait	Mean			
	P_1	P_2	F_1 reciprocals	Hybrid index
Weight (g)	207.0	145.0	179	70
Total length (cm)	020.7	019.2	19.5	35
Standard length (cm)	017.0	015.0	15.7	32

Larviculture of genotype from wild breeders during the parental and next three generations did not show any significant difference but the fourth generation larvae showed faster metamorphosis and higher survival rate.

Selection

Genetic selection was conducted to improve overall quality of the red tilapia in the following traits: production of fry with intense red body color, fast growth and desirable morphological characteristics such as smaller head size, higher body girth and higher weight per unit length. Based on results of the study, red tilapia fry may be produced by spawning individuals which originated from fry with completely red color. The selection of "supergrowers" was found to give rise to faster growing fry as compared to unselected wild stock (Chuah and Nor Azam 1992).

The project is still progressing at the Central Fisheries Research Station (CFRS), Batu Berendam, and quantitative analysis of the response to genetic selection is expected in near future.

Ornamental Fish

A great deal of interest exists among breeders of ornamental fish in genetic variation of color and meristic characteristics. Private breeders in Malaysia and Singapore have developed many new varieties of guppy, tiger barbs and koi (Japanese carp) but due to their own secrecy, published papers are scant. Of significance to mention is the publication from Shahreza et al. (1998) on crosses among three varieties of tiger barb, *Puntius tetrazona* - normal (N), green (G) and yellow (Y). Results of their crosses indicate the dominance of N over G and Y, and there was no segregation of the yellow varieties.

Color genetic studies of ornamental fish are also being conducted by CFRS and will be undertaken by UM (Selvaraj, pers. comm.).

Molecular Genetics

Intensive fish molecular genetic studies were initiated at UPM. Other universities are also planning their research programs on these.

Tan et. al. (1998) developed RAPD (randomly amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) techniques to type for DNA markers in river catfish, *Mystus numerus* and in several strains of *Oreochromis* spp. (Chong et al. 1998; Usmani et al. 1998).

In *M. numerus*, genetic similarity index (SI) values among five sampled populations indicate almost identical results with RAPD and AFLP. High similarity between populations and existence of sufficient heterogeneity within populations were observed. This group now aims to search for molecular markers in the populations which may have influence on quantitative traits.

At UPM, research work has generated a total of 73 RAPD markers in three strains of tiger barbs. Significant polymorphism observed in three populations (N, Y and G) was followed by work on the assessment of genetic variability within and between populations, and estimation of Nei and Li's SI. Results showed significant genetic differences among tiger barb populations (Asma et al. 1998).

Preliminary results on DNA fingerprinting in a Malaysian strain of *B. gonionotus* using a YNZ22 DNA probe on genomic DNA digested with Hae III were reported (Siraj et al. 1998). As there were only a low number of bands shown in the fingerprint, genetic variability in their 15 samples was minimum.

Molecular genetic work is also planned on some ornamental fish by Dr. Selvaraj (pers. comm.) of UM in collaboration with UPM and Central Fisheries Research Institute.

Future Outlook

For a country placing importance on aquaculture, Malaysia cannot afford to neglect the application of genetics in aquaculture. With the problems of artificial propagation being overcome, there is increasing use of hatchery-produced stocks in aquaculture. Hatcheries function as genetically isolated closed units raising their own stock of breeders and producing seed for distribution in grow-out areas. In fish and shellfish, where fecundity is very high with thousands of eggs per female, the breeding can be concentrated on less than 1% of the fish. Management practices thus exert strong selection pressures.

Breeding of fish with high local demand and development of modern hatchery techniques shall further reduce the dependence on imported fry. Already one of the mainstays of the industry, prawn culture, is showing a decline in the quality of stock. The size obtained from wild broodstock is larger than those cultivated from hatchery stock, probably because of inbreeding (Ong 1992a). Here again the selection program for higher body weight and genetic resistance to disease may be practiced.

Restocking of hatchery-bred fish in public waterbodies could reduce the genetic pool more than in closed aquaculture environments. Some rare or endangered fish are being stocked in public waters through hatchery production while commercial fishing continues. Without control of the number of parents to maintain variability, there could be loss of important genetic characteristics such as disease resistance.

Besides inbreeding, changes in the endemic gene pool could also be occurring through introductions of species or varieties for aquaculture. The consequences of such introductions are not yet known.

There is a need for definition of the effective population size and the rate of loss of variability to control inbreeding through hatchery propagation. Consequently, maintenance of a reservoir of genetic variability, so that cultured populations are not vulnerable to change, is important. However, the use of holding facilities is expensive so cryopreservation of sperm and ova if/when possible could be an alternative worth looking into. Moreover, aquatic organisms introduced for culture could be made sterile through hybridization, ploidy or hormonal means.

Fish molecular biology research is gaining importance in UM. This research must be linked with that of institutional or industrial breeders whose cooperation makes it possible to include many fish samples in the experiments, and to develop proper breeding strategies.

Conclusion

Genetic research is no longer an option but a requirement if aquaculture in Malaysia is to improve. However, techniques adopted should be feasible within the infrastructure available at national and farm levels and relatively safe environmentally. Fish species of economic importance should be bred and selected for further genetic improvement. Molecular genetics may play an important role in identification and selection for genetic improvement in the future.

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FISH GENETICS RESEARCH AND DEVELOPMENT IN THE PHILIPPINES

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ABSTRACT

Fish genetics research in the Philippines is still in the nascent stage. It started in the early 1980s with concentration on the Nile tilapia (*Oreochromis niloticus*), which is most popularly used in freshwater aquaculture. Classical breeding programs to develop fast-growing strains of *O. niloticus* were initiated and have resulted in increased growth rate. Other genetic studies were focused more on the production of monosex tilapia using the YY-male technology, hybridization and sex reversal. Selection program is underway for the development of saline-tolerant tilapia for brackishwater aquaculture. Further genetic work is also carried out along with conservation of biodiversity of freshwater fishes.

Introduction

The Philippine archipelago has a wide expanse of water resources, approximately seven times bigger than its land resources. The total marine area is about 220 million ha, including 200-mile exclusive economic zone. Inland fishery resources, which include fresh and brackishwater, swamps, ponds, rivers, lakes and reservoirs, make up 842 247 ha.

The average annual growth rate achieved by the Philippine fisheries during 1987-1997 was 2.2%. While aquaculture and commercial fisheries have shown 5.3% and 4.4% annual growth, respectively, municipal fisheries declined by 1.6%.

In 1997, fish production was 2.7 million t valued at PhP80.7 billion. Of this, 0.885 million t (32%), valued at PhP25.9 billion[b], was from marine fisheries, and 0.958 million t (35%), valued at PhP27.4 billion, was

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from aquaculture. Inland municipal fisheries produced 0.924 million t (33%), valued at PhP27.4 billion (BFAR 1998).

Aquaculture had the highest growth of all fisheries subsectors. Over the last 16 years, its contribution to total fish production tripled, from 12% in 1980 to 35% in 1997. With the decline in production from marine fisheries, aquaculture is playing an increasingly important role in the industry and is being promoted by the government. As a result of technological developments and advances, production from aquaculture is increasing. In 1997, the commodities that contributed to the growth of aquaculture were seaweeds, milkfish, shrimps, tilapia, etc. (Table 1).

Table 1. Major species that contributed to aquaculture production in 1997 (BFAR 1998).

Species	Quantity (t)	%
Seaweeds	627 105	065.5
Milkfish	161 419	016.9
Shrimps/prawns	41 610	004.3
Tilapia (cichlid)	91 831	009.6
Others	35 581	003.7
Total	957 546	100.0

Status of Fish Genetics Research

Fish genetics research in the Philippines has a short history, having started only in 1980. Fish genetics research being undertaken by various Philippine institutions is presented below and summarized in Table 2.

Freshwater Aquaculture Center/Central Luzon State University (FAC/CLSU)

FAC/CLSU, being a host to several genetic projects, is at the forefront of tilapia genetics in the country. The Center, in collaboration with its partner-institution, has implemented several projects in the last five years. Each of these projects is described below.

International Development Research Centre (IDRC)-funded Fish Genetics Project

In this project, within-family selection approach was used to improve the growth of locally adapted *Oreochromis niloticus* strains (Bolivar 1998). The project focused on the development of a selection strategy that will be applicable in conditions with limited

Table 2. Fish genetic research priorities in the Philippines.

Identified research priorities	Aims
1. Tilapia genetic improvement	• Continued selection (different methods; interspecific-intraspecific or a combination of methods): - for growth - growth in particular climatic environments - salinity tolerance - single sex - flesh quality - maturation - disease resistance • YY male technology • Sex reversal • Hybridization • Gene transfer • Guidelines for genetic research and introductions
2. Conservation/characterization	• Identification of indigenous species which are endangered: - *Ludong (Cestraeus plicatilis/Valamugil seheli)* - *Tawilis (Sardinella tawilis)* - *Cyprinids (Puntius sirang)* - *Pigek (Mesopristes cancellatus)* • Advice/plans for *in-situ* conservation • Surveys for establishment of management (genetic) plans • Genetic assessment of effects of stock enhancement • Estimation of inbreeding • Continued monitoring of available biotechnologies, especially biochemical/DNA techniques

facilities. Twelve generations of within-family selection have shown that this approach is effective, as demonstrated by the selection response. The regression of mean breeding values against a number of generations indicated that the expected genetic gain would be about 12% per generation. Based on mixed model methodology, the estimate of heritability in the base population was 0.38. Not much genotype-environment interaction was observed in ponds, tanks and cages. Although the selection was done in tank environment, good response was also observed in hapas and ponds. The study has indicated routine selection activities can be undertaken on small facilities like tanks while the production of stock and grow-out can proceed normally in ponds.

From a managerial perspective, it has been found that within-family selection is easy to manage and inbreeding can be kept to a minimum if a structured mating scheme, like a rotational mating plan is used. Rotational mating has proven to be easy to apply in association with within-family selection scheme where a complete pedigree is maintained. Within-family selection does not require extensive facilities as would be needed for a presumably more efficient selection approach like combined selection. The choice of a selection procedure, particularly for tilapia, is a matter to be decided not only on genetic aspects but also on economic grounds, given the prevalent scale of tilapia industry in Asia, which is highly diverse and small-scale. Small-scale farmers can practice on-farm selective breeding using a simple, low-cost within-family selection scheme. This will empower farmers to use strains of their choice and not to depend on commercial hatcheries.

Genetic Manipulation for Improved Tilapia (GMIT)

Through the application of simple genetic manipulations, the University of Wales, Swansea (UWS), in collaboration with FAC-CLSU and funding from the Overseas Development Administration of U.K. (ODA/UK), has developed a new genetic technology for producing all or nearly all-male progeny in *O. niloticus*. Known as the "YY male technology", this takes the form of a breeding program combining feminization and progeny testing to produce novel males with YY genotypes (i.e., with two male sex chromosomes) instead of the usual XY male genotype (Mair and Abella 1997). These YY males are known as "supermales" and have the unique property of siring only male progeny. The growth performance of the genetically male tilapia (GMT) is 30%-50% higher than mixed sex tilapia. Dissemination of YY technology is being done through 49 accredited hatcheries in the Philippines.

Genetic Improvement of Farmed Tilapias (GIFT)

FAC/CLSU was one of the research partners in the ICLARM-coordinated and implemented Project for the Genetic Improvement of Farmed Tilapia (GIFT). Apart from participation in major breeding experiments, the FAC/CLSU also completed several complementary studies and graduate theses research. Among these were the response of GIFT strain to various feed formulations; growth performance in rice-fish environments and with bagasse (sugarcane waste) use in freshwater aquaculture.

Fish Biodiversity with Focus on Ex-situ Conservation of Freshwater Fishes

For conservation and protection of biological diversity, an inventory of indigenous freshwater fishes in the Philippines was made. This was followed by collection and propagation of these fishes by induced spawning. Artificially bred fishes were cultured in ponds and hapas. Some of the indigenous freshwater fish that were bred successfully in captivity were *Anabas testudineus, Trichogaster pectoralis, T. trichopterus, Clarias macrocephalus* and *Misgurnus anguillicaudatus.*

National Freshwater Fisheries Technology Research Center / Bureau of Fisheries and Aquatic Resources (NFFTRC/BFAR)

For ten years, NFFTRC/BFAR has been a collaborator in UNDP-funded GIFT Project and the ODA-funded GMIT Project. Ongoing activities of the center are:

- Application of YY male technology to an improved breed of *O. niloticus* and fingerling production of GMT from crosses of YY males with different maternal strains of *O. niloticus*, under a collaborative project with UWS with funding from ODA; and
- Tilapia broodstock development for saline waters in collaboration with FAC/CLSU and the Philippine Council for Aquatic and Marine Research and Development (PCAMRD).

University of the Philippines at Los Baños (UPLB)

The Genetic and Molecular Biology Division of UPLB is working mainly on conservation and identification of stocks/genetic variations using biochemical method

and DNA fingerprinting. Some of the studies conducted include comparison of the protein banding patterns of milkfish (*Chanos chanos*), *O. niloticus* and other fish in polluted and less polluted areas in Laguna de Bay. Initial results indicate that heterogenous banding exists in stocks found in Laguna de Bay polluted areas.

University of the Philippines in the Visayas (UPV)

Genetics research at UPV is being carried out in the following aspects:

- survival mechanisms of selected marine finfish larvae (DNA/RNA composition of milkfish larval forms, seabass; DNA/RNA ratio for determining growth of fish);
- database of genetically identified commercial species (being conducted in collaboration with a Venezuelan scientist);
- genetic profiling of commercially important finfish species and species karyotyping (being conducted in collaboration with a Japanese scientist); and
- DNA fingerprinting and electrophoretic identification.

PCAMRD

PCARMD, together with the Centre de Cooperation Internationale En Recherche Agronomique Pour Le Developpement (CIRAD), France, and BFAR, have initiated a four-year project for developing a highly salinity-tolerant tilapia strain for brackishwater environments. The project is financed by the Philippine and French governments and is conducted at the BFAR National Integrated Fisheries Technology Development Center, Pangasinan.

The technique used is repeated backcrossing of F_1 progeny of *O. niloticus* and *O. mossambicus.* From the salinity-tolerant hybrid population, selection for fast growth under saline water conditions will be done, followed by rotational backcrossing scheme protocol.

In France, a similar project using hybrids obtained from crossing fast-growing *O. niloticus* with high salinity-tolerant species *Sarotherodon melanotheron* is also underway. The project is a collaboration among CIRAD, Institut National de la Recherche Agronomique, L'Institut Francais de Recherche Scientifique pour le Developpement en Cooperation, Institut Francais de Recherche pour l'Exploitation de la Mer and Liège University in Belgium.

GIFT Foundation International Inc. (GFII)

For ten years (ending in December 1997), GIFT project has demonstrated how stepwise progression from systematic documentation of genetic resources to their utilization can generate rapid benefits for both farmers and consumers (Abella 1998; GIFT 1998). The project evolved into a self-sustaining tilapia breeding program known as GFII. This nonprofit foundation was established to serve as custodian of the breeding nucleus and to continue the selective breeding started and developed under the GIFT Project. The foundation's research and breeding activities are funded by contributions from hatchery operators who use GIFT broodstock as well as "royalties" collected from the sale of fingerlings by the hatcheries.

San Miguel Corporation (SMC)

SMC, a profit-oriented private company in the Philippines has been doing selection experiments to improve the growth of *O. niloticus*. In 1995, it has developed a special B-Meg strain, called Best 200. This strain showed superior growth compared with commercially available *O. niloticus*.

National Research Priorities

New Philippine National Tilapia Breeding Program (NTBP) of BFAR

The broad objective of the national breeding program (Eknath et al. 1991; Sevilleja et al. 1993) is to enhance genetic resources for aquaculture productivity. The specific objectives are to: (1) sustain a long-term selection program in improving performance traits (growth, survival, maturation, disease resistance and other economic traits); (2) institute broodstock management practices to avoid inbreeding; and (3) disseminate efficiently the genetic gains to target beneficiaries of the breeding program, especially the resource-poor farmers.

Efficient dissemination of genetic gains is possible only when there are organized channels for production and distribution of fish seed to fishfarmers (Morales et al. 1994). The NTBP operates as the National Broodstock Center (NBC) for tilapia in the country. The NBC has the primary function as first-level multiplier/producer

of quality broodstock and fingerlings for distribution to the second-level multiplier stations. The framework of NTBP includes the participation of different Regional Outreach Stations of the Department of Agriculture (DA) and of Local Government Fish Hatchery Units in provinces, municipalities and villages. The program also includes partnership with private tilapia hatcheries assisted by BFAR which will be accredited to serve as the second-level multiplier/ producer of broodstock and fingerlings for the tilapia hatchery operators and grow-out operators, respectively. Various state colleges and universities also serve as collaborators in freshwater aquaculture research, development and extension.

The national fish breeding program was established in the Philippines, following the experience of the GIFT Project. The present efforts of NFFTRC/BFAR and of FAC/CLSU in formulating organizational structure, strategies and management plan for a self-sustaining tilapia breeding program will be further strengthened and given support.

Development of saline-tolerant tilapia

In 1997, tilapia production from 14 000 ha freshwater fishponds was reported at 39 005 t. Brackishwater fishponds with an area of 239 323 ha contributed only 5 939 t (BFAR 1997) because more than half of these are underutilized. There is room for increasing productivity of brackishwater ponds by using other aquaculture species. Tilapia has been identified as one of the species for the purpose, and breeds could be developed to withstand saline and high water temperature conditions.

The need for a salinity-tolerant tilapia species is due to these reasons: (i) increased consumer acceptance of tilapia; and (ii) high demand for tilapia fingerlings due to inadequate supply of milkfish fry for stocking brackishwater ponds where shrimp culture has been abandoned due to disease problems. At present, only *O. mossambicus* and tilapia hybrid can thrive well in brackishwater fishponds.

At FAC/CLSU, a study was undertaken to assess the salinity tolerance of different strains of genetically improved *O. niloticus*. Three strains of *O. niloticus* (GIFT, IDRC and Israel) were mated following a 3 x 3 diallele cross. The saline-tolerant *O. mossambicus*, was used as the reference line.

Progenies from three purebreds and six reciprocal crosses of *O. niloticus* and purebred *O. mossambicus* were tested for tolerance at different salinities. Three salinity tolerance indices, i.e., median lethal salinity (MLS-96), mean survival time (MST) and median survival time (ST_{50}), were used for evaluation. Group variations of these indices were subjected to statistical analysis. The reciprocal effect, heterosis and general combining ability were estimated based on the differences in salinity tolerance of different cross progenies.

Results revealed that salinity tolerance, based on the measurements of MLS-96, MST and $ST_{50,}$ varied among the crosses. Purebred IDRC, IDRC x GIFT, purebred Israel and *O. mossambicus* exhibited highest MLS-96 of 22.43, 22.33, 22.27 and 22.21 ppt, respectively. Crossbred IDRC (female) x Israel (male) (and reciprocal) showed relatively high MLS-96 (20.93 and 20.09 ppt) while purebred GIFT and crossbreds of GIFT (female) x Israel (male), GIFT (female) x IDRC (male) and Israel (female) x GIFT (male) were low-tolerant crosses, and exhibited MLS-96 lower than 20 ppt. For MST and ST_{50}, *O. mossambicus* exhibited the highest tolerance which were 145.16 min and 143.75 min, respectively. Results of the study indicated that IDRC strain is a potential strain for saline water.

Reciprocal effects for MLS-96 and MST were found in the crossbreds between GIFT and IDRC strains. The IDRC (female) x GIFT (male) cross has significantly higher MLS-96 and MST ($P<0.01$) compared to its reciprocal cross. Reciprocal effects were also observed in MLS-96 of GIFT (female) x Israel (male) crossbred ($P<0.01$) and MST ($P<0.05$) and ST_{50} ($P<0.01$) of IDRC (female) x Israel (male) crossbred.

Percentage heterosis was negative for every crossbred. The estimated values for overall crosses were –8.36%, -17.26% and –17.41% for MLS-96, MST and ST_{50}, respectively, indicating no hybrid vigor for salinity tolerance in crossbreds.

Estimates for general combining ability based on the indices measured showed that IDRC strain gave positive values when used as maternal strain and significantly differed from Israel and GIFT strains. This reflects IDRC strain's merit for hybridization.

Based on the results on MLS-96, MST and ST_{50}, *O. mossambicus*, it was observed that the salt-tolerant tilapia that can survive in seawater, cannot survive in salinity over 25 ppt following direct transfer. This could

be attributed to its progenies that were spawned and hatched in freshwater.

Strengths and Limitations in Conducting Fish Genetics Research

Fish genetics research in the Philippines has already achieved a certain level of competency. While limitations exist, these are manageable, and if attended to, the research can be accelerated.

On 24 September 1996, BFAR convened a meeting of research scientists from various Philippine institutions to initiate the formation of the Philippine national network which is the local counterpart of INGA. This national network serves as a forum for information exchange and for facilitating national and international collaborative research.

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ABSTRACT

The paper gives a brief review of the aquaculture genetic research in Thailand, including genetic characterization of populations or species, selective breeding, sex control, genetic manipulation, cytogenetic studies, cryopreservation and genetic engineering.

Introduction

The major inland resources in Thailand available for freshwater aquaculture and fisheries development are composed of 66 rivers, 10 233 lakes and swamps, 685 reservoirs and human-made lakes with a surface area of 566 400 ha. The Gulf of Thailand on the east coast has an area of 320 000 km^2 and a coastline of 1 874 km. The Andaman Sea to the west has an area of 116 280 km^2 and a coastline of 740 km. The distance along both coastlines is 2 614 km, with a total area of 272 000 km^2.

With these natural resources, fisheries industries play an important role in the economy of Thailand. Fish production in 1993 was estimated at 3.385 million t, with a total value of US$3 136.27 million. In 1993, the total value of exported fish and fishery products was valued at 91 018.326 million baht (US$3 640.73 million), ranking fourth among export items. Moreover, the fisheries industries have contributed directly to the development of other related businesses, of which there are about 7 000 units, such as ice plants, cold storage, fish processing, ship building and fishing gear.

Overview

Genetic characterization of populations/ species

Genetic characterization of populations or species in black tiger shrimp *(Penaeus monodon*), banana shrimp (*P. merguiensis*), oyster *(Crassostrea belcheri),* giant freshwater prawn *(Macrobrachium rosenbergii*), snakeskin gourami (*Trichogaster pectoralis*), silver barb (*Barbodes gonionotus*), catfish, tilapia and frog (*Rana rugulosa*) was carried out by molecular genetic studies, using either isozyme electrophoresis, polymerase chain reaction (PCR) and restriction enzyme analysis or microsatellite DNA marker techniques.

Black tiger shrimp

Genetic variation was electrophoretically examined in populations of *P. monodon* from four locations along the coasts of Thailand (Trad, Suratthani, Phuket and Satun) to determine the extent of genetic isolation. The average heterozygosity of the species is relatively low (0.066±0.028) based on 46 loci. Results showed significant difference between populations from

Andaman Sea and Gulf of Thailand, and between those from Trad and Suratthani (Sodsuk 1996).

Studies to characterize microsatellite DNA in *P. monodon* genome and to develop genetic markers for use in various applications, including population genetic studies, selective breeding and genome mapping (Tiptawonnukul 1996) were completed. A total of 131 microsatellites were isolated and characterized from partial genomic libraries by screening with an oligonucleotide probe $(GT)_{15}$. These microsatellites were classified as perfect, imperfect and compound repeats. With 51.9%, imperfect repeats were the predominant category of microsatellites. A total of 97 $(GT)_n$ microsatellites were isolated and the frequency of the $(GT)_n$ repeats in the genome was estimated. The average distance between neighboring $(GT)_n$ microsatellites was 92.8 kb. The most common size class in all categories contained sequences between 30 and 35 repeats in length. PCR primers were designed from the unique flanking sequences of seven microsatellites, two pairs for $(AT)_n$ and five pairs for $(GT)_n$ microsatellites. By testing eight individual shrimps from Kruntung, Andaman Sea and Angsila, Gulf of Thailand, with PCR amplification, it was found that three of seven microsatellite primer sets, namely, Pmo 18, Pmo 36 and Pmo 14, produced fragments which sizes were in the expected range; the others gave nonspecific amplifications. Two microsatellite loci, Pmo18 and Pmo386, yielded scorable PCR products and showed high level of polymorphism indicating their potential use in DNA typing of *P. monodon*.

Randomly amplified polymorphic DNA analysis (RAPD) was used to examine genetic variation of four geographically separated wild populations of *P. monodon* from two regions, Andaman Sea and Gulf of Thailand (Pongsomboon 1996). A total of 75 individuals were tested. A screen of 300 random decaoligonucleotide primers identified seven primers, which were selected for the analysis of DNA. A total of 80 reproducible RAPD fragments ranging in size from 200 to 2 000 bp were scored and 62 fragments (77.5%) were polymorphic. The percentages of polymorphic bands were 57.77, 52.2, 45.6 and 53.4% for samples from Satun–Trang, Trad, Angsila and Medan, respectively. The similarity index (SI) within and between populations and genetic distances was performed based on the RAPD data. The results of SI within population illustrated that the Angsila sample was the most similar among themselves. The results of SI between populations and UPGMA dendrograms showed that the Medan sample was significantly different from the three samples of Thai *P. monodon*. Primer 428 detected a RAPD marker that was found only in samples from Andaman Sea, Satun–Trang and Medan, suggesting the use of this marker as region–specific. RAPD patterns among the four samples gave 214 genotypes. Of these, 160 were population–specific genotypes and 10 were region–specific; 97 of these genotypes were represented by an individual. Chi–square analysis of the genotypes showed that Thai and Indonesian *P. monodon* were significantly different for all primers ($P<0.0001$), and Thai *P. monodon* from Andaman Sea and Gulf of Thailand were also different for primers 101, 268, 428, 457 and 459 ($P = 0.0049$, <0.0001, < 0.0014 and $= 0.0156$, respectively).

cDNA library of abdominal muscles has been constructed in lambda ZAPII vector using *E. coli* XL–1 Blue cost in order to obtain quickly DNA sequences (Boonyawan 1998). A set of 93 partial *P. monodon* cDNA sequences (expressed sequence tags, ESTs) was obtained. Forty-one sequences were found to share significant matching to known gene sequences from other organisms. Their putative identities are: actin, myosin heavy chain, myosin light chain, acylphosphatease, arginine kinase, Ca^{2-} ATPase, cytochrome b, gelsolin, glutaryl–CoA dehydrogenase, heat shock protein, initiation factor, phosphopyruvate hydratase and pyrophosphatase. Two putative actin clones, designated as clones PMM048 and PMM099, were then subjected to complete sequencing. Open reading frames (ORF) of 376 and 377 amino acids were found, respectively. The predicted amino acid sequences showed high degree of similarity to those of insect and crustacean actins as well as of other vertebrate actins. The identities of those sequences were over 90%. The steady state mRNA actins were also analyzed by Northern blot. One 1.2 kb band was found when probed, with each probe corresponding well to its predicted size. When Southern blots, containing *P. monodon*'s genomic DNA digested with *EcoR* I, *Hind* III, *Pst* I and *Xho* I, were probed with DIG labeled probe, 8, 11, 5 and 4 DNA bands larger than 1.2 kb could be detected in autoradiograms. Based on the number of hybridized bands and their intensities, the number of actin genes in the *P. monodon* genome was estimated at five to ten copies per haploid genome.

Banana shrimp

Genetic variation was electrophoretically examined in populations of *P. merguiensis* from three locations along the coasts of Thailand (Chonburi, Suratthani and Satun) to determine the extent of genetic isolation. The average heterozygosity of the species is relatively low (0.066±0.028) based on 26 loci. Results indicated that all three populations are different. The two populations from Gulf of Thailand (Chonburi and Suratthani) were more closely related (Sodsuk and Sodsuk 1998a).

Oyster

Genetic variation was electrophoretically examined in populations of *C. belcheri* from five locations along the coasts of Thailand (Suratthani, Krabi, Ranong, Chantaburi and Trad) to determine the extent of genetic isolation. The average heterozygosity ranged from 0.424 to 0.584, and the mean number of alleles per locus ranged from 2.82 to 3.55, based on 11 loci. The most genetically similar populations were those from Krabi and Ranong, while the most different were those from Suratthani and Ranong (Pongthana et al. 1999a).

Genetic analyses of growth and survival rates were carried out in three *C. belcheri* populations from Suratthani, Ranong and Trad, grown in two natural environments (Ban Don Bay, Suratthani and Kao Youa Bay, Ranong). There were significant genetic effects on growth and survival rates. Significant location effect existed only in survival rate. The growth and survival rates of the genetic groups were site-dependent, and significant genotype by environment interactions were present for both parameters. The difference in ranking of performance in various environments was responsible for this interaction (Pongthana et al 1999a).

Giant freshwater prawn

Genetic variation in natural populations of *M. rosenbergii* was studied to identify races in different locations of Thailand (Yaitavorn 1989). The study was based on analysis of mitochondria DNA (mtDNA) variation, which was isolated from hepatopancreas and cut with restriction enzyme Sau3A1 to generate DNA fragments ranging from 0.2-2.0 kb. These DNA fragments were cloned into vector pUC12 at BamH1 site and transformed into *E. coli* strain JM107. After colony hybridization and Southern blot hybridization, 51 recombinant clones were obtained. These clones were further selected for strongly hybridized signal with α-32 P dATP labeled mtDNA, and used as probes to analyze mtDNA variation in prawns.The recombinant DNA no. 1 showed significant difference in restriction fragment length polymorphism (RFLP) pattern of mtDNA between specimens from Kraburi River and from Bangpakong River by Southern blot hybridization. The specimens from the latter showed strong discrete band at 1.1 kb, but those from the former was at 0.7 kb. By using this clone, RFLP pattern of Bangpakong River was similar to that from Kung Kam Thong Farm. The recombinant DNA no. 1 had inserted fragment about 1.1 kb and restriction endonuclease sites for Rsa1, Hha1, Hae111 and BstU1.

Genetic variation was electrophoretically examined in populations of *M. rosenbergii* from three locations along the coasts of Thailand (Chachoengsao, Suratthani and Songkla) to determine the extent of genetic isolation. The average heterozygosity of the species is relatively low (0.031±0.018) based on 24 loci. Results indicated that all individuals in three areas belong to the same population (Sodsuk and Sodsuk 1998b).

DNA probe was developed using RFLP technique and used for assaying DNA of *M. rosenbergii* (Prakongsai 1991). The extracted DNA has T_m (melting temperature) about 86°C corresponding to 43% G–C content. The genomic library of *M. rosenbergii* was constructed from Sau3AI–digested hepatopancreas DNA of a male prawn and ligated to BamHI site of pUC18. The transformants were selected through ampicillin resistance, their colorless characteristic on the medium containing IPTG and X-gal, and screening by hybridization with total DNA of either male or female DNA of 20 recombinant colonies selected for RFLP analysis. Thirteen contained repetitive sequences and could generate 120 bp repeating fragments as the common RFLP pattern. The repetitive sequences were divided into two types based on their sequence homologies. Sixty-one prawns collected from three provinces were classified into 13 groups based on their HinfI–RFLP patterns against pMR3 probe and suggested strain variation of *M. rosenbergii*. MR3–A fragment of pMR3 could generate HinfI–RFLP patterns and showed homology to DNA of *P. monodon* and *P. merguiensis*, whereas MR3–B and MR3–C hybridized specifically to *M. rosenbergii* DNA.

Snakeskin gourami

Population structures of *T. pectoralis* collected from five locations in Thailand (Samutprakan, Pitsanulok, Suphanburi, Ubonratchathani and Pattanee) were studied by detecting isozyme differences using Tris–Citrate buffer pH 7.0 and 8.0 and Tris–EDTA–Borate buffer pH 8.0 and pH 8.3 to test 14 enzymes, AAT, AK, EST, FUM, GCDH, PGI, HK, IDDH, MDH, ME, MPI, PGDH, PGM and PK, in five organs (muscle, liver, kidney, blood and heart). The highest genetic identity coefficient of 0.985 was found between Samutprakan and Pitsanulok populations; the lowest, 0.923, between Pitsanulok and Pattanee populations. From the estimates of genetic identity and distance coefficients among the five populations, the Samutprakan and Pitsanulok populations had the lowest genetic distance, while the Pitsanulok and Pattanee populations had the highest. Morphometric study was also carried out by comparing the ratio of head length to standard length. The fish from Pitsanulok was different from others. The fish from Samutprakan and Supanburi had the same head length to standard length ratio of 0.28, and the fish from Ubonratchathani and Pattanee had the same ratio of 0.27 (Pongthana et al. 1999b).

Silver barb

Spatial genetic structure of *B. gonionotus* populations in Thailand was reported by Kamonrat (1996). Microsatellite DNA markers were developed from a Thai *B. gonionotus* genomic library and used to study various aspects of its population genetics in Thailand for evaluating management policies on conservation and genetic improvement. Twelve natural populations from three rivers and 29 hatchery stocks from central and northeast regions of Thailand were studied. Genetic variability was high in both groups of populations. Multidimensional scaling analysis of genetic distances revealed the discreteness apparent between watersheds among natural populations, and between geographic regions among hatchery stocks. High genetic variability within populations and significant genetic differentiation between populations both in native and hatchery stocks indicated rich genetic resources of this species in Thailand. However, there were evidences that stock management may pose a threat of losing or altering genetic integrity of both natural and hatchery populations. Mixed stock analysis of the fish sampled from the rivers indicated 75%-96% were from hatchery populations. This high genetic contamination of natural populations was undoubtedly the consequence of restocking programs in which millions of fry/fingerlings of this species are released each year in rivers. Evidence of reduction of genetic integrity between regions was also observed due to stock transfer. The results suggested an urgent need for genetically based stock management policies for both natural and hatchery populations.

The potential use of microsatellites for broodstock improvement in aquaculture was also studied (Kamonrat 1996). Pedigrees of individuals were successfully established in a large communal rearing by using one to five microsatellites. The ability to identify individuals allowed a complicated genetic experiment and selective breeding to be conducted in places where facilities were limited. Results are considered to be more reliable because environmental variances are accounted for as fish are grown together from birth. In this study, heritability of growth traits in three stocks was estimated where all families were reared together. The estimates ranged from 0.193 to 0.523, suggesting that selective breeding in this species should result in good progress. However, heterozygosity in the largest individuals was greatly reduced, indicating that rapid inbreeding is very likely in simple means of selection strategies.

Catfish species

Genomic identification of catfish species by PCR and restriction enzyme analysis of the gene encoding the immunoglobulin M heavy chain constant region were carried out by Thongpan et al. (1994). Nuclear DNA was isolated from blood of catfish representing three families (Clariidae, Pangasiidae and Ictaluriidae) for analysis by PCR and restriction enzymes. The purity of DNA (A_{260}/A_{280}) from all fish samples was in the range of 1.8 to 2.0 primers, specific for the CH4 exon of the gene encoding the immunoglobulin heavy chain of channel catfish (*Ictalurus punctatus*) used. Nuclear DNA when amplified with these primers yielded a single band of about 300 base pairs for *Clarias macrocephalus, Pangasius gigas, P. hypophthalmus* and the hybrid of *P. gigas* x *P. hypophthalmus*. However, the same primers yielded two DNA bands of about 300 and 340 base pairs in *C. gariepinus* and in the hybrid of *C. macrocephalus x C. gariepinus*. Hence, the genetic identity of *C. gariepinus* could be identified at molecular level. Nucleotide sequences of the amplified DNA were identified for *I. punctatus, C. macrocephalus, P. gigas* and *P. hypophthalmus*. Based on the DNA

sequence data, a restriction enzyme, Hpal, was used to further clarify the common single band of *P. gigas, P. hypophthalmus* and their hybrid. Digestion with this restriction enzyme yielded one DNA (300 bp) for *P. gigas*, two bands (100 and 200 bp) for *P. hypophthalmus*, and three bands (100, 200 and 300 bp) for the hybrid. These findings would aid in identifying genetic contributions in hybrid, androgenetic, gynogenetic and polyploid catfish.

Tilapias

Biochemical characterization of blood-serum protein of tilapias was carried out by SDS - polyacrylamide gel electrophoresis (PAGE) (Pongsri 1995). Blood-serum protein of *Oreochromis niloticus, O. spilurus,* and hybrids between *O. niloticus* and *O. aureus* were characterized by SDS-PAGE. Using 8% gel concentration and silver staining, the electrophoretic patterns provided adequate information for the classification of the species and hybrids studied. SDS-PAGE is an applicable technique for genetic and taxonomic studies of tilapias.

Selective breeding

Selection and heritability estimates

Estimates of selection response for body weight and total length were observed in *O. nilotius* and *C. macrocephalus* (Table 1), while heritability estimates for growth and disease resistance were observed in *P. sutchi, Labeo rohita, C. macrocephalus, B. gonionotus, Aristichthys nobilis* and *Saccostrea cucullata* (Table 2).

Hybridization

Inter- and intraspecific hybridization was carried out in oysters, catfish species, tilapia and giant freshwater prawn.

Oysters. Hybridization in commercial Thai oysters (*C. belcheri, C. lugubris and S. cucullata*) was carried out via interspecific and intergenetic hybridization (Charoensit 1995) to explore the possibility of producing hybrid oyster with superior traits. Growth rate, shell morphology and karyotype between hybrids and their parental lines were compared.

Interspecific hybridization of *C. belcheri* x *C. lugubris* was successful only up to spat stage. Growth rates of hybrids and their reciprocal were significantly lower than those of their parents. Intergenetic hybridization was successful only when female *C. lugubris* crossed with male *S. cucullata.* Of all three replications, growth rates of the hybrid were significantly higher than those of *S. cucullata,* but not significantly different from those of *C. lugubris*. Shell morphology of the hybrid was intermediate between the two parental types.

Table 1. Estimates of selection response for body weight (BW) and total length (TL) of *O. nilotius* and *C. macrocephalus* in earthen pond.

Species	Traits	Selection method	Generation	Selection response
O. niloticus	BW	Within family	3	17%
C. macrocephalus	BW	Individual	4	50.5 g
C. macrocephalus	TL	Individual	4	0.88 cm

Diploid chromosome numbers of all three oyster species and the hybrid between female *C. lugubris* and male *S. cucullata* were all the same at 20. Two types of chromosome, metacentric and submetacentric, were recognized. Ratios between these two chromosomes were 16:4 for *C. belcheri* and *C. lugubris*, 14:6 for *S. cucullata*, and 15:5 for the hybrid between female *C. lugubris* and male *S. cucullata.*

Catfish species. Effects of strain crossing of *C. macrocephalus* on growth and resistance *to Aeromonas hydrophila* were studied (Prarom 1990). Local strains of *C. macrocephalus* from Songkla (s) and Saraburi (c) were collected and semi-diallele cross was conducted. Results showed that progenies from crossing of female from the South and male from the Central grew better than other group (sc > cc, ss, cs). Heterosis was observed for body weight and length at ages 2, 3 and 3.5 months. No heterosis was observed in reciprocal cross. Although there were no differences in disease resistance among the crosses and no heterosis, however, progenies from strain crossing had a trend to resist to *A. hydrophila* better than within strain. No correlation was found between: growth and disease resistance, growth and survival rate, and survival rate and disease resistance.

Interfamilial hybridization between *C. macrocephalus* and *P. sutchi* yielded three types of hybrids (Sittikraiwong 1987). The first group, with a single long dorsal fin and relatively *Clarias*-like, made up 18.27%. The second, having two dorsal fins and relatively *Pangasius*-like, was 79.69%. The third group, 2.03% of the hybrids, was actually *Clarias*. Karyotype studies revealed that *C. macrocephalus* had

Table 2. Heritability estimates for growth and disease resistance of six aquatic fish species.

Species	Trait	h^2	Reference
P. sutchi	TL at 99 days old	$h^2_D = 0.052 \pm 0.171$	Leeprasert (1987)
	TL at 126 days old	$h^2_D = 0.195 \pm 0.227$	
	TL at 182 days old	$h^2_D = 0.173 \pm 0.204$	
	Tl at 240 days old	$h^2_D = 0.062 \pm 0.440$	
	BW at 99 days old	$h^2_D = 0.056 \pm 0.273$	
	BW at 126 days old	$h^2_D = 0.139 \pm 0.199$	
	BW at 182 days old	$h^2_D = 0.122 \pm 0.213$	
	BW at 240 days old	$h^2_D = 0.126 \pm 0.424$	
L. rohita	TL at 118 days old	$h^2_D = 0.075 \pm 0.155$	Supsumrarn (1987)
	TL at 202 days old	$h^2_D = 0.046 \pm 0.074$	
	TL at 285 days old	$h^2_D = 0.893 \pm 0.094$	
	BW at 118 days old	$h^2_D = 0.102 \pm 0.107$	
	BW at 202 days old	$h^2_D = 0.024 \pm 0.044$	
	BW at 285 days old	$h^2_D = 0.093 \pm 0.088$	
C. macrocephalus	TL at 9 months old	$h^2_R = 0.39$	Jarimopas et al. (1989)
	BW at 9 months old	$h^2_R = 0.84$	
C. macrocephalus	TL at 210 days old	$h^2_R = 0.23$	Chamnankuruwet (1996)
	BW at 210 days old	$h^2_R = 0.31$	
C. macrocephalus	Resistance to *Aero-monas hydrophila*	$h^2_S = 2.34 \pm 0.07$ $h^2_D = 0.15 \pm 0.22$	Rasatapana (1996)
	BW at 53 days	$h^2_S = 2.01 \pm 0.95$ $h^2_D = 0.31 \pm 0.51$	
B. gonionotus	TL at 111 days old	$h^2_D = 0.012 \pm 0.055$	Jitpiromsri (1990)
	TL at 170 days old	$h^2_D = 0.044 \pm 0.246$	
	TL at 231 days old	$h^2_D = 0.168 \pm 0.541$	
	TL at 276 days old	$h^2_D = 0.202 \pm 0.332$	
	BW at 111 days old	$h^2_D = 0.217 \pm 0.090$	
	BW at 170 days old	$h^2_D = 0.067 \pm 0.322$	
	BW at 231 days old	$h^2_D = 0.055 \pm 0.626$	
	BW at 276 days old	$h^2_D = 0.291 \pm 0.517$	
A.. nobilis	TL at 124 days old	$h^2_D = 0.019 \pm 0.159$	Nukwan (1987)
	TL at 208 days old	$h^2_D = 0.078 \pm 0.122$	
	TL at 292 days old	$h^2_D = 0.038 \pm 0.109$	
	TL at 362 days old	$h^2_D = 0.014 \pm 0.039$	
	BW at 124 days old	$h^2_D = 0.077 \pm 0.186$	
	BW at 208 days old	$h^2_D = 0.069 \pm 0.128$	
	BW at 292 days old	$h^2_D = 0.048 \pm 0.156$	
	BW at 362 days old	$h^2_D = 0.004 \pm 0.043$	
S. cucullata	LW at 15 months old	$h^2_R = 0.184$ (50 oysters/net)	Thavornyutikarn (1994)
	LW at 15 months old	$h^2_R = 0.148$ (150 oysters/net)	

Notes: TL = total length; BW = body weight; LW = live weight; h^2_D = heritability estimated from Dam's variance; and h^2_R = realized heritability.

26 pairs of chromosome: nine metacentric, 10 submetacentric and seven acrocentric, with the fundamental chromosome number of 90. *P. sutchi* had 30 pairs of chromosome: nine metacentric, 11 submetacentric and 10 acrocentric, with the fundamental chromosome number of 100.

The *Clarias*-like hybrid had a chromosome number of 82 (allotriploid, 3n = 82) by obtaining two genomes (2n = 52) from *Clarias* and one genome (n = 30) from *Pangasius*. The chromosome set consisted of 27 metacentric, 31 submetacentric and 24 acrocentric chromosomes, with the fundamental number of 140.

The *Pangasius*-like hybrid had a chromosome number of 56 (amphidiploid, 2n = 56) by obtaining one genome (n = 26) from *Clarias* and one genome (n = 30) from *Pangasius*. The chromosome set consisted of 18 metacentric, 21 submetacentric and 17 acrocentric chromosomes, with the fundamental number of 95. The "actual *Clarias*" hybrids were supposed to arise from gynogenesis.

Tilapias. Improvement in salinity tolerance of *O. niloticus* by means of interspecific hybridization with *O. mossambicus* was studied (Thanakijkorn 1997). The hybrid having *O. niloticus* as a mother (NxM) had higher survival rate after salinity challenge test at 20 ppt than *O. niloticus* purebred, but lower than that of the reciprocal cross and *O. mossambicus*. At a 30 ppt salinity, a direct transfer killed all of *O. niloticus* purebred and the hybrid with *O. mossambicus* as a mother. The reciprocal hybrid (MxN) and *O. mossambicus* purebred had similar survival percentages (97.78±2.08 and 99.44±0.79%, respectively). An acclimated transfer at 30 ppt resulted in survival percentages of 34.45±11.00 and 43.30±7.20% respective to *O. niloticus* and hybrid NxM which were significantly lower than those of hybrid MxN and *O. mossambicus* (98.33±7.20 and 99.44±0.79%, respectively). Growth rate of the hybrid NxM was comparable to that of Nile tilapia while that of the hybrid NxM and Java tilapia was also comparable and lower than that of the first two groups. No statistical differences were observed in carcass percentages of the four groups. Based on the direct transfer results, backcross hybrid MNxN showed the highest salinity tolerance which was comparable to that of *O. mossambicus*. No significant differences were observed among salinity tolerances of the remaining crosses (NxNM, NMxN, NxMN) and *O. niloticus*. Carcass percentage of the backcross hybrids tended to be higher than that of the purebreds. Differences in some morphological traits were observed among hybrids and parental species.

Giant freshwater prawn. The life history and development of larvae in intraspecific crosses of *M. rosenbergii* from Kraburi River, Ranong province, and Chaopraya River, Ayutthaya province, were studied (Rattikhansukha 1993). Four treatment groups, pure lines and their crosses, were included. Differences in egg and larval sizes, postlarval incubation time and larval number were observed. The morphological differences indicated that the ratio between dactylus of the second claw and body weight of female could be used to separate the stock with 73.24% positive prediction. The ratio between head wet weight and propodus of the second claw of males could be used to separate the stock with 79.28% positive prediction.

Sex control

Snakeskin gourami. All-female *T. pectoralis* fry were successfully induced by oral administration of 17β-estradiol (EST) at 200 mg/kg diet to the two-week old post-hatch fry for the duration of 3 weeks (Pongthana et al. 1995a).

Catfish species. Effects of 17α-methyltestosterone (MT) on growth, survival and sex reversal of *C. macrocephalus* were studied by Na-Nakorn et al. (1993). Growth was not affected at 30 days ($P>0.05$) but was retarded at 60 days. Growth of treated groups was increasingly suppressed with increased dose and duration of treatment. Survival rate decreased at 30 days ($P<0.05$), but was unaffected from 31 to 60 days. Partial sex reversal was indicated by presence of ovotestes in some fish treated with MT for 60 days. In general, sex reversal to maleness was not accomplished, and with increasing dose and duration there appeared to be paradoxical feminization.

Wattanakul (1993) experimented on the effects of 17β-estradiol and 11β-hydroxyandrostenedione on sex reversal of *C. macrocephalus*. Sex reversal to female was accomplished in the groups treated with 30 and 55 mg/kg of 17β-estradiol, and not with the 5 mg/kg group. Few intersex gonads were observed in the groups receiving 30 and 55 mg/kg, and few sterile gonads in the 55 mg/kg group. Oral administration of 11β-hydroxyandrostenedione (at 5, 30 and 55 mg/kg feed) to 20 and 30 days old gynogenetic fry resulted in normal growth and survival rates compared to the untreated control group. Only the group treated with 55 mg/kg at 20 and 30 days showed sex reversal.

No reduction was observed in survival, growth rate or GSI (ovarian size in relation to total body weight) relative to control groups in all female *C. macrocephalus* produced through administration of 17β-estradiol. Hormone-treated fish also produced eggs which had no apparent reduction in viability compared to controls in fertilization trials using sperm of *C. macrocephalus* and *C. gariepinus* (Pongthana et al. 1994).

Studies on effects of 17α-MT on sex reversal of *C. gariepinus* revealed the highest female sex ratio of 85.43±9.06% in three-week old post-hatch fish

immersed in dosages of 20 µg/l for 4 weeks. Average survival rate was 55.83±5.20%. The highest male sex ratio of 59.66±10.48% was observed in three-week old post-hatch fish at dosages of 50 µg/l for 3 weeks. Average survival rate was 45.56±31.51% (Pongthana and Tangthongpairoj 1999).

No effect on sex ratio was detected when *C. gariepinus* (three and four-week old post-hatch) were immersed in water treated with 11β-hydroxyandrostenedione (HAS) (Pongthana and Tangthongpairoj 1999).

Tilapia. Thassananukulkit (1979) reported inducing sex reversal using MT and increasing production of male *O. niloticus,* at 60, 40 and 20 mg/kg in diet to fry aged 3-4 weeks - male percentages of 84.5, 81.0 and 72.0%, respectively, as against 57.5% in the control group. Due to hormone effects, abnormal males were found in 1.0, 3.0 and 0.5% cases, respectively, and hermaphrodites, in 2.5, 3.5 and 2.5% cases, respectively. For hermaphrodites, the ratios of the length of the fins (dorsal, pelvic and anal) to the total body length were relatively close to those males. The morphology of hermaphrodites' genital papillae was between that of male and female. The growth rates of fish were significantly different among the three hormone levels and the control. The MT 40 mg/kg diet produced the highest coefficient of condition (3.34), compared with that for other treated groups (3.16 and 3.20) and control group (2.95). Respectively, the survival rates were 96.1, 96.4, 94.7 and 97.8%; and the feed conversion rates were 1.76, 1.80, 1.61 and 1.81.

Sunsetplaty. Feminization was induced by immersion treatment of three-day old *Xiphophorus variatus* fry in 17β-estradiol at a dose of 100 µg/l for 28 days resulting in 82.25% female fish (Kungcharoenvattana 1999). Masculinization was induced by immersion treatment of three-day post-hatch fry in 17α-methyltestosterone at a dose of 50 µg/l for 30 days (Pongthana et al. 1999c).

Studies on the sex determination mechanism indicated that females are homogametic XX and males are heterogametic XY. Trials to indirectly produce all-males were carried out by crossing YY males with normal XX females resulting in average male ratio of 95.37±17.97% (Pongthana et al. 1999c).

Siamese fighting fish. The effects of MT at different concentrations on change of morphology, sex organ, survival rate, mating and behavior of *Betta splendens* females were studied (Suthitep 1982). A total of 1 200 six-week old female fish were cultured in freshwater with $0.5x10^{-4}$, $1.0x10^{-4}$, $1.5x10^{-4}$, $2.0x10^{-4}$ and $2.5x10^{-4}$ ppm of 17α.MT. Changes were observed in terms of body color. Compared with the control, dorsal fin was longer and body standard length was shorter. The ratio between the length of dorsal fin and standard length had linear correlation to the amount of hormone applied with 99% statistical significance. The survival rate decreased with the increase of hormonal concentration. The treated female behaved as an ordinary male. The development of the ovary was abnormal; the ovary lobes were rudimentary, unequally developed and contained abortive eggs and fluid. All treated females were sterile.

Sex reversal was induced by fluoxymesterone (commercially known as Halotestin) treatments (Tangthongpairoj et al. 1988). Three-day old fry, fed with *Moina* sp. immersed in 200 ppm Halotestin for 20 min and treated for 14 days, were morphologically male as distinguished by color and fin shapes. Their gonads couldn't develop to the secondary oocyte stage.

Marine shrimps. Study on the effects of exogenous male hormone, 17α-MT and testosterone proprionate on spermatogenesis and microscopic structures of *P. indicus* showed that 17α-MT at a dose of 1 mg/100g body weight is able to maintain spermatogenesis significantly (Chansawang 1994).

Genetic manipulation

Gynogenesis

Silver barb. Neomale or XX-male (genetically female but functionally male) silver barb (*B. gonionotus*) were produced by hormonally masculinized gynogenetic diploid fry using 17α-MT at 25 mg/kg in the diet, for a duration of 4 or 5 weeks, starting from two weeks post-hatch. Gynogenetic diploid fish was induced by UV irradiation of sperm at 196 uW/cm^2, cold shocking of 2°C for 10 min, starting from 1.5 min after fertilization (Pongthana et al. 1995b). The neomales produced all female offspring when crossed with ordinary females (Pongthana et al. 1999d).

Catfish species. Induction of gynogenesis in *C. macrocephalus* was studied by Leuangpromporn (1990). Irradiation of *P. sutchi* sperm using either 15 or

30 watts UV–tube at distance of either 20 or 30 cm (intensity of 201.38-604.14 erg/mm^2–second) and 2 or 7 min exposure could inactivate sperm resulting in the highest hatching rates of haploid gynogenetic fry. Experiments to study the effects of temperature, time after insemination and shock duration on polyploidization in *C. macrocephalus* showed triploid fish (3n = 81) in the group, which was shocked at 7°C for 20 min. In *C. macrocephalus*, gynogenesis could be induced using heterologous sperms irradiated with UV–ray. The hatching and survival rates of diploid gynogenetic fish were low especially from sperm treated with 30 watts UV–tube and 20 cm distance (intensity of 604.14 erg/mm^2–second). Gynogenetic fish of *C. macrocephalus* obtained from the group using lowest dose of UV–ray (15 watts, 20 cm and 2 min) grew faster than the others, including hybrid fish. This was probably due to paternal effect.

Gynogenetic diploid in *C. macrocephalus* was induced by UV irradiation of sperm at 4.8x10^4 ergs/mm^2, cold shocking the fertilized eggs at 7°C for 14 min starting from 4.5 min after fertilization which resulted in 60% viable diploid gynogenetic fry (Na-Nakorn et al. 1993).

Rungsin (1994) studied the optimum condition for induction of diploid gynogenesis in *C. macrocephalus* by retention of second polar body. UV–irradiated sperms of *P. sutchi* was used to activate the eggs. Cold shock (7°C for 20 min) was most effective when applied to activated eggs at 3.5-5 min after mixing eggs with irradiated sperm. Cold shock at 8-10°C (initiated 4.5 min after activation, lasting 20 min) was equally effective and gave satisfactory hatching of diploid gynogenesis. Shock duration of 14 min was found to be optimum to induce diploidization of gynogenesis (at shock temperature of 10°C, initiated 4.5 min after activation).

Diploid meiotic gynogens were induced in *C. macrocephalus* aimed at creating all female stocks (Na-Nakorn 1995). Induction was performed using UV-irradiated sperm of *P. sutchi* followed by either cold or heat shock. Cold shock (7°C, lasting 14 min) gave significant higher percentage of meiotic diploid fry than that of heat shock. Six-month old meiotic gynogens were all female and showed slightly inferior growth to male and female control. Survivals of the gynogens (73%) were slightly inferior to the mixed sex control (87.3%). The gonadosomatic index of the gynogens and the control female were 5.66 and 8.82, respectively.

Gynogenesis was induced in *C. macrocephalus* by mixing eggs with UV-irradiated sperms of *P. sutchi* following either cold or heat shock (Vichsonkul 1996). Cold shock at 6-11°C (initiated 4.5 min after activation, lasting 4 min) was equally effective and gave satisfactory hatching rates of diploid gynogenesis (30.65-48.24%). Heat shock at 42°C for 1 min was more effective than other high temperature treatments (35, 37, 40 and 42°C for 1-7 min). Heat shock (42°C for 1 min) was most effective when applied to activated eggs at 3.5-5.5 min after mixing eggs with UV-irradiated sperms. Cold shock (7°C for 14 min) gave significantly higher percentage (2.52-10.88%) of diploid gynogenesis (13.40-30.47%) than the heat shock (42°C for 1 min).

Ploidy manipulation

Generally, it is believed that polyploid fish has higher growth rate and stronger adaptability than those of diploid. The techniques of inducing polyploid were studied in catfish and oysters.

Catfish species. Polyploid in *C. macrocephalus* was induced by cold shock at 7°C for 25 min starting after fertilization, resulting in 80% polyploidy (Na-Nakorn and Legrand 1992). Survival rates of diploid and triploid fish were not significantly different in the first 2 months. But in the third, fourth and fifth months, triploid fish showed lower survival rates and body weight compared to diploid group. Food conversion ratio (FCR) of diploid was better than that of triploid in the first month. Both diploid and triploid fish showed similar FCR in the second and fourth months. There was no significant difference between both treatments as regards carcass percentages and resistance to *Aeromonas hydrophila* (Lakhaanantakun 1992).

Oysters. Polyploidy was induced in small oyster (*S. cucullata*) by cold shock at 4°C for 6 and 15 min starting from 20 min after fertilization, resulting in 40% polyploid (Jarayabhand and Jindalikit 1992). Triploidy was successfully induced in the mangrove oyster (*C. lugubris*) with 0.1 mg/l cytochalasin B for 15 min starting 15 min after fertilization resulting in 77% triploids (Roongratri and Youngvanichset 1988).

Polyploidy was induced in *C. belcheri*, by thermal shock (Silapajarn 1994). The polyploidization using heat shock at 35, 38, 40 and 42°C with 5 and 10 min treatment duration 10, 30 and 40 min after insemination had average triploidy of around

0-43.3%, and survival at the straight hinge stage was around 1.19-27.85%. The lowest polyploidy was achieved at shocking temperature of 35°C ($P<0.01$), while the lowest survival rate occurred at 42°C ($P<0.01$). The cold shock at 3, 7 and 10°C seawater temperature, 10, 30 and 40 min after insemination with 15 and 20 min treatment duration increased the triploidy to 8.45-35.28% and survival rate at the straight hinge stage was 0-20.3%. The lowest polyploid was obtained at 10°C ($P<0.01$). The survival rate of treated larvae declined with the decrease in shocking temperature. Oyster treated with heat shock at 35°C starting 30 min after insemination for 5 and 10 min performed better in terms of survival at the straight hinge stage and of growth at the setting stage ($P<0.05$).

Cytogenetic studies

Cytogenetic studies (chromosome number, karyotype and arm number) have been carried out in some freshwater fish species (Table 3).

Cryopreservation

Catfish species

Chairak (1996) studied cryopreservation of milt of *C. macrocephalus*. 0.85% NaCl (282 mOsm/kg, pH 7.0) and modified Cortland's #1 solution (MC#1-2, 292 mOsm/kg pH 7.0) were suitable extenders in terms of sperm motility and fertilizing ability. These two extenders were used for studying the toxicity (decreasing percentage of motile sperm) of either glycerol, dimethylsulfoxide (DMSO) or methanol at the final concentrations of 0, 6, 8, 10, 12, 14 and 16% in both extenders and motility of the sperm was determined at 30, 60 and 120 min after diluting and storing. Glycerol at all concentrations elicited higher toxicity than DMSO and methanol. Freezing sperm in 8, 10 or 12% DMSO and 6, 10 or 14% methanol before storing in liquid nitrogen by the programmable freezing unit at rates of -10, -20 and -30°C/min showed that DMSO was a better cryoprotectant than methanol. In another experiment, 8, 10 and 12% DMSO was used to freeze the sperm in above liquid nitrogen vapor at rates of -5 and -10°C/min. The results indicated no significant difference among treatments and between two thawing temperatures (50 or 70°C).

Red snapper

Hirunchaiyapuck (1996) reported that sperm characteristics of *Lutjanus argentimaculatus* are similar to most teleosts with round head and lack of acrosome. Average sperm density was 20.94×10^6 cells/ml with sperm motility ratings between 9 and 10. Average sperm motility and live sperm detected by Eosin-Nigrosin stain were 50.99% and 69.43%, respectively. The effect of dilulent on sperm preservation was not significant ($P=0.233$). However, modified Cortland's solution, Alsever's solution and glucose normal saline solution as dilulent gave higher percentage of live sperm than others. The cryoprotectant (DMSO) gave a higher percentage of live sperm than glycerol ($P < 0.001$). A study on freezing methods was also conducted. The pelleting method resulted in the highest percentage of live sperm ($P<0.001$), compared with the rapid and the slow freezing methods. There was no significant difference among thawing methods, i.e., thawing in low temperature, room temperature and high temperature (60°C) ($P = 0.215$). Alsever's solution + 10% DMSO freezing by pelleting method and thawing in 60°C is the best procedure for sperm cryopreservation, resulting in 75.67±10.89% of live sperm.

Genetic engineering

Catfish

Transfer of growth hormone gene into fertilized egg of *C. macrocephalus* by microinjection was carried out by Boonanuntanasarn (1995). Fish derived from microinjected egg had significantly higher growth rate ($P<0.05$). Integration rate of introduced gene in one, two and four-cell stages microinjected fish was 5.05% (5 of 99 samples), 6.45% (2 of 31 samples) and 8.5% (1 of 12 samples), respectively. Detection of the introduced gene in various tissues of microinjected fish indicated mosaicism in various tissues of fish, derived from all stages of microinjected eggs. This study revealed that embryonic development of yellow *C. macrocephalus* at one and two-cell stages were appropriate for gene transferring by microinjection.

Future Plans

Following are the plans for aquaculture genetics research in Thailand:

- Set up national breeding plans for all hatchery-produced aquatic species.
- Develop high-yielding strains of all hatchery-produced aquatic species.
- Study the genetic diversity of aquatic species in Thailand.
- Develop a genebank for germplasm preservation

Table 3. Chromosome number (2), karyotype and arm number of freshwater fish species.

Species	2n	Karyotype	Arm no.	References
Notopteridae				
Notopterus chitala	42	1st+20a	42	Donsakul and Magtoon (1990)
N. blanci	42	21a	42	Donsakul and Magtoon (1990)
N. notopterus	42	21a	42	Donsakul and Magtoon (1990)
Mastacembelidae				
Mastacembelus favus	48	5m+2sm+2st+15a	62	Donsakul and Magtoon (1989)
M. erythrotaemia	48	6m+1sm+17a	62	Donsakul and Magtoon (1989)
M. armatus	48	6m+1sm+2st+15a	62	Donsakul and Magtoon (1992)
Macrognathus siamensis	48	4m+1st+19a	56	Donsakul and Magtoon (1992)
M. circumcinctus	48	7m+1sm+16a	64	Donsakul and Magtoon (1992)
M. aculeatus	48	7m+2st+15a	62	Donsakul and Magtoon (1992)
Cyprinidae				
Osteochilus hasselti	46	7m+12sm	76	Magtoon et al. (1988)
Barbodes daruphani	48	7m+3sm+15a	70	Magtoon et al. (1988)
B. gonionotus	50	-	-	Na-Nakorn and Legrand (1992)
B. schwanenfeldii	50	-	74	Magtoon et al. (1989)
Morulius chrysophekadion	50	-	70	Magtoon et al. (1989)
Labeo erythrurus	48	7m+5sm+4st+8a	72	Donsakul and Magtoon (1993)
L. bicolor	50	10m+2sm+1st+12a	74	Donsakul and Magtoon (1993)
L. rohita	50	7m+3sm+2st+13a	70	Donsakul and Magtoon (1993)
Osteochilus melanopleura	50	10m+5sm+1st+1a	96	Donsakul and Magtoon (1995)
Puntioplites proctozysron	50	10m+3sm+3st+9a	76	Donsakul and Magtoon (1995)
Paralaubuca riveroi	48	8m+9sm+2st+5a	82	Donsakul and Magtoon (1995)
Rasbora sumatrana	50	13m+8sm+1st+3a	92	Donsakul and Magtoon (1995)
Catlocarpio siamensis	96	9m+27sm+3st+9a	168	Magtoon and Donsakul (1989)
Probarbus jullieni	96	11m+7a	132	Magtoon and Donsakul (1989)
Tor soro	100	9m+7sm+3st+31a	132	Magtoon and Donsakul (1990)
B. orphoides	50	7m+8sm+2st+8a	74	Magtoon and Donsakul (1990)
B. stoliczkanus	50	11m+11sm+2st+1a	94	Magtoon and Donsakul (1990)
Siluridae				
Kryptopterus cryptopterus	92	4m+5st+37a	100	Donsakul (1991)
K. bleekeri	64	10m+3sm+1st+18a	90	Donsakul (1991)
K. bicirrhis	64	10m+5sm+2st+15a	94	Donsakul and Magtoon (1996)
Ompok bimaculatus	50	17m+1sm+1st+6a	86	Donsakul (1991)
Wallago miostoma	56	13m+2sm+13a	86	Donsakul and Magtoon (1996)
W. attu	88	8m+1sm+2st+33a	106	Donsakul and Magtoon (1996)
W. dinema	62	10m+5sm+4st+12a	92	Donsakul and Magtoon (1996)
Clariidae				
Clarias macrocephalus	54	9m+10sm+7a	96	Sittikraiwong (1987)
C. batrachus	104	1m+1sm+50a	108	Saetung (1991)
Belontiidae				
Trichogaster pectoralis	46	23a	46	Donsakul and Magtoon (1988b)
Osphronemidae				
Osphronemus goramy	48	24a	48	Donsakul and Magtoon (1988b)
Helostomatidae				
Helostoma teminckii	48	24a	48	Donsakul and Magtoon (1988a)
Anabantidae				
Anabas testudineus	46	1sm+22a	48	Donsakul and Magtoon (1988a)
Pangasiidae				
Pangasius larnaudii	60	12m+10sm+2st+6a	102	Magtoon and Donsakul (1988)
P. sutchi	60	10m+6sm+2st+12a	92	Magtoon and Donsakul (1988)

Notes: m – metacentric; sm – submetacentric; t – telocentric; st – subtelocentric; a - acrocentric.

of aquatic species.

- Distribute genetically improved broodstocks and seeds to public and private sectors.

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REVIEW OF FISH GENETICS AND BREEDING RESEARCH IN VIETNAM

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ABSTRACT

A series of studies on fish genetics and breeding programs were conducted in Vietnam in 1998. These programs included genetic control and manipulation of sex ratio; production of monosex *Oreochromis aureus* and *Barbodes gonionotus*; chromosome analysis of *Macrobrachium rosenbergii*; induction of triploids in *Clarias macrocephalus*; generating transgenic *Carassius auratus;* dechorionation of one-cell stage embryos for DNA microinjection into *Misgurnus anguillicaudatus;* and breeding of *Cyprinus carpio, O. niloticus* and *B. gonionotus*. Details of the results of these programs are presented below.

Introduction

The Ministry of Fisheries (MOF, 1998) estimated the total fish production in Vietnam in 1998 at 1.67 million t, of which 538 000 t came from aquaculture, which contributed nearly 35% of total animal protein intake of the nation.

In Vietnam, traditional fish culture has been practiced as early as hundreds of years ago (Dan et al. 1997). Fish culture in ricefields and village ponds was first promoted by rice farmers to supplement their nutritional requirement. The main species cultured are local carps and other indigenous fish. During the 1970s and 1980s, with the introduction of exotic fish species from other countries and the success of induced fish breeding, aquaculture production in the country very much improved (Thien and Dan 1997). From 1990 to present, with the development of shrimp industry and sea farming, aquaculture contributes considerably to fish production for domestic consumption and export. It has become an important economic sector of the country.

Aquaculture is moving from extensive to semi-intensive culture system and commercial scale. The main components of production, such as quality of seed, feed and fish health management, need to be improved. Of these, seed quality is the most important factor to enhance productivity. Unfortunately, the deterioration of genetic quality in several cultured fish species has been very striking since the late 1970s (Thien 1996). Genetics research should be undertaken to create high-quality strains, particularly of valuable species, to improve aquaculture production and upgrade market value of the products. In recent years, the government has provided more funding for research on selective breeding and genetic technology. However, due to lack of qualified human resources and institutions, the research programs are limited, scattered

and poorly coordinated. A review of these programs, with their preliminary results, is presented.

Genetic Control and Manipulation of Sex Ratio

Use of YY-male technology in *Oreochromis niloticus*

Three major applications of YY-male technology in production of monosex male tilapia were conducted in 1998 at the Research Institute for Aquaculture No.1 (RIA-1), Bac Ninh, Vietnam. These were: (1) introduction of normal females and YY-males of the Egypt-Swansea strain *O. niloticus* in Vietnam to produce genetically male tilapia (GMT); assessment of relative performance of GMT and their parental stock in the country's aquaculture systems; (2) investigation of the potential for production of interstrain GMT by crossing YY-males of the Egypt-Swansea strain with females of other available strains, e.g., Thai-Chitralada strains and the genetically improved GIFT strain; and (3) development of YY-males in the well-performanced *O. niloticus* strains, Thai-Chitralada and Vietnamese strains, in the country's aquaculture systems (Tuan 1998).

Sex ratios in intrastrain GMT of the Egypt-Swansea strain (ES, GMT) from 25 families were variable, ranging from 39 to 100% with a mean of 83.2% male, which is lower than expected. Two on-station trials on comparative growth performance of the Egypt-Swansea GMT and five genotype/treatment combinations were underway with fish communally stocked in a series of earthen ponds. There were no significant differences in final harvest weights and survival rates between ES, GMT and sex-reversed male of Thai-Chitralada, GIFT and Vietnamese strains.

Comparative growth evaluation of ES, GMT with mixed-sex tilapia (MST) of available local strains and sex reversed male tilapia (SRT) of the Thai-Chitralada and GIFT strains on farm conditions was conducted. A total of 67 farms (63 integrated, two brackishwater and two sewage) in nine different provinces, with 88 ponds, were stocked with GMT, or SRT or MST control. Results from these trials should be available soon. However, early indications show that the growth performance of ES, GMT is not significantly better than that of local stocks.

Hybrid GMT by crossing YY-males of ES, GMT with GIFT females was investigated. Average percentage of males in hybrid GMT was 98%, and hybrid GMT performed well in pond aquaculture condition. Hybrid GMT was heavier at harvest compared to other genotypes, such as ES, GMT; GIFT, SRT; and Vietnam, SRT (Table 1).

Steady progress was made with the development of YY-males in Thai-Chitralada strain. Several batches of progeny from crosses of seven XY-females and seven males were grown and a number of males have been progeny tested. Furthermore, successful diethylstilbestrol (DES) treatments were applied to progeny from XY x XY cross, and treated females were being on-grown prior to progeny testing to identify YY-females.

Work was initiated on the development of YY-male technology in the Vietnam strain of *O. niloticus,* by application of feminization treatment to several families. This treatment was successful, and fry were grown prior to progeny testing to identify XY-females. Furthermore, a database of family sex ratios was established to determine the inherent variability of sex ratio in this strain of *O. niloticus*. To date, 31 families have been sexed, with sex ratios ranging from 10.2-94% male, with an overall significant skew to male.

Production of monosex *O. aureus*

Progress is being made in the development of GMT-producing broodfish in *O. aureus*. Production of monosex *O. aureus* began with DES treatments of fry from several families produced early in the season. Two concentrations of DES were used, 500 and 1 000 mg/kg, which produced sex ratios of 63% and 71% female, respectively). These results were not significantly different from that of the control (66%

Table 1. Growth performance of hybrid GMT and other genotypes.

Strain	Mean weight (g)		Survival rate
	Stocking	Harvest	(%)
Vietnam ES/GMT	1.3	119.3	84.0
GIFT ES/GMT	1.5	149.3	76.1
ES/GMT	1.6	111.9	87.4
GIFT,SRT	1.6	118.3	83.7
Vietnam SRT	1.7	128.3	81.1

Notes:
Vietnam ES/GMT - Vietnam female x super male of Egypt - Swansea strain. GIFT ES/GMT - GIFT female x super male of Egypt-Swansea strain. ES/GMT - genetically male tilapia of Egypt-Swansea strain. GIFT, SRT - sex-reversed male of GIFT strain. Vietnam SRT – sex-reversed male of Vietnam strain.

female), indicating poor rates of sex reversal. Further progenies were treated with an alternative estrogen, ethylnylestradiol, at same doses. The high dose of 1 000 mg/kg^{-1} caused total mortality but fry from the lower dose of 500 mg/kg^{-1} survived (86.9%-94.3%, with a sex ratio of 63.1% female). It is hoped that sex-reversed ZZ-females can be identified and their progeny subjected to a second generation of feminization treatment.

All-female production of *Barbodes gonionotus*

At RIA-1 in 1998, progress was made in all-female production using technique for induction of gynogenesis and hormone sex reversal in *B. gonionotus.* The sperm was diluted with 0.85% salt solution to concentration of 8 x 10^8 sperm/ml, and 10 ml of diluted sperm was irradiated in a small petri dish for 60 seconds using ultraviolet lamp. A cold shock at 2^{o}C was applied 90 sec after fertilization at 30^{o}C to eggs of *B. gonionotus* fertilized with UV-irradiated sperm for 10 min. Non-irradiated sperm was used in the diploid control group, and one group of eggs fertilized with irradiated sperm was not cold shocked, serving as a haploid control group.

In haploid control group, all hatchlings at two days old eventually died. On the other hand, the average survival rates in diploid control and mitotic gynogenesis groups were 66.7% and 11.5%, respectively. Sex ratios in gynogenetic fish were 91.3-97.4% with a mean of 95.8% female.

Androgen-treatment (17α-methyltestosterone or MT) was orally applied to gynogenetic fish to produce sex-reversed male *B. gonionotus*. Gynogenetic fish were fed with 25 mg MT/kg diets for 5 weeks. Percentages of males in androgen treated-fish varied from 8.1 to 36%, with a mean of 16.7% male (Thang 1998).

Chromosome analysis and sex-specific marker in *Macrobrachium rosenbergii*

In 1998, at the Research Institute of Animal Husbandry (RIAH), Hanoi, an attempt to analyze chromosome in *M. rosenbergii* was made. Chromosomes were prepared from cells collected from abdominal muscle of 50 individuals. A metaphase of *M. rosenbergii* showed variation in chromosome numbers. The method for preparing the prawn chromosome needs to be refined (Sac 1998).

Also in 1998, the University of Hanoi carried out sex determination in *M. rosenbergii*. Through heat shock, Chelex and kit methods, DNA was extracted from shell and muscle. DNA quantity in male and females was assessed. Concentration of DNA obtained from female was remarkably higher than those from males.

The extracted DNA was cut by 18 different restriction enzymes for analysis of restriction fragment length polymorphism (RFLP). Restriction enzyme Mnl I and Pvu I cut the DNA of male prawn at specific nucleotide sequences, whereas these restriction enzymes did not cut the DNA of female.

Random amplified polymorphic DNA (RAPD) technique was used to detect DNA variation between male and females. Several random primers were used: M13 (F), M13 (F and R) and human Y-gene specific primers. However, variability of RAPD profiles generated by these primers in male and females was not sex-specific (Luong et al. 1998).

Induction of triploids in *Clarias macrocephalus*

Induction of triploids in *C. macrocephalus* was conducted in the Southern Branch of Vietnam-Russia Tropical Center and the Mekong River Delta Research Station of RIA-2 for two years (Nga et al. 1998).

Matured breeders were spawned using hormone injection and stripping method. Dry fertilization of eggs was applied. To induce triploid embryos, 2-3 min after fertilization, the eggs were cold shocked at 3-5^{o}C for 30-40 min. (Table 2).

After 28 days of rearing in tanks, the survival rate, mean weight and total length of cold shocked and normal fry were similar (Table 3). Table 4 shows the growth and survival rates between triploid and normal *C. macrocephalus*.

Using cold shock to induce triploid catfish gave preliminary results of 36.2% larval survival and 48.1% triploids. Growth and survival of triploid (3n) catfish from fry to adult stages are normal as those of 2n catfish. After 5 months of rearing in grow-out ponds, the final weight of triploid fish was 16.4% higher than that of normal fish.

DNA microinjection in *Carassius auratus* and *Misgurnus anguillicaudatus*

An initial study on gene transfer in transgenic *C. auratus* was undertaken recently at the Institute of Biological Technology (IBT), Hanoi (Do et al. 1998). After injection of hormone to female and male

Table 2. Treatment parameters and results in inducing triploid *C. macrocephalus*.

Treatment	Timing after fertilization (Min)	Temperature (°C)	Treatment period (min)	FertilIzation %	Hatching %	Survival %	Triploids %
1	3	5	40	71	70	49.7	69
2	2	5	40	70	67	46.9	30
3	3	3	40	64	74	47.4	70
4	2	3	40	54	42	22.7	54
5	3	5	30	58	50	29.6	25
6	2	5	30	60	71	42.6	21
7	3	3	30	51	60	30.6	42
8	2	3	30	40	51	20.4	74
Control	0	0	0	84	80	67.2	0

Table 3. Survival and growth of *C. macrocephalus* fry treated by cold shock and normally reared in cement tanks.

Treatment	Density (fry/m^3)	Survival (%)	Mean weight (g)	Total length (cm)
Cold shocked fry	2 500	67.8	0.80	3.2
Control fry	2 500	66.0	0.76	3.1

Table 4. Growth and survival of triploid and normal *C. macrocephalus* in grow-out stage.

	Mean weight (g) in five-month grow-out period					Survival (%)
	1st	2nd	3rd	4th	5th	
Triploid catfish	12.0	35.4	70.3	112.6	155.5	65
Normal catfish	11.2	30.7	58.4	095.1	132.7	68

breeders, eggs and sperm were collected by stripping. Fertilization was done by dry method. About 25-30 min after fertilization, a one-cell embryo was formed. The chorion membrane of one-cell stage embryos was removed using trypsin enzyme solution with 0.075% concentration. After dechorionation, the embryos were microinjected with human Growth Hormone (hGH). The ADN solution with 40 ng/µg in Tris-EDTA was used for injection. The eggs were then incubated in Holtfretter solution at temperatures of 22-30°C. The hatching rate of injected eggs without chorion membrane was 20%. The survival rate of the three-day old larvae was 35% and of the 30-day old larvae, 3%. These fish are being reared for further study.

The above method of DNA microinjection was also initially applied in loach (*M. anguillicaudatus)* at IBT, Hanoi. The chorion membrane of one-cell stage embryos was removed before microinjection, using trypsin enzyme solution. The suitable concentration of solution, timing of treatment and other parameters are in Table 5. The 0.075% trypsin enzyme solution had the highest survival rate of larvae. The fry of *M. anguillicaudatus* obtained from dechorionation are being reared for monitoring of survival up to market size.

Breeding Programs

Family selection of *Cyprinus carpio*

Mass selection of *C. carpio* was carried out at RIA-1 for the last 10 years, from 1986 to 1996. Six selected generations were produced, of which growth rate in the fifth generation increased by 33% compared to the base population. However, the realized heritability (h^2) of body weight of fish gradually declined to nearly zero in the sixth generation (Thien 1996).

To improve the quality of the fifth and sixth generations, family selection has replaced mass selection. Initially, nine families from the sixth generation were used in family selection at RIA-1 in 1997. Under the Project Genetic Improvement of Carp Species in Asia coordinated by ICLARM, 14 other families were added in 1998 (Thien et al. 1998).

In 1997, nine families were bred completely by induced and stripping method. Based on the survival rate of fry and fingerlings in nursing/ rearing and growth of fish in grow-out stage, the progeny of three families have been selected for the next generation. The selected stocks may reach sexual maturation in March 1999. In 1998, induced breeding and artificial fertilization were conducted in 22 families, but successful in 14 families only. Results of nursing, rearing and grow-out of progeny are in Table 6.

Selection was focused on single trait-growth rate. However, survival of fish in the juvenile stage was

Table 5. Results of dechorionation in one-cell stage embryos of *M. anguillicaudatus*.

Trypsin solution (%)	Treatment period (min)	No. of eggs treated	Hatching rate (%)	Survival of larvae, after 3 days
0.025	1	215	18.6	90.0
0.050	1	104	47.1	88.2
0.075	1	80	80.0	93.7
0.100	1	160	8.1	92.3
Control	0	100	86.0	100

Table 6. Survival rate and growth of juvenile and adult *C. carpio* (progeny of 14 families).

	Survival (%) of			Final mean weight (g)	
Family no.	Larvae	Fry	Fingerlings	In pond	In hapa
1	75.6	55.4*	90.6	291.4	85.4*
2	80.2*	37.2	92.0*	364.4*	84.3*
3	81.3*	66.8*	95.0*	296.5	80.2
4	81.2*	59.7*	91.7*	309.2*	78.8*
5	77.9	34.4	82.0	308.5	73.5
6	65.5	34.4	96.0*	304.5	85.3*
7	71.5	44.5*	92.3*	319.5*	71.5
8	85.9*	63.7*	91.0*	341.6*	90.6*
9	67.6	26.0	93.3*	262.6	86.9*
10	73.2	40.0	64.3	306.3	96.2*
12	75.4	37.0	80.7	317.7*	75.6
13	83.2*	10.0	90.0	303.4	84.1*
14	80.5*	12.9	88.3	293.1	86.4*
15	83.3*	43.9*	90.2*	339.1*	94.1*

considered the most important parameter for selection since quality of seed was evaluated based on the survival rate.

Results showed that families had high survival during larval, fry and fingerling stages and faster growth during grow-out stage. Families 4, 8 and 15 were the best among 14 families with high viability in juvenile stage and fast growth from fingerling to market size. Families 2, 3 and 7 were better than the rest, suggesting that these could also be selected for the next generation.

Selective breeding of *O. niloticus*

During the last decade, tilapia culture in northern Vietnam lost its importance due to degradation of existing species, including *O. niloticus* imported from Taiwan in 1973.

O. niloticus has started regaining its role in aquaculture in the region since 1994 with the introduction of GIFT and Thai strains. These strains have faster growth, larger market size compared to local strains and are rapidly accepted by the producers and consumers.

Poor tolerance to low temperature is a major problem for tilapia culture in northern Vietnam. During cold months, pond water temperatures fluctuate between 10 and 22°C. Occasionally, water temperatures drop below 10°C. In such situations, high mortality occurs particularly in shallow ponds.

Thus, the selection of a strain that is fast-growing and has better cold tolerance is essential to improve tilapia production in northern Vietnam, which is the main objective of the breeding program (Dan et al. 1998). In 1998, family selection of such a strain began at RIA-1. About 100 families of GIFT strain, each including one male and two - three females of the fifth generation were received from the GIFT Project in 1997. Over 400 fish were used as initial materials for the selection. From these, communal stocking for breeding in hapas was carried out in May 1998. The fry were reared in hapas up to fingerling size (8-10 g). A total of 40 000 fingerlings of this size were harvested in July 1998.

Cold challenge test for fingerlings was undertaken under laboratory condition. The fingerlings were acclimatized at 25°C for a day then water temperature was reduced gradually at the rate of 0.5°C/hour. The fish were tested under low temperature until 50% of these died. A total of 4 000 fish were stocked in earthen ponds and reared from July to December 1998. Nearly 3 000 fish were harvested and from these, 200 males and 400 females were selected for overwintering in hapas-in-pond since mid-December 1998 for the selection program in 1999.

Selective breeding of *B. gonionotus*

The program was initiated in early 1998 at Cai Be Fishery Research Station of RIA-2. The base population was evaluated and selected as initial materials for the breeding program (Hao et al. 1998).

Between early January and end of February 1998, the broodstocks were collected from six different locations (six strains) in the southern region for stocking and evaluation in Cai Be station. Four wild stocks were collected from the river systems of Dong Nai, Ben Tre and branches of Mekong River, and two stocks from the farms at Can Tho and Tien Giang. The size of fish ranged from 175.6 to 371.3 g.

Six stocks were conditioned in earthen ponds for maturity. Induced breeding, through hormone injection, was done within the same stock and crossing between two different stocks. There were two breedings and success was achieved on the second time, when over 70% of larvae survived.

The fry obtained from six stocks and three crossings were reared in earthen ponds and cement tanks for evaluation and comparison of performance. After four months, the fish reached 6.5-7.8 g, and there was no significant difference in growth rate among the progenies.

Fin clipping and tagging with the use of fluorescence were tried. Survival and retention of color marks after tagging need to be tested again.

The broodstocks and their progenies are being reared in controlled condition for further evaluation.

Future Plans

The following research activities are ongoing or will be carried out at RIA-1 and other relevant institutions:

- Genetic control of sex ratio in *O. niloticus* (Thai, Vietnamese and GIFT strains), using YY-male technology (RIA-1);
- Mass seed production of all female *B. gonionotus*, based on the success of producing neo-male (x x) (RIA-1);
- Sex reversal of *M. rosenbergii* using hormone treatment technology (RIA-1);
- Induction of triploids in *C. macrocephalus* and consideration for production (Southern Branch of Vietnam-Russia Tropical Center);
- Gene transfer of some fish species (Institute of Biotechnology);
- Cryopreservation of some species (RIA-1); and
- Use of DNA technique for diagnosis of diseases in fish.

The following breeding programs are planned:

- Family selection of GIFT strain *O. niloticus* for second generation. (RIA-1);
- Mass selection of *B. gonionotus* (RIA-2); and
- Family selection of *C. carpio* for second generation (RIA-1).

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FISH GENETICS RESEARCH AT ICLARM - THE WORLD FISH CENTER[a]

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ABSTRACT

ICLARM - The World Fish Center has a mandate to improve the food security of people in developing countries by providing better management advice and methods to conserve current fish stocks and developing efficient aquaculture technologies. ICLARM pursues this through its various programs which include, among others, research on germplasm enhancement and breeding, biodiversity and genetic resources.

A major research project aimed at developing methods for genetic improvement of finfish used Nile tilapia (*Oreochromis niloticus*) as a test species and was followed through six generations of selection. The improved strain (popularly known as GIFT strain) outperformed in growth and survival traits the widely farmed tilapia strains in the Philippines and other parts of Asia. The overall potential impact of the GIFT strain has been assessed in Bangladesh, People's Republic of China, Philippines, Thailand and Vietnam.

The lessons learned and experiences gained from the GIFT project are now being applied for genetic improvement of six species of carp in Asia (Bangladesh, China, India, Indonesia, Thailand, and Vietnam) and the indigenous tilapia species in Africa, in collaboration with national partners.

ICLARM addresses research and policy issues that have impact on the management of aquatic genetic resources. The Center also contributes to capacity building of national institutions in the areas of germplasm enhancement and breeding and conservation of genetic resources through the International Network on Genetics in Aquaculture (INGA) and project-related training programs.

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Introduction

The mandate to improve the well-being and livelihood of present and future generations of poor people in the developing world through improved production, management and conservation of living aquatic resources has been the basis for ICLARM's work for the past two decades. As the only fisheries Center of the Consultative Group on International Agricultural Research Systems (CGIAR), ICLARM has a global role in providing better management advice and methods to conserve genetic resources and develop efficient technologies for aquaculture/fisheries management necessary to augment fish productivity and to meet the world's growing demand for fish.

Pullin et al (1991) summarized the results of studies on tilapias by ICLARM and research partners in the early/mid 1980s. The overall observation was that Asian farmed tilapia stocks had poor growth performance and genetic quality. This could be due to introgressive hybridization of farmed stocks of Nile tilapia (*Oreochromis niloticus*) with feral populations of the much less desirable species for farming – the Mozambique tilapia *(O. mossambicus*). The workshop on Tilapia Genetic Resources for Aquaculture organized by ICLARM in Thailand in 1987 confirmed this and concluded that the presently available farmed stocks of *O.niloticus* in Asia were inadequate for genetic improvement programs because of their small founder populations, inbreeding, and introgression of genes from other less desirable species (Pullin 1988; Pullin and Capili 1988). Further, it was found that the indigenous tilapia populations in Africa are under threat due to indiscriminate fish transfers and habitat disturbance. There was obviously an urgent need to document tilapia genetic resources for the twin goals of conservation and for future aquaculture development in the region.

The above findings formed the basis of ICLARM's major strategic research on fish genetics. The Center realized that interactive research is essential in developing countries for sustained fish productivity and that this is best done in collaboration with its partner institutions.

Germplasm enhancement and breeding and the conservation of biodiversity and genetic resources are the two major thrusts of ICLARM's genetics research. The genetic enhancement thrust aims at developing techniques/methods for improving fish breeds, disseminating the methods developed and training developing country scientists in their use. The Center's major effort on conservation of fish genetic resources is directed at enhancing the capacity of national programs for fisheries and biodiversity management and in demonstrating how to document aquatic genetic resources for their sustainable use and conservation.

Germplasm enhancement and breeding

Genetic improvement of farmed tilapias. Investigations of the potential for genetic enhancement of tropical finfish through selective breeding using *O.niloticus* as the test species, was the first major strategic quantitative genetics research undertaken by ICLARM. It was anticipated that successful results with this species would result in not only better breeds of tilapia for farmers, but also new breeding methods for use with other tropical finfish species.

ICLARM in collaboration with its partner institutions in the Philippines (Bureau of Fisheries and Aquatic Resources, Freshwater Aquaculture Center of the Central Luzon State University, and the Marine Science Institute of the University of the Philippines) and Norway (Institute of Aquaculture Research Ltd.), implemented the Genetic Improvement of Farmed Tilapia (GIFT) project to develop effective methods for producing improved breeds of *O. niloticus* for low cost sustainable aquaculture in developing countries. In view of the poor quality of cultured stocks in Asia, pure strains of *O. niloticus* were collected from diverse countries: Egypt, Ghana, Senegal and Kenya. These were used as founder stocks along with the four existing strains in the Philippines, known as Singapore, Thailand, Israel and Taiwan strains (Eknath et al 1993). Based on the growth performance of the different strain combinations, individuals from the best performing groups were selected for a selective breeding program. Over ten years (1988-1997), selection was undertaken through five successive generations of the base population. Superior performance in terms of growth and survival under on-station and on-farm conditions was observed even after one generation of selection (Eknath 1993). The first generation of the improved strain (which came to be known as GIFT strain) grew 26% faster in on-station trials than the previous generation and 75% faster than the most commonly farmed strain in the Philippines (Eknath 1995). On average, the genetic gain per generation across five generations of selection was about 12-17% (Fig. 1) (Eknath and Acosta 1998; Eknath et al 1998).

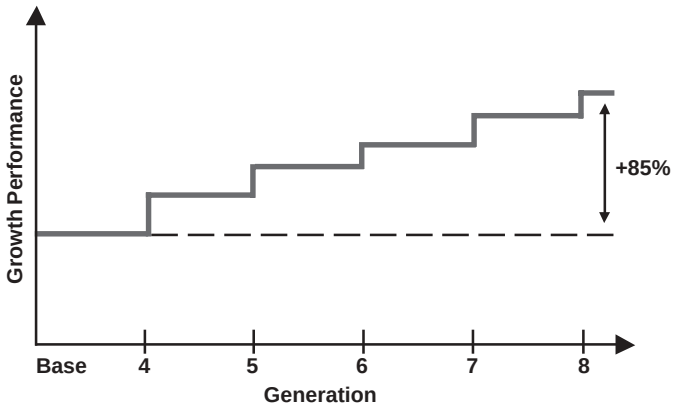

Fig. 1. Observed response to selection across five generations of selection for growth performance in *O. niloticus* (Source: Eknath and Acosta 1998)

Dissemination and evaluation of improved tilapias. Before disseminating the improved GIFT strain to farmers, ICLARM evaluated its performance during 1994-96 in terms of productivity and economics in different farming systems. The environmental impacts were also investigated in five countries: Bangladesh, China, Philippines, Thailand and Vietnam (Dey and Gupta 2000). The second generation of selected GIFT fish were found to have a higher growth rate than local strains: the difference ranging from 18% in China to 66% in Bangladesh. This, combined with better survival, indicated that the yield potential for a given farm could be increased by 54% to 97% depending on local conditions. Further, the use of the GIFT strain resulted in lowering the production costs by 20%-30%. In the Philippines, it was estimated that a 40% increase in productivity could be achieved from adoption of the GIFT strain. Such improvements would increase profitability to the producer by 84% (ICLARM 1998). Studies undertaken to analyze the overall potential impact of the GIFT strain in Bangladesh, China, Philippines, Thailand and Vietnam indicated that the consumers in these countries gain around 23% to 59% of the total economic gain due to the use of GIFT strain (Table 1). The study further concluded that the adoption of the improved strain will increase tilapia production and consequently the total fish production in a country, enhance profitability of fish farming, decrease the market price of tilapia, increase consumption of tilapia by producers and consumers and increase the welfare of the country's economy as a whole (Dey 2000).

Establishment of GIFT Foundation. The research and cooperation between the different project partners did not end with the GIFT project, but led to the establishment of GIFT Foundation International Inc.. ICLARM and its national research partners in the Philippines established the Foundation as an independent non-stock, non-profit organization primarily to continue the selective breeding initiated by the GIFT Project and to commercialize the GIFT strain through partnerships with the private sector.

Genetic improvement of tilapias in Africa. Within Africa, ICLARM's work has been focused on the application of technologies for genetic improvement and conservation of native tilapia stocks in the region. At ICLARM's Regional Research Center for Africa and West Asia in Abbassa, Egypt, native tilapias (*O. aureus*, *Sarotherodon galilaeus* and *Tilapia zilli*) were screened for traits (growth, yield, food conversion efficiency, cold tolerance) of local commercial importance (Brummett 2000). This was followed by a program including the evaluation of mass

Table 1. National level economic gain (equivalent variation) due to technical change in tilapia production

Country	Technical change scenario	Producer (USD)	Consumer (USD)	Total (USD)	% share	
					Producer	Consumer
Bangladesh	I	420 661	305 222	725 883	58	42
	II	514 986	379 525	894 511	58	42
China	I	60 789 451	21 636 295	82 425 746	74	26
	II	75 213 499	26 950 480	102 163 979	74	26
Philippines	I	26 474 674	7 886 059	34 360 734	77	23
	II	34 652 090	10 339 814	44 991 904	77	23
Thailand	I	6 838 918	9 864 826	16 703 744	41	59
	II	8 499 515	12 225 114	20 724 629	41	59
Vietnam	I	1 469 424	884 863	2 354 287	62	38
	II	1 751 932	1 059 597	2 811 530	62	38

Note: Scenario I – early adoption; low adoption but high productivity improvement for adopter
Scenario II – late adoption; high adoption but low productivity improvement for adopter
Source: Dey 2000

selection to improve growth and food conversion efficiency of *O. niloticus* and *O. aureus*. Currently, these species are being bred for another round of evaluation and alternative selection experiments (ICLARM 1999b, 1999c, Brummett 2000).

In collaboration with Auburn University, Alabama, USA, a project has been initiated to evaluate the potential usefulness of marker- assisted selection and quantitative trait loci (QTL) mapping in genetic enhancement of tilapias in Africa (Liu 2001; ICLARM 2000a).

The research at ICLARM in Abbassa is complemented by a collaborative project being coordinated by the International Network on Genetics in Aquaculture (INGA)/ICLARM on genetic enhancement and conservation of tilapia genetic resources in Côte d'Ivoire, Egypt, Ghana and Malawi. With start-up funds from the International Development Research Centre of Canada, the participating institutions (Centre National de Recherche Agronomique, Côte d'Ivoire; Central Laboratory of Aquaculture Research, Egypt; Water Research Institute, Ghana; and University of Malawi, Malawi,) are undertaking the following activities: evaluation of local tilapia populations suitable for genetic improvement/aquaculture; genetic characterization to determine the population structure and genetic diversity of native tilapia stocks; documenting traditional knowledge of tilapia species/ stocks and farming systems; identifying breeding goals and initiating studies for genetic improvement.

To further address the specific requirements of African aquaculture, ICLARM will initiate a project aimed at transferring the selective breeding technology developed in the GIFT project to Sub-Saharan Africa and Egypt. This will be accomplished through the establishment of a Regional Tilapia Genetic Enhancement Center in Egypt, training of Egyptian and sub-Saharan African scientists by Philippine scientists and scientific exchange between Egypt, Philippines and other regions of Africa (ICLARM 2000b).

ICLARM is seeking funds to organize a conference on biosafety issues pertaining to the dissemination of genetically enhanced fish, including genetically modified organisms (GMOs), with special reference to the tilapias within Africa.

Genetic improvement of carps. The selective breeding technique developed for improving the growth and productivity of *O. niloticus* in Asia is now being applied to the other major farmed tropical finfish in Asia, the carps, which make up 43.6% of global aquaculture production (FAO 1998). Bangladesh, China, India, Indonesia, Thailand and Vietnam are the major carp producers in Asia and contribute more than 90% to the global production of carps.

ICLARM in 1997 initiated a project for the genetic improvement of carps, in collaboration with its partner institutions in Bangladesh (Bangladesh Fisheries Research Institute and Bangladesh Agricultural University), China (Shanghai Fisheries University and Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences), India (Central Institute of Freshwater Aquaculture, National Bureau of Fish Genetic Resources and University of Agricultural Sciences), Indonesia (Research Institute for Freshwater Fisheries), Thailand (National Aquaculture Genetics Research Institute and the Department of Fisheries), and Vietnam (Research Institute for Aquaculture Nos. 1 and 2). The first phase of the project focuses on assessing the current status of carp genetic resources including their systematic documentation and evaluation, developing criteria for prioritizing carp genetic research, identifying research priorities and approaches including species, traits, farming systems and breeding strategies and initiating location - specific strategic research and training based on identified research priorities for development of high yielding carp strains (Gupta et al. 1997). Extensive surveys of carp producers, consumers and hatchery operators have been completed in all the six participating countries to prioritize species, traits and farming systems for genetic improvement. Carp genetic resources in the participating countries have been documented. Genetic enhancement of six species of carps: common carp (*Cyprinus carpio),* silver barb (*Barbodes gonionotus*), rohu (*Labeo rohita*), catla (*Catla catla*), Mrigal *(Cirrhinus Mrigala)* and Chinese bream (*Megalobrama amblycephala*) is in progress. Initially the selection is for growth. Some of the improved strains are presently being tested in farmers' fields (ICLARM 1999a).

Biodiversity and Genetic Resources

Conserving fish biodiversity and protecting the environment are two of the major research goals of ICLARM. Since knowledge of genetic diversity and population structure of commercially important species

are vital for the management of farmed and wild populations, ICLARM and its research partners are implementing studies in Africa and Asia to develop methods for assessing genetic diversity. Two projects for documenting genetic resources for their sustainable use and conservation were initiated in 1997. These studies focus on black-chinned tilapia (*Sarotherodon melanotheron*), a coastal species inhabiting brackish-water lagoons and water courses in West Africa and on an Asian carp, *B.goniontous*, a species that is rapidly gaining popularity for culture among resource-poor fish farmers in South and South East Asia .

In collaboration with Zoologisches Institut und Zoologisches Museum, University of Hamburg, Germany and Water Research Institute, Ghana, studies are in progress to assist in the conservation and sustainable use in aquaculture and fisheries of *S. melanotheron* in West Africa (Pullin 2000). Samples of this species were collected over the entire range in Senegal, Sierra Leone, Liberia, Côte d'Ivoire, Ghana, Togo, Benin and Congo. Extensive data were obtained on the genetic diversity of *S. melanotheron* populations and examined using molecular genetic methods and partly with morphometric analysis. Preliminary analysis revealed 17 control region mtDNA haplotypes that clustered samples into three regional groups: (i) Senegal – Liberia; (ii) Côte d'Ivoire – Ghana; and (iii) Bas Kouilou, Congo (ICLARM 1999b). Data were also gathered on the biology, ecology and use of *S. melanotheron* in West African coastal lagoons and watercourses. Culture trials of genetically determined Ghanaian *S.melanotheron* populations are in progress (ICLARM 1999b).

In Asia, research on the genetic diversity of *B. gonionotus*, a species that is gaining importance for low-input pond culture and integrated systems, is being undertaken in collaboration with the University of Wales, Swansea, U.K. Samples were collected from wild populations in central, northern and southern Thailand and in central, east and west Java, Indonesia and from wild and farmed populations in Lao PDR and Vietnam. Initial results from molecular genetic and meristic studies, indicate that *B. gonionotus* is a single species across its range. Samples collected for comparison included *B. altus*, *B. belleroides* and *B. schwanenfeldii* (ICLARM 1999b).

ICLARM addresses research and policy issues that have impacts on the management of aquatic genetic resources through its contributions to the work of: the Convention on Biological Diversity (CBD) and its Subsidiary Body on Scientific, Technical and Technological Advice and Clearing House Mechanism; Global Biodiversity Fora; Species 2000; Food and Agriculture Organization of the United Nations (FAO) Fisheries Division and Commission on Genetic Resources for Food and Agriculture; and the World Conservation Union (ICLARM 1999b). In 1998, ICLARM, in collaboration with FAO, convened a Bellagio Conference entitled 'Towards Policies for Conservation and Sustainable Use of Aquatic Genetic Resources'. The main output of this meeting was a consensus statement which was distributed at the Fourth Conference of the Parties to the CBD held in Slovakia in May 1998 (Pullin et al 1999).

ICLARM also participates in the CGIAR's System-wide Genetic Resources Program and System-wide Information Network on Genetic Resources.

Networking in Genetics Research

ICLARM coordinates the genetics research network - INGA. The network has been playing an important role in national, regional and international genetics research aimed at improving production from aquaculture operations and conservation of aquatic genetic resources and biodiversity (Gupta and Acosta 1999). With a present membership of 13 countries from Asia, the Pacific and Africa and 12 advanced scientific institutions as Associate Members, the network is undertaking the: (i) development of national breeding programs; (ii) initiation of regional research programs for genetic improvement of carps and tilapias; (iii) transfer of germplasm among member countries, following strict quarantine protocols and material transfer agreements; (iv) assistance in formation of national genetics networks; and (v) strengthening of national research capacity (Gupta and Acosta 2001). Details of the network activities are elsewhere in this proceedings.

Training of National Scientists

Through INGA and project - related training programs, ICLARM will endeavor to form a cadre of scientists in INGA member countries in the area of quantitative genetics and selective breeding. Since this area is relatively new for the majority of national institutions in developing countries in Asia, Africa and the Pacific, ICLARM/INGA, in collaboration with Norway's Institute of Aquaculture Research Ltd.

(AKVAFORSK), has organized training programs for scientists from national institutions in these regions. ICLARM/INGA will also address the specific training needs of national institutions in Africa through training programs that will be organized under the project 'Transfer of Selective breeding technology for aquaculture improvement from Asia to Sub-Saharan Africa and Egypt' (ICLARM 2000b).

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ISRAELI AQUACULTURE GENETIC IMPROVEMENT PROGRAMS

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ABSTRACT

Genetic improvement programs in Israel have focused mainly on common carp, tilapias, and recently on marine fishes. A breeding program to develop tilapia strains that are cold tolerant and fast growing in fresh and saltwater environments is in progress. Efforts are also being made to produce disease-resistant carp seed. Selective breeding program for sea bream and sea bass has been initiated. Interspecific hybridization experiments were also carried out to improve their culture performance.

Investigations on color pattern inheritance, chromosome-set and sex manipulations of various aquaculture species have been conducted or are in progress. Genetic engineering and transgenesis of a few candidate genes; development of DNA markers and the use of QTL mapping to support the fish breeding programs are underway. Other efforts include development of methods for gamete cryopreservation of common carp, marine fishes and shrimp and initiation of gene bank.

Introduction

The history and status of aquaculture genetics in Israel were reviewed a few years ago (Hulata 1995). The current report reviews trends and progress of the second half of the 1990s, and provides the vision and perspective for the next couple of years. Selective breeding work is currently focused mainly on tilapias (*Oreochromis* sp.) and gilthead seabream (*Sparus aurata*). Edible and ornamental common carp (*Cyprinus carpio*) are used, among other species, in the application of biotechnological methods.

Breeding programs

A program aiming at breeding new, synthetic populations of tilapia specifically adapted to temperate climates and saline environments was initiated in 1995 at the Department of Aquaculture (ARO, Volcani Center). It is based on plant breeders' approach, and takes benefit of the ease of producing interspecific hybrids among tilapias. The use of interspecific composite (or complex) crosses is an established practice in plant breeding for achieving wide genetic and phenotypic variability. This approach is based on the

creation of an artificial center of origin (ACO) via composite interspecific crosses. The ACO contains wide genetic diversity and presents opportunities for genes to recombine and interact with other genes originating from different species, combinations and interactions which are impossible in any of the pure species. Israeli cut flower breeders recently applied an adaptation of this approach, termed Multiple Re-Speciation, to develop new cultivars of carnations.

The ACO (Hulata et al. 1999; Agresti et al. 2000) was produced by inter-crossing four tilapiine species: *Oreochromis niloticus* [wild type (*On*) and red (R*On*) strains], *O. aureus* (*Oa*), *O. mossambicus* (*Om*), and *Sarotherodon galilaeus* (*Sg*). All hybrids were obtained by natural spawning, except for the *S. galilaeus* x *Oreochromis* sp. F_1 hybrids that were produced by artificial fertilization. A series of cold-water challenge tests were performed, involving *O. mossambicus*, *O. aureus* and their F_1 and F_2 hybrids, to study the genetic basis of cold tolerance in tilapias (Cnaani et al. 1999, 2000). All of the different two-way (F_1 hybrids) crosses required to establish the synthetic stock of tilapia (ACO) have been obtained, as well as a set of three four-way-crosses (4WC) derived from five strains in four species and two genera. Full-sibs of the [(*Om* x *Oa*) x (*Sg* x *On*)] 4WC have been successfully bred to enable mixing of the inherited gene blocks; thus, we are ready to start selective breeding for cold tolerance and growth rate in freshwater and for growth rate in saltwater, from this base population. Selection will be based on a combination of individual and family performance; an approach that was used successfully for the GIFT breeding program.

A tilapia breeding program is also conducted at the Nir David Fish Breeding Farm, aiming at creating a fast growing, salinity tolerant, uniformly red tilapia hybrid (Lahav and Ra'anan 1997), and other commercial strains.

A first step in a breeding program to improve growth rate in gilthead seabream (*Sparus auratus*) carried out at the National Center for Mariculture (IOLR, Eilat) was strain evaluation (Knibb et al. 1997a). The research team then moved on to prepare for family selection, encountering difficulties in efficiently producing single-pair offspring groups (full- and half-sibs) and leading to the conclusion that family mating designs are inappropriate for the group spawning *S. aurata* (Gorshkov et al. 1997). Progeny testing, where single males were stocked with groups of females, which more closely simulate the natural group-spawning behavior of the species, yielded genetically related groups more successfully. The small number of families obtained (4 each for full- and half-sibs) did not allow obtaining reliable estimates of heritability for growth, yet large (14-29%) sire components of the offspring weight variance were evident (Knibb et al. 1998a). Mass selection proved more effective and resulted in significant heritability estimates for growth (Knibb et al. 1997b; 1998a,b). A new program has recently been initiated to assess strains and crossbred among strains of cultured sea bass (*Dicentrarchus labrax*) in Israel. This involves comparative assessment of the performance and culture potential of a new Egyptian and existing domesticated strains, in order to identify the most suitable to commence a long-term selective breeding program for sea bass.

Interspecific hybrids between *S. aurata* and red seabream, *Pagrus major* (both belonging to the Sparidae family) have been produced (Knibb et al. 1998a). The hybrids developed only vestigial gonads at the age 2 and 3 years and were sterile. Subsequently, similar vestigial gonads were observed in offspring of the reciprocal crosses. No consistent growth (and survival) superiority until sexual maturity was detected in the reciprocal crosses, compared with parental species. Hybridization between European sea bass (*D. labrax*) females and striped bass (*Morone saxatilis*) males was carried out. Viable hybrid larvae were produced, but surprisingly 28% were triploids, and apparently only triploids survived to age of 6 months. At the age 8 months, surviving (triploid) hybrids showed poor growth compared to diploid *D. labrax*. Interspecific sterile hybrids of marine cultured fish might be of commercial interest when production of fertile fish is restricted for ecological reasons (Gorshkov, pers. comm.).

A severe viral disease has affected Israeli (edible and ornamental) common carp (*Cyprinus carpio*) stocks a couple of years ago. Commercial farms as well as the Dor station, which is holding the breeding nuclei of the parental stocks used for producing the commercially cultured crossbred, have reported massive mortalities. No final census is available yet of surviving broodstocks, and it may well be that years of genetic improvement work had been lost. So far it is not clear whether survivors from affected ponds can inherit tolerance to this disease to their offspring. Farmers are currently trying to produce resistant seed by hybridizing surviving carp broodstocks with Crucian carp (*Carassius carassius*), even at the cost of obtaining a slower growing fish. It is possible that a breeding

program for common carp will have to be restarted to cope with the catastrophe.

Color inheritance

Investigations on color pattern inheritance are being conducted in various aquaculture species, including ornamental (koi) carp (Gomelsky et al. 1995; 1996; 1998a; David et al., work in progress); goldfish (Rothbard et al. 1999a); grass carp, *Ctenopharyngodon idella* (Rothbard and Shelton 1999); tilapia (Shirak et al. 2000) and guppies, *Poecilia reticulata* (Froyman and Hulata, work in progress).

Various mutations which affect body coloration (e.g., 'golden', 'albino' and 'ebony') were isolated in *S. aurata*, some having pleiotropic effects (Knibb et al. 1996; 1998a). While 'ebony' homozygotes are semi-lethal, heterozygotes show strong heterosis for growth.

Chromosome-set and sex manipulations

Methods of chromosome-set manipulations are employed for genetic improvement of various aquaculture species: ornamental (koi) and edible common carp, black carp (*Mylopharyngodon piceus*), tilapias (*O. aureus* and *O. niloticus*), white bass (*M. chrysops*), gilthead seabream (*S. aurata*), European sea bass (*D. labrax*) and white grouper (*Epinephelus aeneus*). Work on edible common carp, carried out at the Dor station (ARO), was directed towards establishing broodstock producing all-female populations (Cherfas et al. 1996). This involved sex reversing XX gynogenetic females to males (Gomelsky et al. 1994), and using these XX males for breeding. Such all-female seed was released to commercial farms and resulted in 10-15% yield improvement over existing commercial stocks. Triploid common carp were produced and their culture potential evaluated (Cherfas et al. 1994). The survival in shock treated progenies (predominantly triploids) was about 70% of that in diploid control. Most of the one-year-old triploid males and females had undeveloped gonads and were sterile. Triploids grew slower than their diploid sibs under all investigated conditions. The results did not reveal the expected positive effect of sterility on somatic growth in triploid common carp, thence the potential of sterile triploid common carp for aquaculture is questionable.

Work on ornamental (koi) carp was aimed at investigating the inheritance of color patterns (Gomelsky et al. 1995; 1996; 1998a). Manipulations are aimed at producing inbred lines, and as a tool in application of DNA-markers for establishment of improved koi broodstock (research in progress). Successful meiotic and mitotic gynogenesis was achieved when the eggs were exposed to, either, heat/cold, or to pressure shocks. An interesting phenomenon detected during the investigation was the presence of males among gynogenetically produced fish. These, when sib-mated with their sisters, produced all-female progeny [Rothbard (Gan Shmuel Fish Breeding Center), pers. comm.]. The objectives of androgenesis were to produce homozygous koi individuals (XX and YY) of both sexes, and to preserve koi genetic traits in a sperm bank. Androgenesis of koi was induced in edible carp eggs, immersed in carp ovarian fluid to avoid stickiness, by UV-irradiation followed by insemination with koi sperm. Low numbers (2-12) of diploid androgenotes, recognizable at the fry stage by light pigmentation, were obtained (Rothbard et al. 1999b).

Studies involving black carp (*M. piceus*) are aimed at producing triploid, presumably sexually sterile fish, as well as mono-sex female or male populations (through application of chromosome-set manipulations, e.g. gynogenesis, androgenesis, triploidy and tetraploidy). Combined technology of gynogenesis and hormonal sex-reversal, enables production of XXX-triploid fish that are completely sterile, unlike the XXY- or YYX-triploids that possess fragments of testes, and are thus able to produce some active sperm (Rothbard et al., 1997). The black carp is a high quality edible fish, which is also highly valued for its ability to control snail populations [e.g., the golden snail (*Pomacea* sp.), zebra mussel (*Dreissena polymorpha*), or snail infestation in the Israeli National Water Carrier system, causing filter and pump clogging](Rothbard and Rubinstein 1999). The methodology enables producing ecologically and economically important exotic species that can potentially serve for biocontrol, without endangering local fish fauna.

Gynogenesis and sex-reversal were successfully induced in *Morone* sp. in an attempt to obtain broodstocks producing monosex populations to avoid limitations on transfers of this exotic species (Gomelsky et al. 1998b; 1999). This project was unfortunately terminated before reaching commercial application.

A meiogynogenetic line of *O. aureus* was established and gynogenetically propagated for 5 generations at the Faculty of Life Sciences, Bar-Ilan University. A successive increase in viability recorded over these 5

generations was interpreted as a gradual elimination of lethal genes. Mitogynogenetic *O. aureus* were produced (Shirak et al. 1998) using third generation meiogynogenetic females from this stock. Three generations of gynogenetic *O. niloticus* were also produced. Males from the gynogenetic *O. aureus* line were used for hybridization with gynogenetic *O. niloticus* females, resulting in consistent production of 100% male hybrids (Avtalion, pers. comm.).

Triploids and meiotic gynogenetic *D. labrax* and *S. aurata* were efficiently produced using temperature shocks (Gorshkova et al. 1995; 1996; 1998). Gorshkova et al. (1996) found nearly 20% males in meiotic gynogenetic *D. labrax* cohorts. Recently, a high incidence (up to 60%) of severe cranial bone deformities were recorded in gynogenetic cohorts at age 10 months (Knibb et al. 1998a). Fertilizing heat-shocked *S. aurata* eggs with untreated heterologous sperm from *Pagrus major* produced triploid hybrids (Gorshkova et al., 1995). However, no growth superiority of triploid hybrids, compared to either parent, was evident for fish up to age 2 and 3 years. *D. labrax* is characterized with sexual dimorphism, with females tending to grow faster than males, so there is considerable interest in culturing only females. Monosex populations were produced by administration of estradiol (yielding all-females) and 17a-methyltestosterone (yielding all-males) to mixed-sex juveniles (Gorshkova et al. 1996).

Karyological analyses of the major maricultured species under cultivation in Israel are being conducted. Recently, series of experiments were undertaken to attempt the cytological technique for examination of early embryogenesis for the purpose of understanding the genetic reasons of high egg and larval mortality in the white grouper (*E. aeneus*). The proportion of cytogenetically abnormal embryos carrying different types of chromosomal aberrations vary significantly in different spawnings of the parental fish. Cytological monitoring of early embryogenesis is carried to support the selective breeding program and the genetic management (stock identification and production of monosex populations) in the white grouper and other cultured marine species (Gorshkova et al. in preparation).

No karyological evidence for a chromosomal mechanism responsible for sexual differentiation was found in European sea bass. However, males usually have a heteromorphic pair of subtelocentric chromosomes, while females usually have a homologous pair of those chromosomes. Temperature manipulation experiments are considerably effective in changing sex ratio in offspring (at the age of 11 months). While data could not identify any particular genetic and/or environmental reason for the observed deviations of sea bass sex ratios, they do illustrate possible plasticity of sex determination in this species (work in progress; Gorshkov and Gorshkova, pers. comm.).

Genetic engineering and transgenesis

Investigations on a few candidate genes have been initiated and/or are in progress. Most noteworthy is the work on the effect of growth hormone (GH), engineered for expression using various promoters, on common carp and gilthead seabream growth (Cavari et al. 1993a,b; Moav et al. 1995; Fine et al. 1996; Hinits and Moav, 1999). Moav (Dept. of Zoology, Tel Aviv university) and coworkers have demonstrated 20% growth improvement in transgenic carp reared in experimental earthen ponds. Current efforts are aimed at constructing new expression vectors to improve efficiency of producing transgenic fish. Further potential economically important genes considered for genetic engineering in marine fish include genes for D^5 and D^6 fatty acid desaturase enzymes (Knibb et al. 1996).

DNA markers and QTL mapping

Collaborative work with US scientists (Hulata et al. 1999; Agresti et al. 2000) using the [*Om* x (*Oa* x R*On*)] cross resulted in mapping 191 AFLP and 26 UNH microsatellite markers to 24 linkage groups. Twenty UNH microsatellite markers were used to search for quantitative trait loci (QTL) associated with cold tolerance and body weight in an F_2 family of *O. aureus* x *O. mossambicus* hybrid. Two markers putatively associated with cold tolerance and three markers putatively associated with body weight were found. Two of these markers are located on the same linkage group, which is apparently a chromosomal region, which affects growth and survival in tilapia (Cnaani et al. 1999, 2000). In a similar study (Agresti et al., in preparation) using cold tolerance-tested [(*Om* x *Oa*) x (*Sg* x *On*)] 4WC, one UNH microsatellite and two AFLP markers from the female gave evidence of association with body weight; these were all from different linkage groups. Seven markers (3 UNH microsatellite and 4 AFLP) from the male also were found to be associated with body weight and six of them were restricted to

two linkage groups. Based on shared microsatellite markers, 12 composite linkage groups have been identified from the combined mapping data of Kocher et al. (1998), Agresti et al. (2000) and the 4WC family. These likely represent 12 of the 22 tilapia chromosomes.

Attempts are also being made at detecting sex linkage and non-Mendelian segregation of microsatellite DNA markers in a meiogynogenetic family of *O. aureus* (Palti et al. 1999, 2000), and at analyzing genetic variation in immunological parameters associated with stress resistance (Palti et al. 2000, and work in progress).

Work is currently under way for development of DNA markers in edible and ornamental (koi) carp. This work is aimed at detecting linkage between DNA markers and color patterns in the ornamental carp (David and Rothbard, pers. comm.).

Studies are under way to apply molecular biology to support the classical breeding program (specifically for determining parenthood in mass-spawning of *S. aurata* and *D. labrax,* and for genetic testing programs) by developing AFLP and microsatellite DNA profiling techniques (Gorshkova, pers. comm.).

Gamete cryopreservation and gene bank

Methods have been developed at the National institute of Oceanography (IOLR, Haifa), for cryopreservation of carp sperm (Lubzens et al. 1993; 1997), leading to the formation of an operating 'sperm bank' for *C. carpio* (mainly ornamental carp) in Israel. Subsequently, methods for cryopreservation of gilthead seabream, mullet (*Mugil cephalus*) and grouper (*E. aeneus*) sperm have also been developed (Lubzens, pers. comm.).

Attempts are currently directed towards developing cryopreservation methods for gilthead seabream (Lubzens and Pekarsky 1998) and marine shrimp (*Penaneus semisulcatus*) embryos. Successful cryopreservation of yolk-laden eggs containing developing fish embryos has yet to be achieved. Obstacles encountered so far are the low permeability of cryoprotectants into the embryos and sensitivity of fertilized eggs to concentrations above 0.15M of DMSO or methanol. Attempts are currently being made at evaluating possible cryopreservation of ovarian oocytes, as an alternative (Lubzens and Pekarsky 1998).

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GENE MAPPING, MARKER-ASSISTED SELECTION, GENE CLONING, GENETIC ENGINEERING AND INTEGRATED GENETIC IMPROVEMENT PROGRAMS AT AUBURN UNIVERSITY

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ABSTRACT

Auburn University's fish genetics program has four major research projects in aquaculture genetics, namely: (1) selective breeding, (2) genomic mapping and marker-assisted selection (MAS), (3) gene cloning and (4) transgenic fish production, all with the long-term goal to produce fish broodstocks with improved economic traits. In addition to aquaculture interest, the university has a population genetics program with the goal of proper management and preservation of natural fisheries resources. However, this program will not be discussed in this report.

The aquaculture genetics projects were developed based on long-term efforts of selective breeding programs. These projects are dependent upon one another so that the maximum benefits will be obtained by using the integrated genetic improvement approaches. For instance, gene mapping will produce more economically important genes for cloning and application in biotechnology or gene-assisted selection (GAS), and produce markers that are linked to important performance traits for MAS. Biotechnology should use strains that are already developed through selective breeding to obtain the best performance traits. Further genetic improvements by gene transfer into an already superior strain provide the best "starting point" for genetic engineering. Regardless of the selection approaches to be used, traditional or marker-assisted, selection of one trait should not take the expense of another. Thus, a comprehensive selection index should be developed based on relative economic importance of each trait. This report focuses on genomic mapping, marker-assisted selection and genetic engineering. Auburn University has a strong selective breeding program and the genetics team works collaboratively in all projects, directed by a philosophy of integrated genetic improvement. For the most part, this paper will only discuss some of the progress on the work with catfish and, to a less extent, tilapia.

Current Status of Catfish and Tilapia Aquaculture

Catfish is the most important cultured fish in the US and accounts for over 50% of all US aquaculture production. The catfish industry is valued at over US$2 billion and production in 1999 should exceed 750 million pounds. It is the only agricultural sector with a steady annual growth rate of over 8%. In Mississippi, Alabama, Arkansas, Louisiana, Georgia and several other southern states, catfish is one of the top agricultural commodities. Tilapia culture is relatively small in the country, although ranked as the second or third largest cultured finfish.

Despite the development of the aquaculture and catfish industry in the US, a large trade deficit, US$3-6 billion, exists annually for seafood products. This deficit is still increasing. Aquaculture appears to be more and more important, especially considering the collapsing natural fisheries (Anon. 1996, 1997). According to the US Department of Agriculture (DA) estimates, the demand for seafood is increasing steadily and wild fisheries will be able to supply only 25-30% of the additional demand (USDA ERS 1995). Future projections predict a steadily widening gap between the world's demand for fish and the ability of the oceans to meet it (Anon. 1996). Development of a profitable, productive, environmentally sound and sustainable aquaculture industry, therefore, provides an alternative to the already overexploited, collapsing natural fisheries.

Despite its importance, the catfish industry is still a young industry and suffers from various production problems. A recent survey by Auburn University Extension Systems indicated that disease problems are ranked as the top concern of the catfish industry. "To keep my fish alive" is the greatest wish of all farmers (Jensen, unpubl.). Disease problems cause over 30% loss of the industry each year, equivalent to a loss of several hundred million dollars annually. Other concerns include traits in growth, feed conversion efficiency, carcass and fillet yields, tolerance to low dissolved oxygen, tolerance to poor water quality, reproductive success and harvestability. Broodstocks with enhanced culture traits are urgently needed for a sustainable catfish aquaculture. However, most, if not all, economic targets are polygenic and quantitative and controlled by quantitative trait loci (QTL). Understanding genomic organization and genetic linkage and QTL mapping is required for marker-assisted selection, improvements of economic traits through biotechnology and retrogression of beneficial genes from channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*). Auburn University's research addresses all three areas.

The research goal is to provide necessary scientific and technological information for improving the catfish broodstocks by MAS, introgression of important QTLs from channel catfish and blue catfish and genetic engineering using beneficial genes. To reach this goal, initial steps are to construct genetic linkage maps using various polymorphic markers and establish linkages of QTLs with markers. This linkage information should be useful for MAS programs. Development of the catfish genetic linkage map has been identified as one of the most important research for aquaculture by the USDA Aquaculture Steering Committee and it, among mapping of four other aquaculture species, was just approved by USDA as a regional project (NE-186) (Liu and Dunham 1998; Waldbieser et al. 1998).

Second, physical maps of catfish will be constructed so that major genes for important QTLs can be eventually isolated and used for improving broodstocks through the more effective GAS or biotechnology. Beneficial genes from various sources have been used to improve genetic stocks of catfish and develop technology needed for production of improved stocks through genetic engineering (Hallerman et al. 1990; Yoon et al. 1990a, 1990b; Hayat et al. 1991; Chen et al. 1992, 1993; Dunham et al. 1992; Gross et al. 1992; Powers et al. 1992; Chatakondi et al. 1994, 1998a, 1998b; and Dunham 1995).

Third, linkage analysis using polymorphic DNA markers and channel catfish x blue catfish interspecific hybrids should produce information on QTLs that can be introgressed from both species to generate synthetic breeds that can grow faster, have higher feed conversion efficiencies and higher carcass yields, and are more resistant to diseases and other environmental stresses.

In spite of dramatic development in many livestock animals, genomic mapping of aquaculture species is still at its infancy (Liu and Dunham 1998; Waldbieser et al. 1998). However, gene mapping in catfish is timely since well-developed, efficient marker systems are now available. The plan is to generate a catfish gene map with a resolution of 2-5 cM from the USDA-supported project, which would have been virtually

impossible, or required much more resources several years ago. In addition, background research concerning genetics of economic trait loci (ETL) has been completed in the university's laboratory and elsewhere (Dunham and Smitherman 1983a, 1983b, 1987; Tiersch et al. 1990; Dunham et al. 1993b; Tiersch and Goudi 1993; Wolters and Johnson 1994; Dunham 1996; Wolters et al. 1996; Liu et al. 1998a, 1998b, 1998c). The catfish genetics research and breeding programs, heritability studies and availability of large numbers of markers, make mapping of important ETLs in catfish immediately feasible. Development of linkage maps is certainly important, but QTLs will also be mapped for four important economic traits that will be immediately useful for MAS programs in catfish, in addition to generating a genetic linkage map of catfish.

Genetic improvement of catfish is a proven method of addressing production problems such as diseases. Previous research in the areas of traditional selective breeding and molecular genetics has resulted in genetically improved catfish (Dunham et al. 1983a, 1983b, 1987, 1990, 1992; Dunham 1996) and four releases of it to the industry. In addition to disease resistance, growth, feed conversion efficiency and processing yields are also addressed. Faster growth rate would allow for more crops per unit of time, thus saving management cost while increasing productivity. Feed accounts for 60% of the variable costs of catfish farming and, therefore, any improvement would significantly improve profit margins; processing yields are important since a 1% increase translates into US$20-30 million net income annually.

Research Progress in Genomic Mapping

Economic traits, genetic variation and heritability

Domestic channel and blue catfish exhibit significant phenotypic and genetic variation for economic traits such as disease resistance, growth rate, feed conversion efficiency (found highly correlated with growth), environmental stress tolerance, carcass yield, seinability and reproduction (Dunham et al. 1982, 1983, 1984, 1985, 1987a, 1990, 1993; Dunham and Smitherman 1983a, 1983b, 1987; Bondari 1984; Hallerman et al. 1986; Wolters and Johnson 1994; Dunham 1996; Wolters et al. 1996). Auburn University established a catfish genetics research program in 1969 to evaluate traditional selective breeding and molecular genetics for improving these quantitative traits. Growth rate and feed conversion efficiency have been improved by as much as 50% through selection (Bondari 1983; Dunham and Smitherman 1983a, 1987; Rezk 1993; Padi 1995); intraspecific crossbreeding (Bondari 1983, 1984; Dunham and Smitherman 1983b); interspecific hybridization (Dunham et al. 1990; Dunham 1996); and genetic engineering (Dunham et al. 1992, 1995; Dunham 1996). Disease resistance has also been improved primarily through interspecific hybridization (Dunham et al. 1990; Argue 1996; Dunham 1996; Wolters et al. 1996), intraspecific crossbreeding and strain selection (Wolters and Johnson 1994). Tolerance to low oxygen was enhanced primarily by interspecific hybridization (Dunham et al. 1983; Dunham 1996); seinability, by interspecific hybridization (Dunham 1996) and strain selection; and carcass yield by strain selection (Dunham et al. 1984), hybridization (Dunham 1996; Argue and Dunham 1998) and indirect selection (Rezk 1993; Dunham et al. 1985). Heritabilities and genetic correlations have been calculated (Argue 1996; Argue and Dunham 1998). The rationale for creating genetic maps of catfish is to increase the efficiency of selection (Waldbieser et al. 1998). Breeders wish to find molecular markers correlated with genetic loci controlling economic traits and use these markers to select superior broodstocks. Because traits, such as growth rate, are relatively easy to measure with traditional selection, a genetic map will be more useful to select fish for traits for which measurement is difficult or expensive (e.g., disease resistance) and is lethal to broodstocks (e.g., carcass composition). Such map will also be useful for introgression of alleles into channel catfish from other species with which hybrid production is feasible such as blue catfish.

Polymorphic marker development and evaluation

In the last three years, various polymorphic markers were evaluated for their usefulness in catfish gene mapping. These markers include allozymes, restriction fragment length polymorphism (RFLP), expressed sequence tags (EST), random amplified polymorphic DNA (RAPD), microsatellites, amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNuPs). Allozyme markers are type I markers and should be highly useful as anchorage points for comparative mapping. Total numbers of polymorphic loci are small and polymorphic rates are

low at each locus. RFLP markers are codominant markers. However, previous knowledge is required, such as probes for Southern analysis or sequences for polymerase chain reaction (PCR) facilitated RFLP analysis. Only a few RFLP markers are available for use in catfish mapping. We do not anticipate major increases in numbers of RFLPs because developing RFLP markers is slow and costly. Our results using EST from a pituitary cDNA library indicated low polymorphism between channel and blue catfish (about 10%). After sequencing and PCR analysis of 100 cDNA clones, 11 EST markers were obtained (Karsi et al. 1998; Liu et al. 1999e). RAPD markers were evaluated (Welsh and McClelland 1990; Williams et al. 1990) for their application in catfish gene mapping. Polymorphic rates are low among strains of channel catfish, but high between channel and blue catfish (Liu et al. 1999a). Therefore, RAPD should be only useful for mapping using the interspecific hybrid system. Inheritance of RAPD markers was normal following Mendelian expectations (Liu et al. 1998b). To date, 142 primers have been tested and 682 polymorphic markers have been identified using the channel catfish x blue catfish hybrids (Liu et al. 1998b, 1999a). Reproducible results with RAPD markers of sizes 200-1 500 bp were obtained. These markers should be useful for gene mapping analysis in the future.

Microsatellites are abundant, highly polymorphic, easy for genotyping with PCR and are reliable because of their codominance. The progress on developing microsatellite markers from channel catfish is summarized in Table 1, and efforts on this are being continued. Six microsatellite-enriched, small-insert libraries were constructed with channel catfish genomic DNA using the procedure of Orstrander et al. (1992). Two of the six libraries were screened using radio-actively labeled (CA)15 and (GA)15 oligonucleotide primers. A total of 1 530 microsatellite-containing clones (900 CA and 630 GA) were purified. Plasmid DNA containing microsatellites was prepared from 890 clones (500 [CA], 390 [GA]). To date, 590 clones have been sequenced, of which 403 generated enough flanking sequences to design PCR primers (Liu et al. 1999d; Tan et al. 1999, in review). In more than 100 clones, microsatellite sequences were immediately at the cloning site making them useless. There were 403 pairs of primers for their performance in amplifying channel catfish genomic DNA. Of this total, 257 pairs successfully amplified genomic DNA and generated PCR products of expected sizes. High levels of heterozygosity were observed.

As will be discussed below, the channel catfish x blue catfish hybrid system offers great advantages for gene mapping. However, one requirement for using microsatellite markers with the interspecific system is that the microsatellite flanking sequences must be conserved between the two species. This issue was addressed by determination of amplifiability of blue catfish DNA using PCR primers designed from channel catfish microsatellite sequences. Over 90% loci were amplified from both species using channel catfish primers. In fact, most of the microsatellite flanking sequences have been conserved across the genus borders of Ictalurid catfish (Liu et al. 1999d). This indicates that more than 230 microsatellites will be useful for mapping with the catfish interspecific system. The conservation of microsatellite loci between channel and blue catfish is important for practical applications. This will allow a comprehensive linkage map to be constructed using the interspecific hybrid system and various types of markers: allozymes, RFLP, EST, RAPD, AFLP, microsatellites and SNuPs.

In addition to type II microsatellite markers, type I microsatellite markers were also developed. Type I markers represent genes of known functions, thus, these are more useful for comparative gene mapping. A pituitary cDNA library was enriched for microsatellite-containing clones. Fifty clones of cDNAs containing microsatellite CA or GA repeats were obtained, which belong to 15 unique cDNAs after sequencing. Most clones harbor microsatellite sequences at their 3' nontranslated regions (NTR) or 5'-NTR, but two clones harbor CCA and CA repeats in their coding regions (Liu et al., in review).

AFLP markers (Vos et al. 1995) combine the strengths of RFLP and RAPD markers and overcome their problems. The approach is PCR-based and requires no probe or previous sequence information as needed by RFLP. It is reliable because of high stringent PCR in contrast to RAPD's problem of low reproducibility. The weakness is that they are dominant markers thus on average half of which are useful for a given backcross reference family. For gene mapping in

Table 1. Summary of progress on microsatellite marker development from *I. punctatus*.

Microsatellite-enriched library	6
Microsatellite clones purified	1530
Clones plasmid DNA prepared	890
Clones sequenced	590
Loci primers ordered	403
Loci PCR worked	257

catfish, AFLP polymorphic markers are highly abundant between channel catfish and blue catfish. They are inherited in the interspecific hybrids as dominant markers and segregated normally according to Mendelian ratios (Liu et al. 1998c). Some 64 primer combinations were tested and over 3 000 AFLP markers suitable for genetic mapping of catfish were produced (Liu et al. 1999c).

SNuP markers were evaluated several months ago. To date, the university has sequenced 100 each of cDNAs from the channel and blue catfish muscle cDNA libraries. Preliminary results indicate that SNuPs (type 1 in this case since they are cDNAs) are abundant using the catfish interspecific hybrid system.

In summary, feasibility of using seven types of polymorphic markers in gene mapping of catfish was evaluated. Microsatellite and AFLP are the two most useful types of markers for catfish mapping. Mapping the available microsatellite and AFLP markers should generate a map with less than 2 cM resolution.

Resource/reference families

Although channel catfish is the major cultured catfish, channel catfish x blue catfish hybrid system offers great advantages. The F_1 hybrid is fertile and in fact has produced F_2, F_3 and various backcrosses (Argue 1996; Liu et al. 1997; Dunham and Argue 1998a, 1998b, 1998c, in press). They are a major resource for QTL mapping and for MAS. Backcross progeny designed for this QTL mapping project was successfully produced. Sixteen backcross families (eight from channel and eight from blue backcrossed with heterozygous F_1) were produced. These families are reared in 0.1acre ponds ready for QTL evaluation. The interspecific hybrid system for gene mapping of catfish is advantageous because: (1) high rates of polymorphic markers are assured to exist between blue and channel catfish; (2) several important ETLs are possessed by blue catfish, mainly, the disease resistance gene(s) to enteric septicemia of catfish (ESC), carcass yield genes and genes controlling better seinability (Dunham et al. 1993); mapping these important genes is of great importance by itself; (3) mapping ETLs in blue catfish is important to selective breeding programs using backcrossing to introgress beneficial genes from blue catfish into channel catfish; and (4) drastic phenotypic variation of the hybrid system offers tools to be exploited for easy QTL evaluation and segregation.

Genotyping

Genotyping, using AFLP and microsatellite markers, is in progress now. It is reasonable to assume that in about a year, a linkage map for catfish should be produced.

Research Progress in QTL Mapping and Marker-assisted Selection

Two types of experiments toward marker-assisted selection have been initiated. The first is within the efforts for genetic linkage mapping. Phenotypes were evaluated for all the reference families to be used for mapping with the following traits: growth, body conformation (including body length, width and depth; head length, width and depth; caudal width and depth), disease resistance to ESC and to columnaris. The selective genotyping approach is used to have a strong phenotype selection pressure on the potential genotype differences. The top and bottom 12.5% of the individuals in each trait will be genotyped. It is reasonable to expect that markers linked to QTLs controlling these traits will be differential between the best and the worst performers.

The second approach is a whole-genome QTL scan from outcrossing populations. Again, if markers are linked to a specific QTL, they should harbor variable alleles between the best and the worst performers. To date, markers that are linked to QTLs controlling growth and QTLs controlling feed conversion efficiency have been identified. These identified markers, upon confirmation, will be highly useful for marker-assisted selection programs. Equally important, they will be useful for checking the success of traditional selective breeding programs. Selection for growth, for instance, should produce populations that are highly enriched with alleles linked with fast-growing QTLs.

Research Progress in Gene Cloning Efforts and Aquaculture Genomics Other than Mapping

Genomic research has two ultimate applied goals: marker-assisted selection and cloning genes of economic importance. The Auburn University laboratory has been active in isolating channel catfish genes involved in growth, development and reproduction (Tang et al. 1993; Liu et al. 1997; Karsi et al. 1998).

In the last four years, genes for gonadotropin alpha-subunit (Liu et al. 1997), gonadotropin beta-subunit I and beta-subunit II (Liu et al. 2001) were cloned. Two classes of Tc1-like transposable elements named IpTc1 and IpTc2 (Liu et al., in press) and a class of nonautonomous transposons from channel catfish were isolated and characterized (Liu et al. 1999b). Another class of highly repetitive elements, named as *Xba* elements, was characterized, accounting for about 5% of the catfish genome (Liu et al. 1998a). Over 300 cDNA clones were sequenced for EST marker development. These efforts are being continued, and in the near future, it can be expected that over 1 000 catfish genes will be cloned and partially sequenced.

Research Progress in Transgenic Fish

The Auburn University laboratory was one of the pioneers in the world to transfer foreign genes in fish (Dunham et al. 1987b; Dunham 1990; Liu et al. 1990a, 1990b, 1991). It has demonstrated the effects of the transfer of growth hormone genes into warmwater fish in terms of growth, protein content, carcass yield, oxygen tolerance, disease resistance, fat reduction, and enhancement of flavor and texture (Zhang et al. 1990; Hayat et al. 1991; Dunham 1992; Dunham et al. 1992; Powers et al. 1992; Chen et al. 1993; Chatakondi et al. 1994). The laboratory has also demonstrated that transgenic catfish does not pose an environmental risk in regards to growth without supplemental feed, predator avoidance and reproduction (Dunham 1995, 1996). There is greater potential for genetic improvement through the use of genes identified from Auburn's mapping project, which applied traditional selective breeding and molecular genetics. Maximum progress will likely be made by combining the tools of selective breeding with genetic engineering (Dunham 1992), using information obtained from genomic research.

Auburn University is currently working on homologous transfer of growth hormone gene from channel catfish into channel catfish, with promoters of various kinds, including metallothineine, beta-actin, elongation factor 1 and histone H3 (Moav et al. 1991, 1992a, 1992b). Also ongoing is work on gene transfer of antibacterial peptide genes to fish to enhance resistance against bacterial pathogens.

The two most significant problems in transgenic studies are high levels of mosaicism and inactivation of transgene expression after gene transfer. The university is developing expression vectors using the border elements to prevent gene inactivation, and using the Sleeping Beauty transposase system to enhance temporal integration. Upon development, these technologies should circumvent much of the current problems in transgenic fish production. As discussed above, the gene mapping projects will identify and essentially clone more economically important genes, including disease-resistant ones.

Genetic Enhancement of Tilapia in Africa

In collaboration with the International Center for Living Aquatic Resources Management, a tilapia project entitled "Genetic Enhancement of Tilapia in Africa by Combined Selection, QTL Mapping and Marker-assisted Selection" is being undertaken. The goal is to genetically enhance Nile tilapia (*Oreochromis niloticus*), the most widespread farmed tilapia in sub-Saharan Africa, to increase production, efficiency, profit margins and quality of life of the poor fish farmers and consumers. Thus, the project will also meet the needs for food security in Africa and initiate decreasing child and infant malnutrition. The specific objectives of this research are:(1) determination of heritabilities and genetic correlations for important performance traits such as growth, feed conversion efficiency, stress tolerance and sexual maturation; (2) development of selection indices; (3) gene mapping of important QTL; (4) initial evaluation of the feasibility of marker-assisted selection; and (5) training of African scientists in quantitative and molecular genetic techniques. This three-year project is beginning.

Future Perspectives

Radiation hybrid mapping

Although the idea of irradiation and fusion gene transfer was published over 20 years ago (Goss and Harris 1975), the technology was underutilized until resurrected by Cox et al. (1990) for applications in genomic research by combining it with efficient genotyping using PCR. Because of its extreme power for gene mapping, radiation hybrid (RH) panels are recognized as a milestone in human genomic research and is now regarded as the ultimate tool for correction of marker orders for linkage mapping in mammals (Womack et al. 1997). Extremely fine chromosome maps have been constructed in humans

(e.g., Schuler et al. 1996, reviewed by McCarthy 1996), mouse (McCarthy et al. 1997), and bovine (Womak et al. 1997). Recently, RH panels have been reported for rat, baboon, dog (Priat et al. 1998), pig (Yerle et al. 1998), chicken and zebrafish (Chevrette et al. 1997; Kwok et al. 1998). No RH panels are available for aquaculture species yet. In catfish, RH panels are desperately needed as an initial genetic linkage map is nearing. Undoubtedly, the development of RH in catfish will accelerate its genomic mapping and progress in genetic improvement of catfish broodstocks. A tilapia RH panel is under development elsewhere.

Physical mapping using large insert libraries

The second step after construction of a genetic linkage map is to focus on chromosomes that contain important QTLs controlling disease resistance, growth, feed conversion efficiency and processing yields. Catfish have 29 pairs of homogenous chromosomes (LeGrande et al. 1984) which make it extremely difficult to microdissect for development of chromosome-specific markers. Therefore, the strategy is to first identify the chromosomes containing important QTLs of interest. Then, large insert libraries, such as BAC library, will be used to tag markers linked to specific QTLs to specific BAC clones. Once the anchorage points are found, contigs of the BAC clones can be established rapidly surrounding the linked markers. This would allow development of chromosome-specific or regional markers for fine mapping of QTLs from the associated BAC clones. The work on physical mapping started in late 1999.

Research Teams and Facilities

A major strength of Auburn University's research is that it combines the expertise of two complementary research teams specialized in molecular genetics for molecular marker development, evaluation, and genotyping, gene cloning, sequencing and expression vector development, and in catfish aquaculture for strain selection, phenotypic evaluation, fish culture and maintenance, QTL measurement and evaluation, population and quantitative genetic analysis, transgenic fish production and rearing, and evaluation of environmental impact of transgenic fish.

Auburn University has the best facility in the nation for simulated commercial conditions for catfish genetic research such as QTL mapping. A large earthen pond facility, with 128 experimental research ponds, is available for evaluation of performance under closely simulated commercial conditions. This is important since genotype-environment interactions can occur in genetic evaluations of catfish requiring pond experiments to obtain realistic results.

Auburn University's Fish Genetics Research Unit also has the best facility to develop transgenic fish for aquaculture application. A large earthen pond facility with 28 ponds, secured with nets and filters approved by USDA for transgenic fish studies, is available for evaluation of performance of transgenic fish. This is the only government-approved outdoor testing facility for transgenic fish in the world. Its features include concrete stabilized walls, chain-link fence and barbed wire surrounding the complex, bird netting, common drain line, quadruple filtration, static conditions and French drain located in a seepage pond. The ponds are located 35 feet above the 100-year flood level, and a 17- acre barrier pond is located below these ponds.

A 6 500 ft^2 hatchery contains 300 tanks for indoor spawning, incubation and initial QTL evaluations.

The 6 500 ft^2 Molecular Genetics and Biotechnology Laboratory includes a computer room, main laboratory (radioactive safety approved), refrigerated room, dark room and tissue culture room. Two offices are attached to the laboratory. The recently acquired automated DNA sequencer (LI-COR IR^2 system) is equipped with genotyping apparatus for both microsatellite and AFLP analysis and genotyping software. The laboratory also has all the equipment for molecular biology: molecular cloning, PCR, restriction analysis, gel electrophoresis, DNA sequencing, blotting, hybridization, gene expression studies of RNA and proteins, radioimmunoassays (RIA), ELISA and Western blotting. Other major equipment include: four PCR cyclers, a microinjection system with Brinkman MM33 micromanipulator controlled by a Medical Systems Corporation pico-injector Model PLI-100, a Backeon Model 2000 for electroporation, a Beckman DU20 spectrophotometer, a Sorvall RC-6B clinical centrifuge, a Beckman J2-21 centrifuge, a Beckman GS-15 centrifuge, five microfuges, computerized low and high voltage power supplies for electrophoresis, two 37°C CO_2/humidified incubators, two -80°C freezers, one -20°C freezer, one analytical balance, one 37°C shaker incubator, one 37°C incubator for dish culture of *E. coli*, UV and visible transilluminators with camera, a Leitz microscope, two dissecting microscopes, one ice

maker, autoclave, three biosafety cabinets, one of which is devoted as a radioactive work station, CAT assay tanks and a scintillation counter. The following computer hardware are available - Power Mac 7100, Power Book Mac and two IBM computers, with two laser printers. Available are DNA analysis software packages, such as DNAStar and DNASIS and free access to GenBank, EMBO and other databases, and to GCG software packages with minimal cost. Auburn University also has excellent core facilities for oligo synthesis, sequencing, TaqMan PCR, confocal microscope and EM facilities with minimal costs.

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GENE BANKING AND COMMON CARP BREEDING PROGRAM IN HUNGARY

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GORDA, S and L. VARADI. 2001. Gene banking and common carp breeding program in Hungary, p. 119-122. *In* M.V. Gupta and B.O. Acosta (eds.) Fish genetics research in member countries and institutions of the International Network on Genetics in Aquaculture. ICLARM Conf. Proc. 64, 179 p.

ABSTRACT

The development of a live gene bank of domesticated carps was started in the Fish Culture Research Institute, Szarvas, Hungary, in 1963, when common carp *(Cyprinus carpio)* strains were collected from different regions of the country. Later, the stock of the live gene bank was enlarged with common carp strains from different parts of Europe and Asia. At present, there are 20 Hungarian and 15 foreign races and strains in the gene bank. Besides the commonly cultivated carps, wild races are also maintained; those are native in the two major Hungarian Rivers, Danube and Tisza. The broodstocks of 15 foreign races and strains are also available in the gene bank, mostly from Europe, e.g., the Czech mirror and scaly carps, the Croatian Nasic and Poliana carps, the scaly carp of Ukraine and the Russian Ropsha carp that has a long history in production.

Some Asian common carp races can also be found in the gene bank: scaly carps from Vietnam and Thailand, wild carp from River Amur, and Japanese koi carp. Besides common carps, the pure lines of silver carp *(Hypophthalmichthys molitrix)* and bighead carp *(Aristichthys nobilis)* are also available here.

The original goals of collection of common carp strains were: (i) to compare their characteristics within identical environmental and farming conditions; (ii) to use them in the selection and hybridization work; and (iii) to produce and supply high-value races, strains and parental lines of hybrids to preserve the strains with their original genetic structure as a potential gene reserve.

Introduction

In the early stage of the institute's experimental work, comparison tests on the productivity and characteristics of the collected Hungarian strains were carried out. The performance of strains was determined on the basis of the performance of their progenies. Significant qualitative and quantitative differences have been found among the various strains.

Broodstocks of strains are kept in eight earthen ponds of 1 ha each, with an average depth of 1.2 m. Each pond with continuous water exchange throughout the year holds about 200-250 breeders. The breeders are fed ad libitum except for those chosen for reproduction. These breeders are fed with special feed having high animal protein content, for 2-2.5 months before the spawning season.

The minimum population size of the breeding stock of each race and strain in the gene bank is 50 individuals. When the size of the breeding stock is not less than 50 individuals, the inbreeding coefficient (F) is quite low (F= 0.01%) per generation.

Each strain of the gene bank is renewed in 8-10 years or the population is supplemented. Eggs and sperms of a minimum of 10 females and 10 males are used during reproduction, simulating panmictic population conditions.

For the identification of the given races and strains in the gene bank, group marking is applied. The mirror carps are marked with brand marking, while the scaly ones by fin clipping. PIT tagging system is also used for the identification of individuals.

The establishment of cryopreserved sperm bank was started in 1997, which was based on the male individuals of live gene bank. One male is represented with 15-20 ml (4 x 5 ml straw) frozen sperm in liquid nitrogen. At present, there are cryopreserved sperm of 68 male broodstocks.

Breeding Programs

Using the strains preserved with original gene pool, several crossing combinations and inbred lines were produced. Gynogenesis was also used to produce high-level inbred individuals. Since 1963, more than 400 crossings have been applied to determine the best crossing partners and produce hybrid common carp with high productivity. As the races, strains and hybrids are different in their qualitative and quantitative characteristics, a progeny performance test was elaborated for the evaluation of their productivity.

During the tests, the scale of heterosis effect was analyzed in the crossbred progeny. The progeny groups in the performance tests were kept in identical environmental conditions for reproduction until the end of the investigation. These conditions are similar to commercial fish farms in Hungary. The progeny groups are always produced by artificial reproduction, at the same time, during the natural spawning season. After hatching, the larvae are kept in larval rearing tanks for 2-3 days until the beginning of external feeding. In the first year, the larvae are reared in 1 ha size ponds with a low stocking density, until they reach 100-150 g body weight. At the end of the first growing season, the individuals of the experimental groups are marked one by one, and each group has its own mark (fin clipping and brand marking). After marking, the groups are stocked in the same pond. The weight loss and mortality occurring during winter are evaluated. In spring, at the beginning of the following growing season, the fish groups are stocked in grow-out ponds for market size fish production.

The evaluation of experimental groups is carried out not only in the experimental ponds of the institute, but also in three commercial production farms. In the tests, five main features are evaluated which determine the economic value of races, strains and hybrids. These parameters are survival, weight gain, feed conversion ratio, slaughtering value (dressing yield) and fat content of the fish flesh. To evaluate the overall performance of the progenies, and to make the comparison easier, a point system is elaborated. Thirty, 25, 20, 15 and 10 points can be given as a maximum for weight gain, survival, feed conversion ratio, slaughtering value and fat content, respectively. If all five characteristics of a tested group get the highest score, the sum of the points equals to 100. The maximum point for a characteristic is always given to a group that has reached the highest performance during the test, and the points for the other groups are decreased proportionally.

As a result of the breeding work, three high productivity hybrids were produced, which received a state certificate. The first hybrid was the mirror Szarvas 215, produced in 1977. This hybrid was introduced to commercial production, and has been cultivated with good results in intensive farming conditions and cage culture in Hungary. The second hybrid, the scaly Szarvas P. 31, was produced in 1978. It is a heterozygote scaly hybrid which has also been widely cultivated in Hungarian fish farms.

As a recent result of the institute's breeding work, the third hybrid, the two-line scaly hybrid, called Szarvas P. 34, was produced in 1989. This hybrid inherited its excellent feed conversion ratio and growth rate from the domesticated maternal line, and its high resistance and good ability to select natural food, from the wild paternal line. The hybrid has been produced for the stocking of angling waters, reservoirs and extensively utilized fishponds.

The productivity of parental lines is surpassed by more than 20-25% by the productivity of these hybrids.

Maternal and paternal lines of high productivity hybrids have also been supplied to different countries

(Croatia, Czech Republic, Brazil, Bulgaria, Greece, Lao PDR, Madagascar, Poland, Rumania, Russia and Vietnam), and the hybrids have proven their high productivity and good adaptibility even in tropical conditions.

After the political and economic changes in Hungary in the early 1990s, several new acts and regulations have been issued, among them the Animal Breeding Act which regulates activities related to breeding of cultured animals, including fish, through the Ministry of Agriculture and Regional Policy (MARP), National Institute for Agricultural Quality Control (NIAQC) and approved breeding organizations. There are specific regulations on the operation of insemination stations, embryo-transfer stations, poultry and fish hatcheries, and of export and import of breeders and breeding materials.

Operation of fish hatcheries is controlled by NIAQC. The basic preconditions to operate a fish hatchery, which produces larvae for sale are: license from NIAQC, qualified hatchery manager and certificate from the local veterinary authority. Only pure strains or parental lines of hybrids, with state approval and certificate of origin, can be propagated. The hatchery should keep a hatchery record book containing the number of spawners, certificate of origin, hormone treatment, mass of stripped and fertilized eggs, number of produced larvae and registry number of certificate of origin if the larvae is sold.

At present, there are 20 licensed carp hatcheries in the country and this number is increasing. The operation of hatcheries is checked randomly by NIAQC. Common carp strains can get state approval by undergoing a standardized code of carp performance test supervised by NIAQC. During the first year of the two-year test, the broodstocks of the various strains are transported to a central breeding station where propagation and fingerling rearing take place under identical conditions. During the second year, the fingerling groups are tested together on three fish farms in different regions of the country. The tested traits are survival, weight gain, food conversion ratio, slaughtering value (dressing yield) and fat content.

The feed conversion ratio is tested in five wire-cages of 165 m^2 each, placed in an earthen pond stocked with the test groups of fish. About 25 individuals of known body weight are stocked in each cage and fed with a feeding rate of 5% body weight throughout the growing season. Natural food is available for the fish in the cage, just like the other fish in the pond. The food conversion ratio is also examined under laboratory conditions in closed recycling water system where feeding is based on artificial food only. After second growing season, at the time of harvesting, the fish are counted one by one and survival is expressed in a percentage of the number of fish at stocking. The weight gain is calculated as a difference between weight at stocking and harvesting, when each individual is weighed.

Slaughtering value means the rate of edible meat of the fish mass in percentage. For determination of the slaughtering value, 20 individuals of each group are tested in laboratory conditions. The fat content of the fish flesh is determined from the edible part by acido-butirometer analysis and expressed in percentage. The abnormalities and body proportions are also recorded during the harvesting and examination of slaughtering value.

NIAQC publishes the recorded data for agricultural quality control in the official newsletter of MARP. To date, 17 Hungarian strains (including the above-mentioned three hybrids) have been tested and have license for breeding and marketing of their progenies.

In 1996 the Hungarian Carp Breeding Organisation was established to assist and coordinate breeding programs, organize meetings and workshops, provide consultancy to breeding farms and present the interest of the breeders in various fora.

Other new breeding organizations, legal measures and regulations have contributed to the improvement of carp breeding and product quality in Hungary in the past five years. The exchange of information on these specific aspects would also contribute to the improvement of carp breeding on an international scale.

AQUATIC GENETIC RESOURCE ACTIVITIES OF THE FISHERIES DEPARTMENT, FOOD AND AGRICULTURE ORGANIZATION

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ABSTRACT

The sustainable use and conservation of aquatic genetic resources are key activities of the Fisheries Department in the implementation of the Code of Conduct for Responsible Fisheries (CCRF) and other mandates of the Food and Agriculture Organization (FAO) of the United Nations. Currently, the emphasis is on normative activities under the Regular Programme. However, there are a small number of projects dealing with genetic resources under the Field Programme. Main areas of specialization include:(i) promotion of efficient, sustainable and responsible fisheries sector management at the global, regional and national levels in the framework of CCRF; (ii) global monitoring and strategic analysis of fisheries to provide a sound basis for protection of production potentials, resource trends and impacts of fisheries practices; (iii) promotion of increased contribution of responsible fisheries and aquaculture to world food supplies and food security; and (iv) improvement of biological data on marine resources.

Introduction

The CCRF is the most prominent document providing both the policy framework for FAO, as well as the fundamental reference and guidance document to governments, private sector and most NGOs, regarding aquatic genetic resources in the context of global fisheries and aquaculture and their sustainable development and conservation. Currently, the emphasis within the Fisheries Department is on normative activities under the Regular Programme (Table 1). However, there are a small number of projects dealing with genetic resources under the Field Programme (Table 2) and an increased number of activities in support of the implementation of CCRF at national level.

Regular Program

Within the Fisheries Department of FAO, the Fishery Resources Division (FIR) is the lead division dealing with issues of aquatic genetic resources; the majority of the work is handled by the Inland Water Resources and Aquaculture Service (FIRI) with assistance from the Marine Resources Service (FIRM), the Fishery Information, Data and Statistics Unit (FIDI) and the Fishery Development Planning Service (FIPP). The priorities concerning fishery genetic resources are: (i) Promotion of efficient, sustainable and responsible fisheries sector management at the global, regional and national levels in the framework of CCRF; (ii) Global monitoring and strategic analysis of fisheries to provide a sound basis for protection of production

potentials, resource trends and impacts of fisheries practices; (iii) promotion of increased contribution of responsible fisheries and aquaculture to world food supplies and food security; and (iv) Improvement of biological data on marine resources.

Information on these priorities is distributed to members and interested parties in specialized international fora and in normative publications, such as guidelines, codes of conduct and protocols, technical publications (Fisheries Technical Papers and Circulars), external scientific publications and conference proceedings, and increasingly in the FAO Aquaculture Newsletter and the Fishery Department's internet site on WAICENT.

Promotion of responsible fisheries and aquaculture

The Fisheries Department continues to support the implementation of FAO CCRF and Convention on Biological Diversity (CBD) through a variety of activities, such as participation in specialized meetings of CBD, publication of technical guidelines on fisheries and aquaculture, for example on the precautionary approach to the use of new species in aquaculture, and organization of international fora on fishery genetic resources. The "Towards Policies for the Conservation and Sustainable Use of Aquatic Genetic Resources Conference" was successfully convened in 1998, with the support of the Sustainable Development Department of FAO and the International Center for Living Aquatic Resources Management (ICLARM). The conference aimed to provide guidance on policy development for fishery genetic resources.

Global monitoring and strategic analysis of inland fisheries and aquaculture

Activities under this element concern analysis of new species being farmed or fished and their genetic resources. A specialized online and searchable database on the introduction of aquatic species (DIAS) has been added to FAO's fisheries internet site that provides basic information on exotic species and summarizes important issues. FAO fishery statistics, data from DIAS, and information and illustrations from the Species Identification Programme have been incorporated into FishBase, a relational database distributed on CD-ROM, by ICLARM. With the increase in number of genetically improved species being farmed and the growing interest in genetic engineering, efforts are underway to document new techniques, and evaluate existing legislation that may be required for sustainable use of fisheries and equitable benefit sharing.

FAO statistics on capture fisheries and aquaculture continue to provide valuable information on genetic resources. There are efforts to improve these data through more accurate reporting and inclusion of information on fish stocks and genetic resources. A fisheries global information system (FIGIS) is currently being designed that will integrate many of the components of the department which deal with fishery genetic resources.

Table 1. 1997-1998 budget allocations to regular program elements with components relevant to fishery genetic resources, and estimated weight of these components.[a]

Programme element	Budget (US$000)	Estimated weight of fishery genetic resources components
Promotion of responsible fisheries and aquaculture	1 700	Medium-low
Global monitoring and strategic analysis of inland fisheries and aquaculture	1 300	Medium-low
Increased contribution of inland fisheries and aquaculture to world food supplies	2 200	Medium-low
Improvement of biological data on marine resources	300 (not including staff salaries)	Medium

[a]*Program elements were redefined in 1998 for the 1998/1999 biennium.*

Table 2. FAO field projects that have a significant component on fishery genetic resources.

Project	Budget (US$000) and dates	Estimated weight of fishery genetic resources component
Genetic improvement of tilapia in Venezuela (TCP/VEN/6611)	225 1997-1998	Very high
Research for the management of the fisheries on Lake Tanganyika (GCP/ AF/271/FIN)	1 054 1998 – 2001	Medium-low
Sustainable contribution of fisheries to food security II (GCP/INT/643/ PN)	900 1997- 2000	Medium-low
Regional Aquaculture Development for the South Pacific (GCP/RAS/116/ PN)	797 1997-1998	Low

Increased contribution of inland fisheries and aquaculture to world food supplies

The main activities in this element involve the publication of technical documents and consultations that document, characterize, and assess fishery genetic resources and related technologies important for food and agriculture. For example, through collaboration with the International Center for Advanced Mediterranean Agronomic Studies, a review of genetic technologies in the region was published, and further work involving a survey of the private aquaculture sector is ongoing. In cooperation with ICLARM and the World Fisheries Trust (Victoria, British Columbia), a global information and communication system on aquatic animal diversity and a global strategy for the management of aquatic animal diversity are being formulated.

Improvement of biological data on marine resources

The Species Identification and Data Programme continues to promote upgrading of marine fisheries data and reliable species identification through publication of species inventories, species diagnostic keys, reference system and a readily accessible information system.

Participation in interagency and interdepartmental activities

The Fisheries Department continues to support regional and national fisheries bodies, networks and scientific associations in promoting sustainable use and conservation of fishery genetic resources. Principal external partners include the Secretariat to CBD, SBSTTA (Subsidiary Body for Scientific, Technical and Technological Advice) and expert groups of CBD, as appropriate, ICLARM, the International Network on Genetics in Aquaculture, the Consultative Group on International Agricultural Research System-wide Genetic Resources Programme, the World Fisheries Trust, the Asian Fisheries Society and the American Fisheries Society. Internally, the department collaborates with interdepartmental working groups on biosafety, ethics in food and agriculture, biological diversity, inland water management, integrated coastal area management and integrated pest management.

Several activities and outputs (Annex 1) related to sustainable use and conservation of fishery genetic resources have been generated over the past three years. It is now time to incorporate this background information into planning a global strategy, elements of which will be developed in consultation with domestic animal, crop plant and forestry sectors, as well as within the fisheries sector in the near future. The roles of a global information system and an intergovernmental body, i.e., the Commission, will be included in the resulting strategy.

Table 1 lists the major allocations to program elements within FAO's 1997-1998 Regular Programme budget for the Fisheries Department, in which substantial fishery genetic resources activities are pursued.

Annex 1. FAO-supported technical consultations, training courses and workshops, and publications related to fishery genetic resources and aquatic animal diversity.

Technical consultations supported by FAO

Technical Consultation on Inland Fishery Enhancement, in cooperation with the Department for International Development of the United Kingdom, 7-11 April 1997, Dhaka, Bangladesh.

Genetics and Breeding of Mediterranean Aquaculture Species, in cooperation with the International Center for Advanced Mediterranean Agronomic Studies and the Network on Technology of Aquaculture in the Mediterranean, 28-29 April 1997, Zaragoza, Spain.

FAO Technical Consultation on Policies for Sustainable Shrimp Culture, 8-11 December 1997, Bangkok, Thailand.[a]

Towards Policies for the Conservation and Sustainable Use of Aquatic Genetic Resources, in cooperation with the Rockefeller Foundation, ICLARM and Sustainable Development Department, 14-17 April 1998, Bellagio, Italy.

[a]FAO. 1998. Report of the Bangkok FAO Technical Consultation on Policies for Sustainable Shrimp Culture, 8-11 December 1997, Bangkok, Thailand. FAO Fish. Rep. 572. Rome, Italy. Also, visit http://www.fao.org/WAICENT/FAOINFO/FISHERY/faocons/shrimp/bangk.htm

Regional Workshop on Health and Quarantine Guidelines for the Responsible Movement (Introduction and Transfer) of Aquatic Organisms, 28 January 1996, Bangkok, Thailand.

First Regional Workshop of the FAO/NACA/OIE Regional Programme for the Development of Technical Guidelines on Quarantine and Health Certification, and Establishment of Information Systems for the Responsible Movement of Live Aquatic Animals in Asia, 6-20 January 1998, Bangkok, Thailand.

Ad-hoc Expert Meeting on Indicators and Criteria of Sustainable Shrimp Culture, 28-30 April 1998, Rome, Italy.

Development of an Information and Communication System on Aquatic Animal Diversity – with CGIAR System-wide Genetic Resources Programme and World Fisheries Trust (British Columbia), 16-17 November 1998, Rome, Italy.

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Bartley, D.M. *Ex situ* conservation, genebanks and responsible fisheries, p. 45–56. In B. Harvey, C. Ross, D. Greer and J. Carolsfeld (eds.) Action before extinction: conserving fish genetic diversity. World Fisheries Trust, Vancouver, B.C., Canada.

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ACTIVITIES AT AKVAFORSK OF IMPORTANCE TO THE INTERNATIONAL NETWORK ON GENETICS IN AQUACULTURE

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GJEDREM, T. and T. SVEÄLV. 2001. Activities at AKVAFORSK of importance to the International Network on Genetics in Aquaculture, p. 129-132. *In* M.V. Gupta and B.O. Acosta (eds.) Fish genetics research in member countries and institutions of the International Network on Genetics in Aquaculture. ICLARM Conf. Proc. 64, 179 p.

ABSTRACT

One of the main objectives of the Institute of Aquaculture Research of Norway, Ltd. (AKVAFORSK) since its establishment in 1971 is to study the theoretical, biological and practical bases to start, develop and run breeding programs. The work started with Atlantic salmon and rainbow trout in Norway. It was later transferred to a breeding company, Aqua Gen, which today supplies 70% of the Norwegian industries with improved eyed eggs. AKVAFORSK has been and is offering its competence and experience in developing breeding programs for members of the International Network on Genetics in Aquaculture (INGA) as well as for others. In the future, services will be provided through AKVAFORSK Genetics Center.

The programs so far undertaken by AKVAFORSK have clearly shown that there is a large unexploited potential for genetic gain in all populations of fish and shellfish studied. A genetic gain of 10-20% in selection response for growth rate is usual, and a considerable genetic gain is also obtained by selection for disease resistance and age at sexual maturation. By applying selective breeding, it is possible to increase productivity and sustainability in aquaculture.

Introduction

AKVAFORSK is a nonprofit research institute owned by public shareholders. Its main office is located at the Agricultural University of Norway (AUN), 30 km south of capital Oslo, while the two research stations at Sunndalsøra and Averøy are located in the coast some 500 km northwest of Oslo. As of 1999, the Institute has 85 employees, of whom 28 are scientists and 7 are Ph.D. students. One-third of the budget is basic funding from the Research Council of Norway, and most of the rest comes from competitive-grant research projects. AKVAFORSK scientists teach aquaculture at AUN and at Sunndalsøra College.

The main fields of research are nutrition, quantitative genetics and breeding, and flesh quality; and to a less extent, preventive health care, environmental issues and technology. The main activity in Norway is research on coldwater species, such as Atlantic salmon, rainbow trout and Atlantic halibut; work with oysters and scallops is planned. Internationally, AKVAFORSK

is engaged in many countries in Europe, Southeast Asia and South America. The projects are in the fields of selective breeding and nutrition.

Simple Breeding Programs

Genetic variation has been demonstrated for economically important traits in several species. A selection response in the order of 10 to 20% per generation is obtained for growth rate and, in some instances, also for disease resistance. It can therefore be concluded that it is possible by means of efficient breeding programs to increase productivity and obtain domestication in aquatic species.

The simplest procedure for selective breeding is to use mass selection or individual selection, which has proved to be very efficient under certain conditions: (i) It must be possible to measure or record the trait of interest in live animals. In practice, this is true for body weight or body size. (ii)The trait should have a moderate or high heritability, higher than 20%.

In prolific breeding animals, such as fish and shellfish, inbreeding is frequently encountered. Since the inbreeding depression is considerable for fitness traits as well as for growth rate, steps must be taken to keep inbreeding low. A method to control it in a breeding program has been described for *Cirrhinus mrigala* (Bentsen et al. 1996b), *Barbodes gonionotus* (Bentsen et al. 1996a), *Oreochromis niloticus* (Gjedrem et al. 1997b) and *Chanos chanos* (Gjedrem et al. 1997a). To keep inbreeding low, the key element is to use many parents per generation. Bentsen et al. (1996a, 1996b) described how to produce families and to sample out certain number of progenies from each family to reach about the same number per family during the grow-out period.

From a simulation trial, it is concluded that: (i) in individual selection one should use many families, more than 50 pairs. (ii) since facilities for grow-out testing will be limited, only few animals per family should be allowed.(iii) this strategy will keep inbreeding low, avoid reduction of diversity and secure long-term response to selection.

A breeding program must continually be improved and developed to utilize new knowledge as well as to meet the requirements of the industry for more productive animals. If more traits are to be included in the breeding goal in the future, family selection must often be applied. The estimation of breeding values becomes more complicated as the number of traits increases, making it necessary to optimize different components in the breeding program.

Economic Traits for Genetic Improvement

Traits of economic importance will vary from species to species and among production systems. In most instances, growth rate and disease resistance will be the important breeding goals. Age at maturation is important in species like salmonids and tilapia. Flesh quality will vary among species as well as over time, and is usually difficult to measure, particularly in live animals. Temperature and salinity tolerance may be important in tilapia to expand the areas for cultivation.

During the grow-out period, test animals should be reared under conditions similar to those used by the industry. It must be defined how and when each trait should be measured or recorded. Growth rate, body weight or body length, should be recorded at marketing. This is easy to apply: age of sexual maturation can be recorded as frequencies of mature animals at a certain age, flesh quality can normally only be measured on carcasses. To obtain a measure of disease resistance, challenge tests against specific pathogens have proved to be efficient. Challenge tests could also be used to test for temperature tolerance, while salinity tolerance should be evaluated by growing test animals in water with the wanted salinity while recording survival and growth rates.

Lessons from the GIFT Project

The Genetically Improved Farmed Tilapia (GIFT) Project of ICLARM was started in 1988, and the base population was formed by a complete diallele cross between eight strains of *O. niloticus*. This base population had broad genetic variation and possible inbreeding in the domesticated strains was eliminated. In each generation, more than 100 families were produced while avoiding mating of close relatives. During testing period, efforts were made to standardize the environmental conditions. In each generation, strong selection was practised except in the first. These procedures were key elements that led to the great success of GIFT project.

The selection response in growth rate during five generations averaged 13.2% per generation. This

genetic gain is similar to results obtained for several fish species. These results show that it is possible to double the growth rate in six generations in tilapia, since the genetic gain is cumulative over generations. When these results reach the industry, it will cause a big change in production and productivity. There will be a dramatic reduction in the turnover rate and production per unit will increase. The feed conversion rate will become much lower. This increase in productivity will also increase the survival rate. In summary, the production cost will markedly reduce and total production will increase. Every farmer in the whole country, rich and poor, can obtain this benefit.

The cooperation among all partners in the GIFT project - ICLARM, AKVAFORSK, and the Philippine institutions, Bureau of Fisheries and Aquatic Resources (BFAR) and Central Luzon State University (CLSU) - has been very productive and educational.

Developing Breeding Programs for the Industry

Back in the 1970s, AKVAFORSK formed the theoretical basis for a breeding program for Atlantic salmon and rainbow trout through a series of research projects. It also actually started and developed the program in Norway. After some years, AKVAFORSK invited the industry to participate in running the program, and in 1992, a breeding company, Aqua Gen, was established. From then on, this company has been running national breeding programs for salmon and trout in Norway. AKVAFORSK participates in further developing the breeding program by including additional economically important traits, optimizing the different components of the program and continuously estimating breeding values.

In 1993, the partners in the GIFT project started a breeding program for *O. niloticus* in the Philippines. Later, this led to the establishment of the GIFT Foundation, which is now running a commercial breeding program for *O. niloticus* in the Philippines. GIFT Foundation builds on the GIFT strain and continues the selection for growth rate and late maturation. It is located in the facilities built by the government at Munoz, Nueva Ecija, and has accredited eight private hatcheries for production of fry for the industry.

These are two good examples of how breeding projects have developed into breeding programs run by companies to benefit the industry. The most difficult aspects in this development have been to: (i) establish a breeding organization; (ii) raise financial support for investments and to start the breeding program; and (iii) raise financial support to scale up production and market the improved product.

Other international breeding projects in which AKVAFORSK has been involved with are listed in Table 1.

In cooperation with INGA, AKVAFORSK is involved in designing breeding plans for tilapia, mrigal carp *(C. mrigala)* and silver barb *(B. gonionotus)* in Vietnam, and for Nile tilapia, common carp and milkfish *(C. chanos)* in Indonesia. The collaboration with INGA continues, and AKVAFORSK plans to expand this.

In 1995, ICLARM and INGA organized the Advanced Course in Quantitative Genetics and Breeding for scientists from INGA member countries. Held in Cavite, Philippines, some of the lecturers were from AKVAFORSK. In 1998, INGA held a similar course in India, with lecturers from AKVAFORSK.

AKVAFORSK also plans to offer short courses in these areas. It supervises Ph.D. students in aquaculture in the fields of nutrition, breeding and selection, flesh quality and disease prevention. However, it cannot offer fellowships or financial support.

Table 1. International breeding projects with which AKVAFORSK has involvement.

Country	Species	Period
Completed projects		
Iceland	Atlantic salmon	1987-1993
Philippines	Nile Tilapia	1988-1997
Hawaii	Shrimp[a]	1994-1997
Brazil	Shrimp[a]	1997-1998
Ongoing projects		
India	Rohu carp	1993-
Chile	Coho, rainbow trout, Atlantic salmon	1996-
Columbia	Shrimp[a]	1997-
Vietnam	Nile Tilapia	1999-
Scotland	Atlantic salmon	1999-
Italy	Rainbow trout	1999-

[a]*Penaeus vannamei.*

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OVERVIEW OF FISH GENETICS RESEARCH AT QUEENSLAND UNIVERSITY OF TECHNOLOGY

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MATHER, P.B. 2001. Overview of fish genetics research at Queensland University of Technology, p. 133-139. *In* M.V. Gupta and B.O. Acosta (eds.) Fish genetics research in member countries and institutions of the International Network on Genetics in Aquaculture. ICLARM Conf. Proc. 64, 179 p.

ABSTRACT

This paper documents the focus of applied genetics research on aquatic species at Queensland University of Technology (QUT), Brisbane, Australia. Studies have focused on exotic tilapias and native freshwater crayfish, with emphasis on development of genetic markers and their application to breeding programs designed to improve cultured stocks. Since many cultured stocks have been exposed to significant bottlenecks in the past, studies have also focused on comparisons of levels and patterns of genetic diversity in wild stocks, as they represent a considerable genetic resource which can be used to provide novel genetic traits in culture. These studies undertaken in parallel with those of collaborating institutions using animal breeding approaches (selective breeding) have produced better culture strains of tilapia in Fiji and redclaw in Australia.

Introduction

Genetic projects at QUT on species of interest to the aquaculture industry have focused largely on two groups of organism, tilapias and Australian native freshwater crayfish.

Tilapia Genetics

Tilapias are considered noxious species in Australia and therefore cannot be cultured for legal reasons. Genetic research on tilapias at QUT has focused, therefore, on the development and application of appropriate genetic markers for tilapia breeding programs conducted outside Australia in developing nations. QUT's major research project on tilapias is based in Fiji in collaboration with the Fisheries Division of the Ministry of Agriculture, Fisheries and Forests (MAFF). This project commenced in 1993 and was directed at assisting the Fijians to develop better culture stocks for their tilapia farmers. Initial research was on evaluating the culture performance of the four indigenous tilapia stocks in Fiji (*Oreochromis niloticus* Chitralada strain, *O. niloticus* Israel strain, *O. mossambicus* and a red hybrid) under two production systems (integrated and nonintegrated), and identifying the stock which performed best there under most environmental conditions. This work was completed in 1996 and better stock management practices developed and put into practice to maintain stock quality (Macaranas et al. 1997).

In parallel, a range of molecular markers (allozyme, randomly amplified polymorphic DNA [RAPD], mt DNA and microsatellite loci) were developed at QUT to characterize tilapia stocks and to monitor their genetic integrity over time. The second phase of the project has the objectives of first comparing the relative performances in culture of the best-performing stock in Fiji (*O. niloticus* Chitralda - determined in the earlier work) against the genetically improved GIFT *O. niloticus*. Once the evaluation is complete, QUT intends to take the best-performing stock through three generations of family selection to further improve growth performance. At the end of the program, it is hoped that these will be achieved: a better performing stock for Fijian farmers; a set of markers which will allow monitoring of stock quality over time; and highly trained staff in Fiji who can maintain fry quality. Stock evaluation trials are conducted under near quarantine conditions so that only better-performing stocks are retained for fry production. Other stocks will be culled at a later date reducing their potential for hybridizing with high-performing lines. Since most fry used by farmers in Fiji are provided by the Fisheries Division hatcheries to farmers at low cost, this should allow strict control of fry quality.

Development of genetic markers

Much of the tilapia genetic work at QUT has focused on development and application of genetic markers. Initially, allozyme markers were developed using the cellulose acetate system. Currently, there is an excess of 50 allozyme coding loci which are used to routinely screen *O. niloticus* and *O. mossambicus* stocks (Table 1). As other studies have reported, however, allozyme variation is generally quite low in tilapiine species with percent polymorphic loci estimates commonly below 5% and average heterozygosity per locus estimates often below 10%. The marker work was expanded, therefore, to include DNA sequences that commonly show higher levels of genetic diversity. Thus, the focus in recent years has been on assessing variation in the mtDNA control region and at RAPD and microsatellite loci (Table 2). MtDNA control region sequence variation screened using Temperature Gradient Gel Electrophoresis (TGGE) and variation at RAPD loci have been used to describe phylogenetic and systematic relationships among cultured stocks of *O. niloticus* and wild and feral stocks *of O. mossambicus.* Patterns of variation in the mtDNA control region sequence in wild *O. mossambicus* stocks in southern Africa indicate that disjunct wild stocks have experienced long periods of isolation which has led to separate evolution of stocks in different parts of the species' natural range. The extent of differentiation observed could indicate the presence of discrete evolutionary lineages or even subspecies (Table 3).

The publication by Kocher's laboratory (Lee and Kocher 1996) recently of >150 microsatellite primer sequences for *O. niloticus* as a starting point for developing a saturated gene map for the species has allowed the expansion of the marker work to document microsatellite variation in cultured and wild tilapia stocks. The conditions have been optimized for approximately 30 microsatellite loci in *O. niloticus* and screened variation in both *O. niloticus* and *O. mossambicus*. Primers developed for *O. niloticus* generally work equally well in *O. mossambicus*. After initial trials and characterization of variation at specific microsatellite loci, nine loci with high allelic diversity (>12 alleles per locus) were selected to examine the relationship between average heterozygosity and relative growth performance in the *O. niloticus* Chitralada stock in Fiji, as heterosis has been linked to growth performance in some aquaculture stocks (e.g., Zouros et al. 1980).

Currently, (with collaborators in Fiji) the culture performance of GIFT and Chitralada strains of *O. niloticus* is being compared under identical culture conditions. In parallel, the two stocks for microsatellite allelic diversity have been characterized, and their relative heterozygosity levels as a component of trials have been directed at identifying the best performing *O. niloticus* stock for Fiji. After the best performing stock has been identified, the markers will be used to monitor genetic diversity across three generations of family selection. The same markers will be used over the long term to monitor the genetic health of the culture stocks that are used to generate fry for Fijian farmers. Poor management and husbandry practices can often lead to changes in the genetic characteristics of culture stocks that may compromise their long-term productivity. Since genetic marker technologies have been applied to tilapia culture in Fiji, the quality of fry provided by government hatcheries to farmers has improved noticeably. Despite the increased costs and difficulties with shipping tissue samples for genetic analysis among collaborating partners (Australia and Fiji), the benefits in terms of stock quality have been very evident. This outcome demonstrates that it is not necessary for all groups involved in genetic improvement programs on aquatic organisms to

Table 1. Allozyme systems screened in *O. niloticus* and *O. mossambicus* stocks.

Protein/enzyme	E.C. no.	Locus	Tissue source
Aspartate aminotransferase(AAT)	2.6.1.1	AAT-1	Liver
		AAT-2	Liver
Acid phosphatase (ACP)	3.1.3.2	ACP	Liver
Adenosine deaminase (ADA)	3.5.4.4	ADA-1	Heart
		ADA-2	Muscle
		ADA-3	Muscle
Alcohol dehydrogenase (ADH)	1.1.1.1	ADH	Liver
Aconitate dehydratase (AH)	4.2.1.3	AH-1	Liver
		AH-2	Liver
Adenylate kinase (AK)	2.7.4.3	AK-1	Muscle
		AK-2	Muscle
Aldolase (ALD)	4.1.2.13	ALD-1	Muscle
		ALD-2	Muscle
Aldehyde dehydrogenase (ALDH)	1.2.1.5	ALDH-1	Liver
Creatine kinase (CK)	2.7.3.2	CK-1	Heart
		CK-2	Heart
		CK-3	Muscle
Esterase	3.2.2	EST-1	Heart, liver
(EST)		EST-2	Heart, liver
Fructose-bisphophatase (FBP)	3.1.3.11	FBP-1	Muscle
		FBP-2	Muscle
Fumarate dehydratase (FH)	4.2.1.2	FH-1	Heart
		FH-2	Heart
Glyceraldehyde-3-phosphate	1.2.1.12	GAPDH-1	Heart
Dehydrogenase (GAPDH)		GAPDH-2	Heart, muscle
Glycerol-3-phosphate	1.1.1.8	G3PDH-1	Heart
Dehydrogenase (G3PDH)		G3PDH-2	Muscle
Glucose-6-phosphate		G6PDH	Muscle
dehydrogenase (G6PDH)	1.1.1.49	GPI-1	Heart
Glucose-6-phosphate isomerase (GPI)	5.3.1.9	GPI-2	Heart
L-Iditol dehydrogenase (IDDH)	1.1.1.14	IDDH-1	Liver
		IDDH-2	Liver
Isocitrate dehydrogenase (NADP+)	1.1.1.42	IDHP	Liver
(IDHP)			
Malate dehydrogenase (MDH)	1.1.1.37	sMDH-1	Muscle
		mMDH-2	Heart
		mMDH-3	Muscle
		mMDH-4	Muscle
Malic enzyme (NADP+)(MEP)	1.1.1.40	MEP	Liver
Phosphogluconate dehydrogenase	1.1.1.44	PGDH	Liver
(PGDH)			
Phosphoglucomutase (PGM)	5.4.2.2	PGM	Muscle
Superoxide dismutase (SOD)	1.15.1.1	SOD	Liver
General Protein (PROT)		PROT-1	Muscle
		PROT-2	Muscle
		PROT-3	Muscle
		PROT-4	Muscle

develop and maintain the extensive technical infrastructure and expertise necessary to receive the benefits of application of these technologies to tilapia stock improvement.

Wild tilapia genetic resources

Concurrently at QUT, there is interest in characterizing the levels and patterns of genetic diversity in wild tilapia stocks, as they represent

Table 2. Operon primers used for routine screening of tilapias.

Primers	Molecular sequence (5' - 3')	No. of scorable loci	Informative loci
OPA-4	AATCGGGCTG	9	3
OPA-7	GAAACGGGTG	12	7
OPA-8	GTGACGTAGG	13	7
OPA-9	GGGTAACGCC	13	9
OPA-10	GTGATCGCAG	15	9
OPA-16	AGCCAGCGAA	18	11
OPA-18	AGGTGACCGT	16	12

important genetic resources that can be used to improve cultured stocks in the future. An excellent example of this approach has been the GIFT project. To this end, samples of wild African as well as cultured and feral stocks of *O. mossambicus* have been obtained from outside Africa and genetic variation at protein coding loci and DNA sequences have been characterized. These studies have indicated that cultured and feral stocks of *O. mossambicus* are relatively low in genetic diversity and appear to have suffered extreme genetic bottlenecks in the recent past and have as a consequence lost much of their natural genetic diversity (Table 4). Loss of genetic diversity has most probably affected their performance in culture in the past. In addition, patterns of variation in the mtDNA control region among wild and feral stocks have suggested that the original translocations of *O. mossambicus* from southern Africa probably consisted of only a small number of individuals from the extreme northern end of the species' natural distribution. Regional genetic diversity in wild African populations of this species is otherwise very high (Table 5). All cultured and feral stocks of *O. mossambicus* outside Africa, therefore, have probably originated from a very narrow genetic base which has been further reduced by subsequent translocations.

O. mossambicus is now considered in many parts of the world, where tilapia is cultured, to be a species of low culture potential and even a pest. In many countries, it has been replaced by better performing strains of the *O. niloticus*. Hybrid introgression of feral *O. mossambicus* genes into cultured *O. niloticus* stocks in some countries has also reduced their culture performance (notably in the Philippines and Indonesia). Studies conducted by the university suggest that the potential of *O. mossambicus* as a culture species should be reassessed in the future. This is because existing cultured and feral stocks of

Table 3. Pairwise Fst estimates based on mtDNA haplotype diversity among eight wild African stocks of *O. mossambicus*.

Populations	Lower Shire Rufanes		Limpopo / Incomati Rufanes					Rufanes
	1	2	3	4	5	6	7	8
1. Bangula	-	NS	0.951	0.914	0.914	0.930	0.900	0.876
2. Elephant marsh		-	0.957	0.914	0.965	0.932	0.896	0.945
3. Crocodile			-	0.627	0.975	0.536	0.461	0.962
4. Ohrigstad				-	0.940	0.251	0.294	0.913
5. Sabie					-	0.954	0.925	1.000
6. Arabie						-	NS	0.932
7. Klaseri							-	0.892
8. Rufanes								-

Table 4. Genetic variability estimates in wild and feral *O. mossambicus* stocks

Population	N	Mean no. of alleles/locus	% polymorphic LOCI	Mean heterozygosity Observed	Expected
Feral					
1. Malaysia	49	1.15 (0.36)	4.6	0.05 (0.15)	0.04 (0.13)
2. Singapore	14	1.20 (0.40)	19.5	0.03 (0.08)	0.05 (0.12)
3. Fiji	51	1.05 (0.22)	4.9	0.01 (0.07)	0.02 (0.08)
4. Australia	55	1.02 (0.16)	2.4	0.01 (0.07)	0.01 (0.07)
Wild					
5. Crocodile	50	1.24 (0.77)	12.2	0.03 (0.11)	0.03 (0.12)
6. Ohrigstad	50	1.17 (0.54)	12.2	0.05 (0.15)	0.05 (0.15)
7. Sabie	50	1.24 (0.62)	17.1	0.07 (0.16)	0.07 (0.16)
8. Arabie	50	1.29 (1.00)	14.6	0.05 (0.14)	0.04 (0.14)
9. Klaseri	50	1.24 (0.73)	14.6	0.04 (0.10)	0.04 (0.14)

Notes:
1. *50 allozyme loci were screened in each population.*
2. *The Singapore population shows evidence of hybrid introgression from* O.niloticus *genes*.

Table 5a. Distribution of 26 mtDNA haplotypes in wild and cultured *O. mossambicus* stocks.

Haplotype																											
Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Feral stocks	All																									
Bangula	3											28	2	1	1	1	1	1		2			2	2		
Elephant	1										21						1		1		1	1	1	1		
Crocodile	41	6				3																				
Ohrigstad			42	2					2																	
Sabie				50	6																					
Arabie		5	16			23		1	5																	
Klaseri		3	13		2	29		2	1																	
Rufanes							30																			

Table 5b.

Population	Total haplotypes	N	Haplotype diversity (+/- SD)	Nucleotide diversity (+/- SD)
Feral stocks	1	163	0	0
Bangula	12	45	0.6111 (0.0846)	0.0059 (0.0037)
Elephant	7	50	0.4418 (0.1159)	0.0031 (0.0023)
Crocodile	3	50	0.3159 (0.0079)	0.0079 (0.0046)
Ohrigstad	3	50	0.2841 (0.0771)	0.0148 (0.0080)
Sabie	1	50	0	0
Arabie	5	50	0.6792 (0.0421)	0.0123 (0.0068)
Klaseri	6	50	0.6008 (0.0604)	0.0198 (0.0104)
Rufanes	1	30	0	0

Note: Feral stocks were sampled from Australia, Fiji, Malaysia and Singapore.

O. mossambicus outside Africa probably do not represent the real genetic diversity (and possibly culture potential) that exists in this species. Since some stocks of *O. mossambicus* are known to possess greater tolerance of raised salinity and colder temperatures compared to most cultured *O. niloticus* stocks, re-examining wild *O. mossambicus* stocks as a resource for developing new culture lines particularly in conditions unfavorable to *O. niloticus* may prove beneficial.

Freshwater Crayfish Genetics

A variety of crustaceans are farmed throughout the world with most interest paid to the culture of Penaeid shrimp species. The culture of freshwater crayfish, however, has expanded greatly in recent years in many parts of the world. Australia has a relatively large number of endemic species of freshwater crayfish. Three species belonging to the genus *Cherax* have attracted interest as culture species, i.e., *C. quadricarinatus,* redclaw; *C. destructor,* yabby; and *C. tenuimanus,* marron.

Culture of the tropical redclaw crayfish which has a natural distribution encompassing many northern and western flowing rivers and streams in northern and northeastern Australia began in the early 1980s and has expanded rapidly in Queensland and the Northern Territory. More recently, the culture potential of this species has been recognized more widely, and viable culture industries have been established outside Australia, notably in China, Ecuador and the USA. Production of redclaw in China currently exceeds the total production of the species in Australia.

Development of the industry has not been highly organized, and little attention has been paid to the genetic characteristics of culture stocks. Most producers have obtained their broodstocks from other growers, determined simply by juvenile availability. Virtually all cultured stocks in Queensland are believed to have originated from limited collections of relatively small numbers of wild individuals from only a few river systems in the southern part of Cape York, Queensland. Thus, genetic diversity was probably very

limited in the original culture stocks and has been further compromised by the imposed bottlenecks (due to small sample sizes) during subsequent translocations among culturists. This history of the development of culture stocks suggests that genetic diversity is likely to be generally low and some stocks may even be inbred. Redclaw farmers outside Australia are even more limited in their ability to maximize genetic diversity in their culture stocks as they are unable to access new genetic material other than that available through existing breeders in Australia. The poor genetic status of redclaw culture stocks encouraged QUT to begin research on the applied genetics of this species.

In 1993, QUT commenced a project which sought to describe and quantify genetic diversity in both wild and cultured redclaw stocks with the long-term objective of using the information gained to help develop better culture stocks. Initially, the focus was on developing genetic markers for redclaw and describing levels of variation and the extent of genetic diversity present in both wild and cultured stocks. Following this, comparison of juvenile growth trials commenced under controlled environmental conditions where the relative juvenile growth performance of different wild stocks and their interstock "hybrids" were being examined (Gu et al. 1995). To this end, allozyme, RAPD, mtDNA and microsatellite markers were developed for redclaw and used to quantify genetic diversity in the species (Macaranas et al. 1995). Unlike the tilapia studies, where significant work had been carried out elsewhere which could be used directly in QUT's project, virtually nothing was known of genetic diversity in redclaw before the studies commenced. During this period, collaboration was developed with the Queensland Department of Primary Industry (QDPI) which was trialing artificial selection methods to improve the growth rate of cultured redclaw stocks. Like QUT, QDPI had recognized that existing cultured stocks were of unknown status, had probably originated from limited collections and were probably of poor genetic quality. QDPI, therefore, established base population by collecting new wild genetic material from a small number of river systems from the southern Cape York region of Queensland to establish starting gene pools. Over three generations of mass selection, QDPI has improved the growth rate of cultured stock by 20 to 30% without a noticeable loss of genetic diversity or significantly changing gene frequencies at marker alleles. This result demonstrates that cultured redclaw stocks will respond to practical attempts to improve their culture performance.

In marker studies undertaken in parallel to QDPI selection trials, QUT has examined genetic diversity across much of the natural range of species in Australia and Papua New Guinea. Phylogenetic comparisons utilizing mtDNA cytochrome oxidase and 16S RNA markers indicate extensive stock differentiation across the species' natural range, and even the possibility of cryptic species. Results of analyses of variation at seven microsatellite loci (fast-evolving sequences) go further and indicate that gene flow even among adjacent river systems has been limited historically (Table 6). Limited natural gene flow has produced extensive genetic differentiation both locally and regionally, and to date little of this variation has been exploited in cultured stocks. QUT's laboratory studies of juvenile growth performance have also indicated that different wild stocks grow differently under identical culture conditions. Juvenile mortality can be high and big differences are often observed in growth rates among individuals even within families and among stocks. Both faster-growing and late-maturing stocks have been identified in these studies, and the differences appear to be heritable traits that are not affected greatly by the culture environment. Currently, QUT

Table 6. Microsatellite diversity in four wild and two cultured stocks of redclaw crayfish.

Population		Microsatellite locus				
		CA.27	CA.29	CA.15	CA.28	CA.4
Flinders R.	a	3	2	2	3	5
	h	0.180	0.03	0	0.21	0.70
	P(HW)	0.700	0.00	0	0.50	0.00
Gilbert R.	a	5	5	6	6	9
	h	0.260	0.66	0.660	0.53	0.80
	P(HW)	0.002	0.97	0.069	0.15	0.07
Mitchell R.	a	3	8	11	4	6
	h	0.260	0.56	0.900	0.10	0.33
	P(HW)	0.890	0.00	0.000	0.00	0.00
Weipa	a	4	2	5	4	9
	h	0.540	0	0.700	0.41	0.70
	P(HW)	0.260	0	0.170	0.48	0.66
Park Ridge	a	7	3	6	9	9
	h	0.550	0.20	0.440	0.60	0.61
	P(HW)	0.030	0.16	0.540	0.21	0.06
Yandina	a	7	9	6	10	14
	h	0.460	0.32	0.220	0.58	0.74
	P(HW)	0.000	0.01	0.000	0.00	0.00
Total no. of alleles		10	11	16	15	22

Notes: a - number of alleles; h - heterozygosity; P(HW)- probability of conforming to Hardy-Weinberg.

is comparing relative juvenile growth rates in three genetically divergent stocks and their reciprocal interstock hybrids. The three stocks were collected from three geographically most disjunct wild streams where redclaw occur naturally in Australia. This experiment is assessing the potential that hybrids may show for expressing hybrid vigor or even outbreeding depression. A recent study conducted on the related species, *C. destructor* (yabby), which used a similar approach, identified some crosses which yielded all-male progeny. This may prove to be a significant advantage to the yabby culture industry because male freshwater yabbies commonly grow faster and larger than do females.

Future Directions

QUT's collaborative projects with the Fiji Fisheries Division on tilapia and with QDPI on redclaw genetic improvement have approximately two years to run. Genetic marker studies on both species are well advanced, and progress has been made towards developing faster-growing lines for both Fijian tilapia and Australian redclaw farmers. This year, QUT is commencing a project on strain/stock evaluations on freshwater prawns (*Macrobrachium australiense*) which will utilize similar approaches to those outlined above. It is also currently exploring the possibility of applying the genetic markers developed for tilapia more widely in countries where tilapia culture is practiced through developing collaboration with appropriate agencies. It is a costly and technologically demanding process to develop and utilize marker technology to assist stock improvement so it is important to use it as widely as possible once available. Existing markers and expertise can be applied directly to cultured stocks of *O. niloticus*, *O. mossambicus* and their interspecies hybrids basically anywhere genetic improvement or stock management practices are being trialed for tilapias. It is therefore important in the future to identify appropriate collaborations between organizations to maximize the technologies already developed to improve tilapia (and crayfish) culture. This will reduce the costs of research programs designed to produce better stock, stop unnecessary duplication and help promote aquaculture research in developing nations.

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GENETICS RESEARCH AT THE SOUTHEAST ASIAN FISHERIES DEVELOPMENT CENTER/ AQUACULTURE DEPARTMENT

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ABSTRACT

Southeast Asia is endowed with a variety of species that can be cultured as food fish. Since 1973, the Southeast Asian Fisheries Development Center/Aquaculture Department (SEAFDEC/AQD) has devoted research efforts to establish broodstock for the tiger prawn (*Penaeus monodon*), milkfish (*Chanos chanos*), and other economically important fish and crustaceans in the region. The objective is to ensure the availability and reliability of seed supply for fish culture.

SEAFDEC's pioneering effort on genetics was a collaborative study with the University of the Philippines Marine Science Institute on the genetic variation of milkfish populations in the Philippines. Towards the end of the 1980s, genetic activities, co-funded by the International Development Research Centre (IDRC) of Canada in collaboration with Dalhousie University of Canada, were focused on tilapia.

Introduction

Compared to agriculture and livestock, aquaculture is still behind in the management of genetic resources. Majority of the genetic resources of aquatic animals is still found in wild populations as very little domestication has taken place. Exceptions include strains and varieties of the common carp, rainbow trout and, during the last decade, tilapia.

Genetic variation in milkfish populations

Chanos chanos is one of the most important and exploited marine resources in the Philippines. Twelve populations analyzed for eletrophoretic variation showed significant variations (Macaranas et al. 1990). There are at least two subpopulations in the Philippine waters.

Development of salinity-tolerant strain of Nile tilapia hybrid

The objective of the project implemented at the SEAFDEC/AQD Tigbauan Station was to produce a salinity-tolerant strain of *Oreochromis niloticus* that can spawn and grow in regions with inadequate supplies of freshwater. The procedure involved hybridization of *O. niloticus* with *O. mossambicus* followed by repeated backcrossing of the hybrid with *O. niloticus*.

The mean survival time (MST) and median survival time (ST_{50}), of freshwater-spawned and reared *O. mossambicus* and *O. mossambicus* and *O. niloticus* hybrids showed higher salinity tolerance than *O. niloticus* (Villlegas 1990a). Increased salinity tolerance with age or body size was also evident in the study. The highest growth for *O. mossambicus* was at 15 and 32 ppt while the optimum salinity range for growth of *O. niloticus* was 0-10 ppt (Villegas 1990 b).

Experimental and statistical strain comparison procedures

One major objective of any fish breeding program is to develop genetically improved strains. Since genetic improvement programs are expensive, it is essential that proper strain testing methodologies that can detect an economically important diference between two strains be developed. In aquaculture decisionmaking, the magnitude of difference that the researcher wants to detect can be decided a priori on purely economic grounds. Economic theory or results of previous strain-comparison studies can help indicate the magnitude of differences that would be important between the growth rates of fish. Comparison experiments at SEAFDEC/AQD involve rigorous size grading (collimation), use of full-sib groups and inclusion of internal reference fish in each replicate tank/cage for statistical control.

The usual strain comparison procedure involves analysis of variance (ANOVA). The internal reference procedure uses analysis of covariance (ANCOVA) where growth of the internal reference fish (red tilapia) is the concomitant variable to reduce error variance caused by environmental variation like population density and food supply (Basiao and Doyle 1990a).

Strain comparison in diverse environments

The efficiency of using an internal reference population in detecting strain differences has been demonstrated in high-input, well-managed environments as well as low-input marginal or artisanal conditions. Tilapia strains showed significant genotype effects during a three-week temporary crowding period (Basiao and Doyle 1990a, 1990b) and at the end of the final grow-out period in hapa cages (Basiao et al. 1996). Inclusion of an internal reference strain increased the sensitivity of the statistical test to detect a 7% significant difference among strains. Approximately 450 replicate families would have been needed to detect the significant strain differences if the reference strain had not been included in the experimental design.

Genetic variation in growth was found in *O. niloticus* strains held in freshwater for 2 weeks, acclimated for 4 days in a salinity of 32 ppt and reared in 32 ppt seawater for 2 weeks and then reared again in freshwater for another 2 weeks (Basiao, unpubl.).

Growth and survival of five Asian red tilapia strains were compared in brackish and seawater. Statistical analyses on mean specific growth rate showed that a Philippine strain grew best in seawater while a Thailand strain performed well in brackishwater (Romana-Eguia and Eguia 1999).

Genotype x environment interaction

Genotype x environment interactions (GXE) or the differential response of specific genotypes under different environmental conditions (Falconer 1989) has important implications for selection, strain testing and aquaculture in general. Romana-Eguia and Doyle (1992) using an internal reference fish found indications of GXE interactions among three *O. niloticus* strains reared in low (rice bran) or high (commercial diet) quality feeds. These strains also differ in their growth response under restricted or nonrestricted feeding regime (Romana-Eguia and Eguia 1993). However, ranking of the strains is similar in the two feeding regimes.

Resistance to heavy metal stress

Small fingerlings of two *O. niloticus* strains showed significant strain differences in terms of heavy metal tolerance when exposed to mixtures of zinc, cadmium and mercury (Cuvin-Aralar 1993). This difference was diminished, however, in older and bigger fish. The more resistant members of the population (survivors), which have the ability to adapt to the heavy metal toxicants were able to pass on the resistance to their offspring (Cuvin-Aralar and Aralar 1994).

Selective Breeding and Broodstock Management of Tilapias

Collimated mass selection

An important objective of aquaculture genetics is to provide improved fish that will benefit the private sector and also the small fish farmers. However, genetics research is an expensive endeavor for most developing countries and most results do not benefit marginalized fish farmers. The conventional mass selection on tilapia, which has not shown positive results in early studies, has been modified by the collimation procedure or the early culling of large fry before a two-step directional selection is applied (Basiao and Doyle 1999). The collimation procedure reduces the self-amplifying phenotypic variance in growth and increases the genetic to phenotypic variance at the time of selection. One generation of collimated mass selection resulted in a significant positive response of 3% relative to the control. The realized heritability (h^2) of approximately 16% is comparable with other recent estimates of h^2 in aquaculture populations of tilapia.

Farm-based broodstock improvement

The collimated mass selection procedure developed at SEAFDEC/AQD was pilot- tested in a small hatchery farm in Laguna, Philippines. The objective of this farmer participatory research is to help farmers develop their own tilapia broodstock. The farm trial showed a 7-9% response to selection after one generation of selection (SEAFDEC/AQD 1999). An important implication of a farmer-based broodstock selection is that fish farmers would have more control over their choice of good quality spawners. Dependence on a franchise-dealer type of seed production will be minimized and socioeconomically self-sustaining genetic conservation will be achieved.

Development of high-yield red tilapia strain

The genetic improvement of a Philippine red tilapia strain through introgressive hybridization with the Chitralada strain of *O. niloticus* from Thailand and subsequent application of size-specific mass selection is in progress. One generation of introgression resulted in a significant positive response of 2.5% as measured by the percent differences in the lengths of introgressed red-orange offspring from the normal red tilapia (SEAFDEC/AQD 1999). As an accompanying study, a 2 x 2 factorial experiment to determine the reproductive efficiency of the introgressed red tilapia and the normal red tilapia fed with two formulated diets was done. The frequency of spawning and seed (fry and egg) production were highest in the normal red breeders fed a commercially formulated diet. The introgressed red and normal red tilapia fed the SEAFDEC-formulated feed showed comparable number of spawning. However, fry production was higher in the normal breeders, while egg production was higher in the introgressed red tilapia. The lowest spawning frequency and seed production were observed in the introgressed red breeders given the commercially formulated feed.

The reproductive efficiency of three genetically diverse Philippine red tilapia strains (BFS, FAC and PF) and one Thai red tilapia strain (NIFI) in two seed production systems were compared with the objective of improving red tilapia fry production (Romana-Eguia and Eguia 1999). Seed production in all strains was considerably higher in tanks than in cages. The mean daily seed production in tanks was highest for FAC, followed by NIFI, BFS and PF. In fine-meshed net cages, mean daily seed produced per female was highest for BFS, followed by NIFI, FAC and PF.

Genetic characterization of hatchery-bred Nile tilapia

Genetic variation is the basic resource of any successful artificial selective breeding program. Hence, the level of genetic variation should be monitored in hatchery stocks for their efficient management. This concern is addressed by SEAFDEC/AQD by monitoring the genetic variation of the hatchery-bred tilapia maintained at the Binangonan Freshwater Station using protein and enzyme analysis. Sixteen enzymes and 24 loci were screened in three generations of hatchery-bred *O. niloticus*. The six polymorphic loci detected in the base population were also found in the F_1 and F_2 generations (SEAFDEC/AQD 1999).

Genetic Diversity of Wild and Cultured Black Tiger Shrimp (*Penaeus monodon*) in the Philippines

The genetic variability of wild *P. monodon* collected from four geographical regions (Capiz, Negros Occidental, Palawan and Quezon) in the Philippines was compared with cultured samples collected from Antique and Negros Occidental, Philippines. Fifteen out of the 18 commercially available primers were selected and used to produce randomly amplified polymorphic DNAs.

Initial results showed that the wild samples are more polymorphic (66-71%) than the cultured samples (54%). Wild samples from Palawan showed the highest polymorphism (71%).

Six microsatellites were used to establish possible correlation between genetic diversity among wild *P. monodon* populations and status of mangroves and shrimp culture systems in the Philippines. Two cultured populations were examined as controls. Results indicated that all six microsatellite loci were polymorphic and observed heterozygosity ranged from 46.6% to 100%. Genetic differences between populations, based on genotypic and allelic frequencies, exhibited some degree of association with the status of the mangroves and culture system near the site where the samples were collected. The Negros Occidental population, with the most severe mangrove loss and the most intensive culture systems, was the most significantly differentiated population. It also showed the lowest average number of alleles per locus, suggesting a decreased diversity in the population. The second most differentiated population was the population from Capiz, a province with wide area of extensive culture ponds and few remaining secondary mangroves. The Quezon and the Palawan populations were not genetically different from each other. The two cultured populations showed less genetic diversity and were significantly different from the four wild populations. The potential risk of genetic contamination by the accidental or intentional release of cultured populations of shrimp into the wild should be addressed in the light of these results.

Future Activities

Refinements of on-station and farmer-based research on selective breeding of tilapia will be pursued. Genetic improvement of the bighead carp (*Aristichthys nobilis)* introduced in the Philippines will be initiated. Genetic characterization of hatchery-bred fish and crustaceans and the population genetic structure of indigenous freshwater fishes will be done. These future research thrusts of SEAFDEC/AQD will be done in collaboration with other research institutions and universities in the Philippines.

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OVERVIEW OF AQUACULTURE GENETICS RESEARCH IN THE INSTITUTE OF AQUACULTURE, UNIVERSITY OF STIRLING

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ABSTRACT

The Genetics and Reproduction research group is one of the largest within the Institute of Aquaculture (IOA) at the University of Stirling. The institute works with a broad range of species from diverse geographical areas and habitats and has benefited from collaborations with other institutions in many countries. This overview of activities focuses particularly on areas of relevance to the International Network on Genetics in Aquaculture (INGA) and collaborations with INGA members.

Aquaculture Genetics Research

Molecular taxonomy, population and conservation genetics

Allozyme, mtDNA, ribosomal DNA, minisatellite and microsatellite DNA variation have been used in Institute of Aquaculture (IOA) laboratories in studies on the taxonomy and population genetics of wild organisms of interest to aquaculture, and captive aquaculture stocks. Examples of these studies are molecular taxonomy and population genetics of tilapias (Sodsuk et al. 1995; Rognon et al. 1996); population genetics of *Penaeus monodon* (Klinbunga et al. 1996, 1998a, 1998b, 1999), *Macrobrachium nipponense* (Wong and McAndrew 1994), *Dicentrarchus labrax* (Castilho and McAndrew 1998; Martinez Rodriguez et al. 1998), *Salmo salar* (Taggart et al. 1995a, 1995b; Prodoehl et al. 1997; Stone et al. 1997) and *Catla catla*.

IOA's interest in conservation genetics has focused on the use of cryopreservation and on the potential of androgenesis in gene banking, with tilapia and salmonids as the main experimental subjects (McAndrew et al. 1993; Myers et al. 1995a, 1995b; Rana 1995; Penman et al. 1997). The combination of sperm cryopreservation and diploid androgenesis does allow recovery of viable animals from a sperm gene bank, but successful cryopreservation of eggs is still not possible.

Broodstock management and quantitative genetics

IOA has been involved in collaboration with the University of Agricultural Sciences (UAS), Bangalore, India, and the University of Wales Swansea on broodstock management and selective breeding of *C. catla* and *Cyprinus carpio* in Karnataka, India (Basavaraju et al. 1998). In UK, IOA is involved in

developing broodstock management and selective breeding programs on molecular markers (principally microsatellites), in Atlantic salmon and other cold-water species.

Sex determination and differentiation

Research on sex determination mechanisms and their manipulation to produce, e.g., monosex stocks, has been a major interest for IOA for some time. Tilapia, principally *Oreochromis niloticus*, has been the major model, due to its commercial importance, ease of maintenance under laboratory conditions, amenability to genetic and hormonal manipulations, and the emergence of temperature sex determination (TSD) and autosomal genetic factors affecting sex determination in addition to the major (chromosomal) sex determination.

Recently, the chromosomal location of the sex determining region in *O. niloticus* has been demonstrated (Carrasco et al. 1999a). Fully inbred clonal XX and YY lines in this species have been produced (Sarder et al. in press). Studies now focus on molecular and cytogenetic analysis of the sex determination system and on the role of aromatase in sex differentiation and TSD.

Collaborative research with the National Aquaculture Genetics Research Institute, (NAGRI) Thailand and the Bangladesh Fisheries Research Institute has led to the development of genetically monosex female production in the silver barb (*Barbodes gonionotus*) for aquaculture (Pongthana et al. 1995a, 1999).

Genetic manipulations

Gynogenesis (Hussain et al. 1993, 1995; Myers et al. 1995; Pongthana et al. 1995b) and androgenesis (Myers et al. 1995a, 1995b) have proven to be useful tools in the study of sex determination and the development of monosex culture, the development of clonal lines (Hussain et al. 1998; Sarder et al. in press) and gene mapping, etc.

These tilapia clonal lines have already proved to be extremely useful in studies on nonspecific and specific immune responses, and the institute is planning to use them in research on areas as diverse as nutrition and quantitative genetics.

Studies have also been carried out on induced triploidy in tilapia (Hussain et al. 1995, 1996), rainbow trout (Carrasco et al. 1999a, 1999b) and halibut in the last few years. Although triploidy could not currently be used commercially in tilapia due to the difficulties of collecting adequate numbers of unfertilized eggs, studies elsewhere have shown its potential in limiting reproduction in pond culture. Triploidy is, however, used commercially in rainbow trout. A recent Ph.D. study at IOA (Carrasco et al. 1998) revealed differences in meiotic chromosome pairing between genetic male and female triploids which may account for the differences in the success of maturation of male and female triploids (male triploids are generally capable of producing some aneuploid sperm whereas female triploids are generally sterile).

IOA carried out a policy study on genetically modified fish for the UK Department of the Environment (Woodwark et al. 1994; Penman et al. 1995), which led to drafting guidelines for the UK government.

Genomics

Gynogenesis has proven to be a useful tool in gene mapping, in addition to the more conventional crosses. IOA initially studied gene-centromere recombination distances for allozymes, color loci, etc (Hussain et al. 1994). More recently, highly polymorphic DNA markers, such as microsatellites and AFLPs or RFLPs have been developed and, in collaboration with Dr. Tom Kocher at the University of New Hampshire, a linkage map has been developed using haploid gynogenetic embryos (Kocher et al. 1998) and gene-centromere linkages are being estimated for a wider variety of loci (Markert et al. 1999). IOA has a particular interest in mapping sex determining loci in tilapias and is pursuing a variety of strategies for this.

The genomics capabilities at IOA have been strengthened in the last two years by a new initiative known as “Aquagene”. Prof. Alan Teale and his group come with a background of mammalian genetics, with a past focus on mapping disease resistance genes in cattle and mice, while Dr. John Taggart, Dr. Margaret Cairney and Dr. Mike Leaver are involved in mapping salmonid genomes and identifying ESTs in these species.

IOA is currently developing strategies for the detailed analysis of the genetic basis of a number of traits related to disease resistance, growth performance, nutrition and control of reproduction.

Collaborative Projects with INGA Member Countries

Aquaculture Development and Coordination Program

The Aquaculture Development and Coordination Program (AADCP) was implemented from 1990 to 1994, with funding from the European Union (EU) and the Association of Southeast Asian Nations (ASEAN). Five components in different ASEAN countries were twinned with partner institutions from EU countries (Penman 1995). In component 5 of AADCP (aquaculture genetics), NAGRI in Thailand was twinned with IOA. The two main areas of research were genetic manipulations and population genetics.

DFID Fish Genetics Research Programme

The IOA genetics group has been involved in several collaborative projects with INGA member countries through projects funded by the Department of International Development (DFID, formerly Overseas Development Agency) Fish Genetics Research Programme (see Mair and Beardmore, this vol., for more details). The main projects under this program are on population genetics of *Penaeus* sp. (with University of Putra, Malaysia); monosex culture of *B. gonionotus* (with NAGRI, Thailand, and the Bangladesh Fisheries Research Institute); and aquaculture genetics of Indian and common carps (with UAS, Bangalore, India, and University of Wales, Swansea, UK).

Ph.D. studentships, visiting scientists and others

The IOA genetics group has been fortunate to have had several Ph.D. students from INGA countries in the research group over the last decade. The students came from Thailand, Bangladesh and Malaysia and were funded through international programs (e.g., AADCP) and UK or local government, etc. There were also visiting scientists from a number of countries, including India and Thailand, under various schemes. Every year, there are M.S. Aquaculture students carrying out their research projects in IOA.

IOA hopes to obtain funding for new projects in the near future, and welcomes new collaborations through INGA.

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RESEARCH ON THE GENETICS OF AQUATIC ORGANISMS AT THE SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF WALES SWANSEA

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ABSTRACT

The School of Biological Sciences, University of Wales Swansea (UWS), has for many years, worked on a range of topics relating to the genetics of aquatic species, from fundamental studies on population structure, speciation and molecular evolution to very applied projects for developing better fish for aquaculture.

The university is contracted by the Department of International Development (DFID), U.K. to manage the DFID Research Programme in Fish Genetics which aims to harness the wide range of genetic techniques now available to provide productive inputs to aquaculture and enhancement fisheries in developing countries targeted at the alleviation of poverty and improvement of livelihood among rural poor. While tilapias and carps, being low down in the food chain and appropriate for low-input aquaculture, are the species of most direct interest, recent projects have involved kapenta (*Limnothrissa miodon*), shrimps and groupers.

Currently, five projects are running within the programme. These are focused on: (i) improved tilapia through transgenesis (Southampton and UWS); (ii) improved production in India of common carp (*Cyprinus carpio*) and Catla (*Catla catla*) (Bangalore, Stirling and UWS); (iii) Socioeconomic factors in uptake of new technology (Centre for Development Studies, UWS); (iv) development of genetic technology of monosex production in *Oreochromis mossambicus* for South Africa (Stellenbosch University and UWS); and (v) development and extension of the YY male technology in *O. niloticus* in Southeast Asia (Asian Institute of Technology, Thailand; Central Luzon State University, Philippines; and UWS).

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Introduction

The School of Biological Sciences (SBS), University of Wales Swansea (SWS) has, for many years, worked on a wide range of topics on the genetics of aquatic species, from fundamental studies on population structure, speciation and molecular evolution to very applied projects developing better fish for aquaculture. Although members of the school have worked on a diverse range of species, the major species of commercial interest on which research has been conducted include tilapias, carps, salmonids, bivalve molluscs and *Artemia*. This paper reports progress in some of the more recent research conducted in Swansea in collaboration with associates throughout the world. The major emphasis of recent work has been conducted under the auspices of the British Government Department for International Development's Fish Genetics Research Programme (DFID/FGRP) which has been managed by UWS since its inception in 1990.

DFID Fish Genetics Programme

The overall objective of this programme is to harness the wide range of genetic techniques now available to provide productive inputs to aquaculture and enhancement fisheries in developing countries.

The ultimate aim is to benefit livelihoods of poor people and to aid in the alleviation of poverty through improvement of production in enhanced and maintained populations of fish and shellfish, especially by small farmers. The programme has a dominant theme of sustainability in the widest sense (including environmental impacts of all kinds), paying full regard to the provisions of the Convention on Biological Diversity on access to genetic resources. In practical terms, research uses the wide range of analytical, manipulative and breeding techniques available in genetics to generate better strains of fish. This work fulfils the programme's purpose in aquaculture and enhancement fisheries of improved performance in productivity giving benefits of increased income, food security and food costs. While any approach to improved production carries a risk to sustainability, genetic approaches are intrinsically more likely to support sustainable development than approaches based on increased levels of inputs which frequently have environmentally undesirable effects.

While the global yield from captive fisheries is static, at best, the world output from aquaculture which already forms about 20% of shellfish and finfish production, is increasing at about 10% per annum. In populous developing countries, such as India, China and the Philippines, the yield from aquaculture forms 30-60% of total aquatic production. Aquaculture, thus, has an increasingly important role in world nutrition especially in developing countries.

The range of researchable constraints to which genetics can supply solutions is considerable. Major practical improvements in the diversion of energy from reproduction to growth through control of reproduction, greater resistance to disease and extending tolerance to environmental constraints like temperature, in which genetic factors play a significant role, are to be expected. In general, solutions generate more and better fish for the same inputs through increased efficiency and thus do not impose additional burden on scarce resources (see Table 1). In applying genetics-based technologies to greater efficiency of

Table 1. Examples of targets for improved products in aquaculture and how these can be addressed using genetic techniques.

Target for increased Production	Solution using genetic techniques	Examples in relevant species
Removal of problems associated with early sexual maturity and unwanted reproduction	Production of monosex by developing "supermales" siring only male offspring or "neomales" siring only female offspring or sterile triploids	Tilapia, silver barb
Increased growth rate	Transgenics of growth hormone gene; genetic characterization and breeding programs	Tilapia, salmon, carp
Expanded geographic utilization without environmental risks to ecosystems	Transgenics which are sterile or are inviable in nature; production of triploids	Tilapia (especially for Africa)
Increased environmental tolerances such as cold or salinity	Selective breeding and/or crossbreeding/hybridization	Tilapia hybrids
Improved quality of stock	Development of good broodstock and user-friendly maintenance protocol for this	All species, especially carp
Improved disease resistance	Selective breeding; transgenics	Shrimp

production in the low-input aquaculture systems characteristic of developing countries, the needs of poor families seeking to develop better livelihoods are being addressed in a direct and useful way. The greater efficiency of improved strains should lead, ultimately, to significantly cheaper food for both rural and urban poor.

The scale of benefit to be expected from applications of genetic technologies is very large. The evidence from the many species of cultured crop plants and domesticated farm animals, which are genetically improved, is compelling. Gains in productivity arising from application of techniques such as selective breeding frequently exceed 100%. Even in salmon, the only aquatic species in which systematic long-term breeding has been carried out, the gains in productivity already amount to more than 40%.

While tilapias and carps are the species of most direct interest within the programme, as they feed low down in the food chain and are best adapted to low-input culture or enhancement systems, recent projects have involved species such as kapenta, shrimp and groupers.

In addition to the use of genetic technology, an important need for radically improved and rational genetic management of broodstock in aquaculture is becoming increasingly evident in many areas. Not infrequently, in some societies, traditional custom may act against best genetic practice by, for example, the choice of the smallest individuals in a harvest to be the parents of the next generation because these have least value at market. Thus, establishing protocols for the proper genetic management of broodstock is a factor of considerable importance in both aquaculture and enhancement fisheries. The selection of appropriate techniques chosen from the now formidable array of methodologies available requires that the end product be environmentally neutral (as in use of sterile strains). However, the advent of improved strains takes place within communities and ecosystems. It follows that suitable concurrent studies need to accompany the genetic technology to ensure that environmentally deleterious effects do not ensue. Furthermore, it must be assured that acceptance of such techniques is assisted by good understanding of socioeconomic structures, that the benefits of genetically improved strains are available to support livelihoods of families with few resources and that in general terms the objectives of UK International Development Aid are met in identifying appropriate target beneficiaries. The availability of water is obviously a central need but even poor families can often share in improved aquaculture production, and benefits can accrue to nonproducers through provision of employment and increasing the availability of cheap fish protein.

The dissemination of programme products is assisted by income-generating activities of a UWS company Fishgen Ltd. and its sister organization at the Philippines' Central Luzon State University (CLSU), Phil-Fishgen.

Genetic Projects with UWS Involvement

Table 2 lists the current and recent projects conducted under the DFID/FGRP.

Sex determination and the YY male technology in the Nile tilapia

This project arose from early research work in Swansea on the genetics of the inheritance of sex in tilapia. This work has developed toward the current consensus that, in the commercially important *Oreochromis* species, sex determination is "predominantly" monofactorial, being controlled by sex chromosomes or primary sex determining gene(s). Research using sex reversal, progeny testing and chromosome set manipulation has revealed two alternative "sex chromosome" models. In *O. niloticus*, the female is homogametic XX, the male being heterogametic XY (Mair et al. 1991a) while in the closely related *O. aureus*, the alternative model of heterogametic WZ females and homogametic ZZ males applies (Mair et al. 1991b). There is also substantial evidence for effects of one or more autosomal genes on sex ratio together with an increasingly well-documented effect of temperature in influencing sex. Elevated temperatures (~36ºC) during the period of sex differentiation have been shown to increase the proportion of males in putative monosex female *O. niloticus* and, to a lesser degree, to increase the proportion of females in putative all male progeny (Abucay et al. 1999). Similarly high temperatures have been shown to modify sex ratios *in O. aureus* (Mair et al. 1990). The only recorded effect of low temperatures was to increase the proportion of males in *O. mossambicus* (Mair et al. 1990).

Based on the theory of predominantly monofactorial sex determination, it has proved possible to manipulate sex ratio using a combination of sex reversal and

Table 2. Current and recent projects funded under the DFID Fish Genetics Programme.

Period	Institutions	Project title	Description
1998-2001	University of Stellenbosch (South Africa), UWS	Genetic improvement and utilization of indigenous tilapia in Southern Africa	Genetic characterization and application of the YY male technology to indigenous strains of *O. mossambicus*
1993-1998; 1998-2000	University of Southampton (UK), UWS	Development of sterile high-yielding transgenic tilapia	Work at present confined to UK under strict containment; close liaison with DFID maintained on all aspects of work
1997-2000	Research Institute for Aquaculture #1 (Vietnam); AIT (Thailand); CLSU (Philippines), UWS	Improvement and regional evaluation of the YY male technology	Developing and evaluating regional potential of GMT in the Philippines, Thailand and Vietnam
1997-1999	CLSU (Philippines), UWS	Socioeconomic analysis of dissemination and impact of GMT	Characterizing small-scale aquaculture in the Philippines and evaluating current and potential impact of GMT for alleviation of poverty
1995-1998	NAGRI (Thailand), Fisheries Research Institute (Bangladesh), University of Stirling (UK)	Development of female monosex technology in silver barb	Technology applied and being adopted by farmers in Thailand
1994-2000	University of Agricultural Sciences (India), University of Stirling (UK), UWS	Genetic improvement of Indian and common carps for aquaculture	Emphasis on genetic characterization of catla and the problem of early sexual maturation in local common carp
1992-1995; 1994-1997	NAGRI and AIT (Thailand), CLSU (Philippines), UWS	Two projects on technology adaptation, development and field testing of GMT	Extending the YY male technology developed earlier to other systems and strains and comprehensive on-farm trials
1988-1991; 1991-1994; 1994-1997	CLSU (Philippines), UWS	Three successive projects related to the development and improvement of the YY male technology in tilapia	Successful development and mass production of novel YY males in *O. niloticus* producing fast-growing GMT with mean sex ratios $\geq$ [3] 95% male

progeny testing to identify sex genotypes. Under the auspices of several DFID/FGRP, a major breeding program (combining hormonal feminization and progeny testing) was carried out in *O. niloticus* in which it was found possible to mass-produce novel YY "supermales" (Mair et al. 1997). When crossed to normal females (XX), these YY males have the unique property of siring only male progeny. These progeny are termed genetically male tilapia (GMT) and are normal (XY) genetic males (although some can "naturally" revert to female, giving GMT an average sex ratio of >95% male). The hormone treatments used as part of the process to produce YY males are two generations removed from the fish that are marketed to the consumer so neither the GMT or their YY male parents are hormone-treated in any way. This makes the technology more user and environmentally friendly than the alternative of direct hormonal sex reversal. Furthermore, the technology can be applied in a range of hatchery systems simply by replacing broodfish with YY males although good management is required to prevent contamination of broodstock.

On-station and on-farm trials indicated substantial increases in production (40% increases in yields) using GMT compared to normal mixed sex tilapia (Mair et al. 1995). The foci of current research work on the technology itself are to: (i) improve the understanding of and ability to manipulate feminization processes in tilapia using safer methods, ideally with alternative estrogens to the toxic DES currently used; (ii) improve the understanding of the role of temperature in sex differentiation; (iii) improve the understanding of the role of genetic variance in growth and sex ratio of GMT

including relative performance of intra- and interstrain crosses, G x E interactions and response to selection; (iv) assess the relative performance of GMT and their parental stock in Thai and Vietnamese aquaculture systems; and (v) investigate the potential for producing early season GMT using new species or hybrids in Vietnam.

The outputs of the research work on the development of the YY male technology are now being widely disseminated in the Philippines and Thailand through the distribution of YY male and normal female broodstock to accredited hatcheries. This process is being conducted through financially self-sustaining mechanisms coordinated by UWS and CLSU in the Philippines and the National Aquaculture Genetics Research Institute (NAGRI) of the Department of Fisheries in Thailand. The impact of GMT in Southeast Asia is likely to be considerable. For example, NAGRI estimates indicate that approximately 25% of the tilapia harvested in Thailand in 1999 are GMT. The gains from additional income and dietary protein are not precisely quantified but are considerable. Issues related to the social and economic impact of the introduction of GMT in the Philippines have been addressed in another DFID project.

Social and economic impact assessment of GMT

This project (1997-1999) was carried out as collaboration between the Freshwater Aquaculture Center (FAC) of CLSU and School of Biological Sciences and the Centre for Development Studies of UWS. The overall objective of the project was to improve equitable uptake of the products of YY male technology by all sectors of the tilapia culture industry in the Philippines. There were four major activities:

- Characterization of small-scale aquaculture in the Philippines;
- Assessment of the impact of three genetics-based technologies - GMT; genetically improved farm tilapia (GIFT) and sex-reversed tilapia (SRT) - in hatcheries, and especially, grow-out farms;
- Study of interrelationships between different sectors of the aquaculture industry; and
- Conduct trials of GMT among currently nonbenefiting sectors of the industry.

The project concluded that the current "passive" dissemination process is achieving its original objectives of dissemination of research outputs (namely, improved fish) and financial sustainability of research, development and dissemination. However, it was evident that hatcheries which are benefiting are relatively large-scale, semi-intensive and has commercial operations, and that small-scale hatcheries were effectively excluded from adopting the technology on the basis of cost and awareness. At a premium of only 12.5% over normal mixed sex fish, GMT was the cheapest of the available "improved" fish. Furthermore, there is a relatively widespread awareness of GMT across all sectors of the industry, to a greater extent than the alternative monosex technology of SRT which has been promoted in the Philippines for 20 years. However, the nature of dissemination and lack of targeted promotion are limiting adoption of GMT to medium-scale tilapia growers in the mainstream of the industry, with full access to its resources.

The results provided valuable pointers with regard to the reformulation of the dissemination program. The results from field trials in which two external organizations participated actively (an NGO and a multilateral donor funded project) indicated that nongovernmental organizations (NGO) and people's organizations (PO) can be potentially valuable partners in dissemination efforts targeted at small-scale producers, e.g., improved relations between FAC-CLSU and other institutions, such as NGO.

Private small-scale hatcheries operating outside fingerling trading networks were also identified as having significant potential to enable small-scale grow-out producers to adopt GMT production. Dissemination through state sector hatcheries appeared inefficient and failed to target small-scale producers. These and other findings have been incorporated in a series of recommendations to guide Phil-Fishgen in its dissemination and research efforts.

According to supplementary research, tilapia has an important role in the diet of poor in inland provinces in Luzon. Enhanced production of tilapia can therefore contribute to better food security among the poor of such provinces, as well as protect their entitlement to nonmarket food fish.

Overall, the results from this project suggest that small-scale farmers benefit from adoption of GMT where dissemination is accurately targeted and coordinated with agencies that can provide credit, training and extension support. These efforts can act as catalyst to attract new entrants to aquaculture and to provide extension material. Also indicated is that enhanced

tilapia production associated with the adoption of GMT by small, medium and large-scale producers can help the rural poor by increasing supply of food fish critical to their food.

Genetic improvement of *O. mossambicus* in Southern Africa

Having begun in early 1999, this project is very much in its formative stages. The overall development objective is the enhancement of livelihood of rural poor in Southern Africa through: (i) improved production of fish from existing aquaculture facilities utilizing enhanced varieties of indigenous fish species; (ii) sustainable use of existing resources, infrastructure and expertise with the settlement of small-scale farmers as independent fish producers;(iii) building entrepreneurial capacity and providing employment opportunities in an environment of decreasing demand for farm labor; and (iv) increased availability of affordable fish protein through expanded culture of low- input, low-cost species.

The specific research objectives are as follows:

- Develop an assembly of accessions of up to 15 strains of *O. mossambicus* from throughout Southern Africa; conduct growth performance trials of a minimum of eight strains, under communal stocking in two environments, to include farm cages and earthen ponds;
- Genetically characterize available strains of *O. mossambicus* (minimum of eight) using molecular techniques (microsatellite DNA loci, amplified fragment length polymorphisms and/or restriction endonuclease analysis of mitochondrial DNA haplotypes); strains will be characterized for strain specific markers, levels of genetic variability and population structure;
- Adapt YY male technology to strains of *O. mossambicus* chosen on the basis of growth data and species purity; YY males will be generated using two approaches: (a) the standard breeding program combining feminization and progeny testing (Mair et al. 1997) and (b) the more rapid means using androgenesis which may enable production of a few YY males in 1.5 generations (1-1.5 years) compared to 2.5 (1.5-2.5 years) in the standard breeding program;
- Conduct on-farm trials of GMT in both cages and ponds; comparisons will be made with nonimproved, locally available *O. mossambicus* as controls; and
- Determine potential social and economic impacts of the promotion of improved fish through participatory research with farmers and potential consumers.

Production of transgenic tilapia

Transgenesis is one of the most promising technologies for relatively rapid genetic improvements. It involves the introduction, via techniques of genetic engineering, of a DNA sequence into a recipient organism to confer novel phenotypic characteristics on that organism (Beardmore 1997). Typically, the desired genes are first identified, sequenced and then cloned. Multiple copies of the gene are then introduced to the fertilized eggs, commonly by microinjection. At a later stage of development, cells of the organism are tested to determine whether copies of the transgene have become incorporated into the genome and whether this incorporation is in all cells or only in the cells of some tissues (i.e., a mosaic). After incorporation is determined, the organism can be evaluated to determine if the transgene product is being expressed and in what amounts; and then whether the transgene is inherited and expressed in the next generation via the germ cells. Inheritance of the transgene would be required to develop true breeding lines of the transgenic organism. Among the several attempts to develop transgenic lines of tilapia, one of the most successful has been conducted under DFID, in a collaborative project between the University of Southampton and UWS.

The study, which is presently limited to stringently contained laboratory conditions in the UK, involves the introduction of a fish growth hormone gene into *O. niloticus* (Rahman and Maclean 1999). The major part is conducted at Southampton University with the role of UWS in gene mapping for tilapia. Introduction of the transgene by microinjection incorporation was detected. Initial transmission rate from G_0 to G_1 generation was found to be less than 10% in these lines, indicating a mosaic distribution of the transgene in the germ cells. However, transmission rates from the first to the second generations were found to follow the expected Mendelian ratios. The chinook salmon growth hormone was produced in several generations of the transgenic tilapia indicating expression of the gene, resulting in dramatic growth enhancement with the average weight of the transgenic fish being four times that of their nontransgenic siblings.

Transgenesis appears to offer very considerable potential for enhancement of yield in tilapia. However, the rate of genetic change in transgenics is such that phenotypic and behavioral properties cannot easily be predicted and the introduction of fish for commercial aquaculture faces many constraints. The risks to the environment posed by the uncontrolled introduction of transgenic fish need to be adequately assessed, and many governments are adopting cautious policies with regard to their introductions. In addition, consumer response to these genetically modified organisms (GMOs) in some countries may be very negative to the extent that adoption by farmers may involve significant economic risks. A lot of these constraints may be overcome if guaranteed sterile transgenic tilapia can be produced through efficient methods of triploidization or, in the medium to long-term, through disruption of the physiological pathways of reproduction via the introduction of new antisense transgenes. Very thorough and comprehensive field trials will be required prior to general release of such organisms to aquaculture.

Genetic improvement strategies for carp in Karnataka, Southern India

This project is a collaboration among the University of Agricultural Sciences, Bangalore, India, UWS and the Institute of Aquaculture, Stirling University. The principal objectives of the project are to: (i) investigate the present status of the cultured Indian major carp Catla (*Catla catla*) in Karnataka State, India, and design and initiate a program for the genetic improvement of the cultured strains; and (ii) investigate the phenomenon of early sexual maturation and unwanted reproduction during culture of common carp (*Cyprinus carpio*) in Karnataka and develop solutions through genetic or hormonal manipulations.

Work on catla has slow progress due to the long generation time, and short and unpredictable spawning season in Southern India. Early in the project, fry and fingerlings were collected from domesticated stocks in several major hatcheries in Karnataka and from wild stocks from the Rivers Ganges and Brahmaputra. Fin tissue samples were also collected from other wild and hatchery populations from different parts of South Asia.

A survey of broodstock management practices concluded that the Karnataka hatchery stocks, which had been reproductively isolated since the introduction of the species in the 1950s, were likely to be inbred with rates ranging from 4.7 to 10% per annum. However, the results from genetic characterization using randomly amplified polymorphic DNA analysis (RAPD) and microsatellite DNA loci and restriction fragment length polymorphism (RFLP) of mito-chondrial DNA were ambiguous. Results from RAPD of nuclear DNA did not enable the discrimination of hatchery stocks and indicated that the levels of genetic variation were similar both within and between the wild and hatchery stocks. Analysis of results from four microsatellite DNA loci showed both hatchery and wild stocks to have similar levels of average heterozygosity with most populations in Hardy-Weinberg equilibrium. Furthermore, there were similar mean numbers of alleles at each locus between the hatchery and the wild stocks, contradicting the hypothesis that the hatchery stocks are inbred.

Results from growth trials over three successive years have consistently indicated higher yields from crossbreds between the two major hatchery strains compared to the pure strains, indicating positive heterosis for commercial traits. Inclusion of wild caught stocks in these trials has not yet been possible.

Studies on the feasibility of the application of microsatellite markers for pedigree analysis produced promising results but require further primers to be developed to provide the necessary levels of resolution by evaluating more loci. Work on the development of additional primers is well underway. If microsatellite markers can be used for identification of fish at the family level they may become useful tools in communally stocked growth trials and in farm-based selection programs.

The work on common carp has progressed well. Early on-station and on-farm trials revealed relatively early maturation of local stocks of common carp under a range of culture conditions although spawning did not occur in the absence of aquatic vegetation as a substrate. Fish became mature at less than six months old and as small as 150 g in weight. Furthermore, GSI was as high as 20% in both males and females prior to attaining a harvestable size.

Three approaches to solving this problem have been evaluated. Masculinization protocols have been developed prior to identifying sex-reversed XX males,

which can be used to produce all-female progeny. Protocols for induction of triploidy (with triploid induction rates up to 100%) have also been developed. Growth trials of sterile triploid fish up to the age at which sexual maturation occurs in diploid fish revealed no differences in growth rates although dress-out weights of triploid fish were greater than those of diploids, post-sexual maturation.

Recently a selected strain was introduced from Vietnam and early growth trials have indicated that this has faster growth rates and later sexual maturation than the local strains of common carp. Analysis of standard broodstock management protocols among the dominant state hatcheries revealed potential for selection for early maturation and revised management protocols are presently being developed in conjunction with the Karnataka Department of Fisheries.

Other research

Researchers at UWS are active in many other areas of research outside of those under DFID/FGRP.

Genetic diversity of silver barb

This three-year project (started in 1998) is funded by the DFID Competitive Research Facility, in collaboration with ICLARM, developing the theme of the interrelations of aquaculture and biodiversity (Beardmore et al. 1997). The project aims to collect and collate data on genetic diversity of wild populations of the silver barb, *Barbodes gonionotus* (formerly *Puntius gonionotus*) throughout its natural range in order to document genetic resources. This is being achieved through the application of a suite of biochemical and molecular techniques at the laboratories at UWS, with emphasis on mtDNA and microsatellite loci. These data will be analyzed to determine the levels of genetic variability in the natural stocks of *B. gonionotus* and to identify those populations that can act as valuable reserves of genetic diversity. It is hoped that the degree of resolution of these techniques will be sufficient, when combined with indigenous knowledge, to determine the source of introductions of any stocks that may be feral and non-native.

Samples for *B. gonionotus* were collected from several sites in Indonesia and Indochina, and further sampling trips are planned. To date the control region of the mtDNA molecule has been surveyed in four populations, two from Java, Indonesia, and two from Vietnam, with a number of fixed differences noted between the samples from the two regions. Molecular markers are now being used to survey the populations collected so far. With the isolation of a large number of positive clones from the microsatellite libraries, a large suite of microsatellite markers is anticipated for use, in addition to those already obtained from other researchers.

Collectively, it is hoped that the techniques and approaches used in this project would represent a valuable case study investigating the genetic diversity of a commercially important species. Furthermore, it is intended that the data gathered can be used to recommend a long-term strategy for the conservation and management of this diversity in relation to future introductions and the predicted expansion of the farming of this species.

Future Directions

Many of the research themes highlighted above will continue. DFID/FGRP projects will focus even more tightly on potential impacts of research outputs on the alleviation of poverty. It is likely that most projects will include research components evaluating important social and economic factors related to the role of increased efficiency of aquaculture production achieved through application of genetics-based technologies, on the livelihood of the poor.

Following are other areas of research currently being considered (not necessarily within the framework of the DFID/FGRP):

- In tilapia, studies on the use of clonal lines as internal controls in growth trials, comparisons and integration of different genetic improvement strategies, and research on dissemination strategies of improved fish for the benefit of the poor;
- Surveys on existing broodstock management protocols prevalent for commercially important species in developing countries and their likely impact upon seed quality;
- Strain comparisons of common carp in the tropics; and
- Application of molecular markers in fisheries stock assessment in the Mekong basin.

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GENETICS RESEARCH AT WAGENINGEN UNIVERSITY AND RESEARCH CENTRE, THE NETHERLANDS

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ABSTRACT

An overview of genetics research in the Fish Culture and Fisheries Group, Department of Animal Science, Wageningen University and Research Centre, the Netherlands, is presented. The major model species for genetics research is common carp, *Cyprinus carpio*, L. In carp, several inbred strains have been produced by gynogenesis or androgenesis. Techniques for gynogenesis and androgenesis, hormonal sex reversal and cryopreservation, and the consequences of inbreeding and crossbreeding in terms of genetic and phenotypic variation are discussed. Theoretical considerations for application of gynogenesis and androgenesis in selective breeding programs are illustrated with experimental results and a case study on selective breeding for stress response. The consequences of inbreeding for sex determination and differentiation are also presented.

Introduction

Research by the genetics section of the Fish Culture and Fisheries Group at the Wageningen University, Netherlands focuses on the use of induced gynogenetic and androgenetic reproduction in selective breeding programs. The common carp (*Cyprinus carpio*) is the species mainly used in the studies, although some breeding work with the African catfish, *Clarias gariepinus* is performed. The following are the main areas of research since 1992:

1. Development of a toolbox for gyno/androgenesis, for sex control and cryopreservation of gametes;
2. Development of a theoretical framework for breeding with gyno/androgenesis in fish;
3. Case studies in which the principles laid down in the theoretical framework could be tested; and
4. Development of genetic markers which could be used in combination with gyno/androgenesis to study quantitative trait loci (QTL) associations for traits of interest.

A brief description of the progress in each of these stages is given below.

Principles of Androgenesis and Gynogenesis

Common carp can be reproduced by gynogenesis or androgenesis. In gynogenesis, eggs are fertilized with

genetically inactivated sperm to produce haploid embryos. These embryos are made diploid by either inhibition of the extrusion of the second polar body (2pb-gynogenesis) (Komen et al. 1988) or inhibition of the first cellular division (endomitosis) (Komen et al. 1991). In the latter case, the result is a double haploid (= diploid) homozygous individual. In androgenesis, the same principle is applied but the maternal genome is genetically inactivated and fertilization is performed with intact sperm (Box 1; Bongers et al. 1994). Survival is typically low: around 5-15 % of treated eggs develop as viable fry, which necessitates rigorous screening of the progeny for true parthenogenesis (no contribution from the irradiated genome) and homozygosity. To confirm the parthenogenetic status of the fry, phenotypic markers can be used for which the dominant alleles are carried by the "irradiated genome" and the recessive alleles by the genome which will be reproduced by gyno or androgenesis. True parthenogenonts do not express dominant alleles. Segregation ratios of a phenotypic marker, for which the parent is heterozygous, can also be used as a simple first check for homozygosity of the parthenogenetic progeny. In the studies, S/s (scaled/mirror) was used as dominant marker, and Bl/bl (wild type vs. blond, orange or yellow phenotypes) as a marker for segregation[a] . Homozygosity can also be determined by the use of DNA markers, or, in the case of clones, simply by skin or scale transplantations (Komen et al 1990).

Genetic and phenotypic variation in gynogenetic and androgenetic families

After androgenetic or gynogenetic reproduction, a large expansion of phenotypic variance is generally observed (Komen et al. 1992a). Within one homozygous family, this expansion is the result of increased environmental variance since genetic variance does not increase. Theoretically, the additive genetic relation between parent and offspring equals 1 (Fig.1; Bongers et al. 1998). Therefore, gynogenetic reproduction could be useful for selection purposes when one is interested to estimate breeding values of individual dams (or sires in androgenesis) included in a breeding program. Likewise, gynogenesis can be used to estimate the genetic load of a dam (or sire) included in a breeding program. In this case, the expression of deleterious recessive genes is measured. However, all estimations based on phenotypic variances should be treated with caution since the phenotypic variance can also increase due to treatment effects or increased sensitivity to environmental influences (Bongers et al. 1997a).

Three types of environmental variance (V_E) should be considered within homozygous offsprings:

1. "true" V_E (interindividual variance);
2. V_E, due to developmental instability (DI, intraindividual variance); and
3. V_E originating from embryonic damage (ED) caused by the chromosome manipulation treatment.

To investigate the relative contributions of each of these factors, homozygous (F = 1) androgenetic and gynogenetic families and partly heterozygous (2pb-gynogenesis: F = 0.79) gynogenetic families were observed. All families were produced from genetically identical parents. DI and ED were determined by measuring fluctuating asymmetry (FA) of five bilateral symmetric morphometric characteristics (Bongers et al. 1997c).

The androgenetic groups showed highest FA and variations caused by ED, followed by 2pb-gynogenetic and homozygous gynogenetic groups, respectively (Table 1). It was concluded that increased variation within gynogenetic or androgenetic offsprings is mainly the result of embryonic damage, caused by the chromosome manipulation treatment.

Phenotypic variation in gynogenetic and androgenetic clones

Parthenogenetic reproduction of animals which are homozygous, i.e., animals from a gynogenetic or

Box 1:
Androgenesis in cyprinids is difficult since eggs are activated the moment they come into contact with aquatic solutions. To overcome this problem, the centre developed a technique to irradiate eggs from common carp with UV, by using an artificial ovarian fluid for irradiation medium (Bongers et al. 1994). Eggs are shaken in this medium which ensures an even irradiation and proper genetic inactivation of DNA. Eggs are irradiated to a total dose of 175 mJ/cm^2. Diploidization is achieved with a 40°C heat shock for 2 min, given around the time of the first mitotic metaphase, which is between 26 and 32 min after activation (at 24°C).

[a] *In many carp strains, B1 is still present as a tetraploid locus with duplicated alleles b11 and b12. Only the homozygous recessive genotype b11/b11; b12/12 is colored (Komen et al. 1991)*

Table 1. Fluctuating asymmetry values for the parameter P (= number of pectoral fin rays) and for metric indices A (distance of mouth corner to lower end operculum), B (lower to upper end operculum), C (upper end operculum to eye) and D (eye to mouth corner) in 12 experimental groups. F = coefficient of inbreeding, n = number of animals analyzed, Con = normal fertilizations, 2pb = 2pb-gynogenetic treatment, Gyno = gynogenetic treatment, andro = androgenetic treatment. Treatments with a common superscript do not differ significantly (Duncan's multiple range test, P < 0.05).

	Con (F=0.75)				2pb (F=0.79)		Gyno (F=1.0)		Andro (F=1.0)			
n	60.0	65.0	59.0	58.0	55.0	47.0	60.0	58.0	19.0	31.0	12.0	30.0
P	04.7	03.5	04.3	03.6	05.8	06.2	05.0	04.6	06.7	03.7	07.1	06.4
A	03.3	04.8	03.5	03.3 [(a)]	05.7	07.2 [(ab)]	04.4	05.0 [(ab)]	05.1	07.6	10.1	06.4 [(b)]
B	05.1	04.7	04.3	03.4 [(a)]	07.3	07.6 [(b)]	05.7	07.4 [(ab)]	05.2	08.1	09.2	07.9 [(b)]
C	04.1	03.0	03.7	03.7 [(a)]	06.6	07.1 [(ab)]	04.2	04.7 [(ab)]	06.2	07.4	13.7	04.9 [(b)]
D	04.8	04.1	04.7	04.6 [(a)]	06.5	09.4 [(b)]	04.6	06.7 [(ab)]	07.3	06.9	10.2	06.9 [(b)]

androgenetic sib family, produces homozygous clone lines. Cloning offers the opportunity to capitalize on nonadditive genetic effects. This can be done by using gynogenetically and androgenetically produced individuals from different lines as parents. In this way, the additive genetic superiority of selected gynogenetic individuals can be combined with the effect of heterosis. Among the crossbreds, there should be no genetic variation (isogenic strains), which would promote product uniformity and dissemination of genetic progress from the breeding population to the commercial population (Komen et al. 1993; Bongers et al. 1997a; van der Lende et al. 1998).

To test this last idea, the importance of two types of V_E in three isogenic strains (produced by crossing homozygous inbred strains) and one partly outbred strain of common carp was examined. As all were conventional breedings, V_E due to embryonic damage was absent. True V_E was determined by measuring length, body weight and number of dorsal fin rays. DE was determined by measuring FA as before.

The isogenic strains varied in degree of homozygosity (coefficient of inbreeding F: 0 to 0.99) (Table 2). The strain with the highest F displayed the lowest true V_E. Surprisingly, FA was equal in all isogenic strains and highest in the partly outbred strain (Bongers et al. 1997a).

Gynogenesis and Androgenesis: Theoretical Considerations

Under gynogenetic reproduction, offsprings receive genes only from their dams since paternal genes are not transmitted. In gynogenetic progeny from a non-inbred dam, there is still genetic variation since the gametes produced by the dam differ due to Mendelian sampling. Theoretically, gynogenetic families can therefore be used to estimate heritabilities. In gynogenesis or androgenesis, the total genetic variance V_G is partitioned in V_A, V_D and V_I. Of these, V_D is absent in homozygous families while V_i is probably negligible. However, the additive genetic variance in the offsprings is doubled compared to the non-inbred dam population and is equally distributed within and between gynogenetic sib families:

$$V_{A\text{-tot}} = V_{A\text{-between families}} + V_{A\text{-within families}} = 2fV_A + (1+F-2f)V_A,$$

with f = coefficient of co-ancestry and F = coefficient of inbreeding (Fig.1) (Bongers et al. 1997c; Bongers et al. 1998).

Family sizes for estimating heritabilities are smaller for gynogenetic sib families than for conventional full-sib families. For low heritabilities (<0.35) there is a small advantage in accuracy of the estimated heritability for

Table 2. Coefficient of inbreeding (F) and means plus coefficients of variation (cv) for length (L, mm), body weight (BW) and the number of dorsal fin rays (#D) in four isogenic groups. n = number of animals analyzed. Cv's with a common superscript do not differ significantly (Levene's F, P<0.05).

	E4xR3R8		E4xE5		E4xE4.Y5		E4xFS	
F	0		0.5		0.99		0.375	
n	50		50		50		50	
	mean	cv	mean	cv	mean	cv	mean	cv
L	93.2	11.6	88.0	11.8	83.2	09.6	84.8	13.0
BW	35.0	29.4[b]	29.0	27.2[ab]	26.5	27.2[a]	30.2	31.8[ab]
# D	21.0	05.2[abc]	21.1	11.4[c]	20.9	04.3[a]	21.3	06.6[bc]

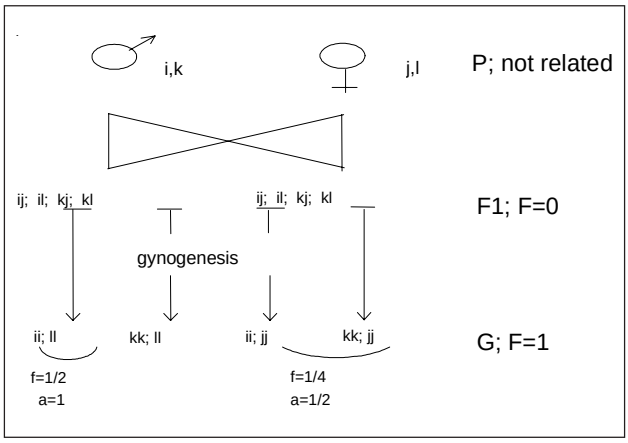

Fig. 1. Determination of genetic relations in gynogenetic offspring. Parents (P) are assumed not to be related. F_1 females are reproduced by homozygous gynogenesis to produce homozygous gynogenetic offspring (G). F = coefficient of inbreeding; f = coefficient of co-ancestry; a = additive genetic relation; a= 2*f.

gynogenetic sib families, but with higher heritabilities there is a clear disadvantage (Bongers et al. 1997c).

Selection for high and low stress responders in androgenetic families

In 1996, a project was started to investigate whether selection for a stress response in common carp is feasible, and how heritabilities can be estimated using androgenetic progenies. The starting population for this study was an F_1 hybrid cross between a domesticated carp strain from Wageningen and 6 males from a feral carp strain caught in a Dutch lake (Fig. 2) (Vandenputte et al., in press). The stressor used within the selection program was a simple cold shock of 9°C for 3 hours (Tanck et al. 2000). A first generation of 33 androgenetic families was produced using the F_1 hybrids and these families were subjected to a cold shock and blood was sampled 20 min after onset of the shock. Furthermore, all individual fish were tested for homozygosity using a panel of 11 microsatellite markers.

Heritability estimates on a group of 512 homozygous animals ranged from 0.60 ± 0.16 for cortisol response to 0.09 ± 0.05 for weight at 12 months. From these 33 families, 12 potential high and low responder homozygous males were selected for further androgenetic reproduction. The progenies from these males are expected to be homozygous clones and genetically identical to their respective "fathers".

In 1998, new clones of common carp were produced from the 12 homozygous males which were selected for

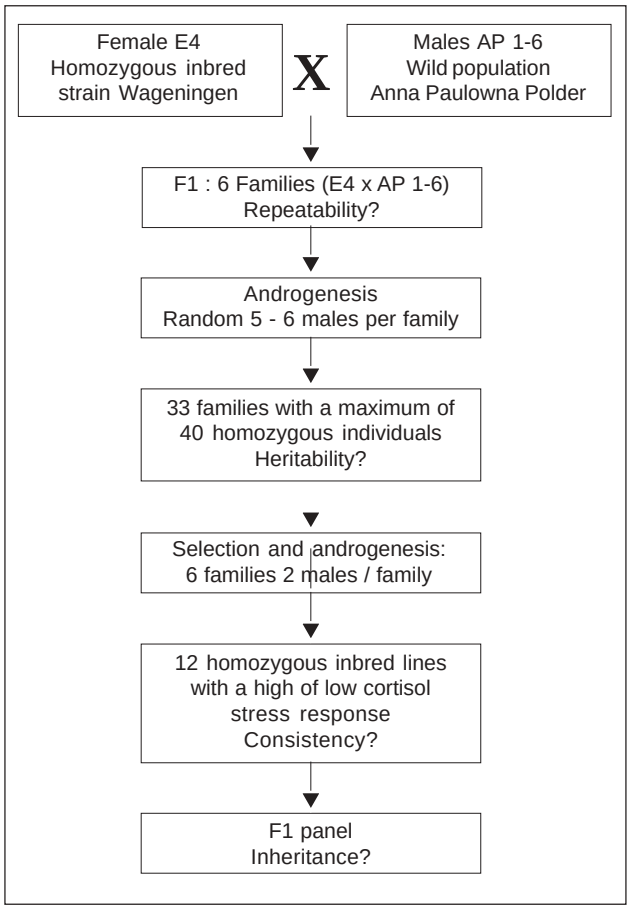

Fig. 2. Breeding strategy with androgenesis.

a high or low cortisol response during a temperature shock. From the 12 homozygous clones, four were selected, which descended from the two sires with the lowest and the two sires with the highest estimated breeding values. These clones were given a standard cold shock of 3 hours to induce a cortisol response and again sampled 20 min after onset of the shock (Komen et al. 2000). The aim was to

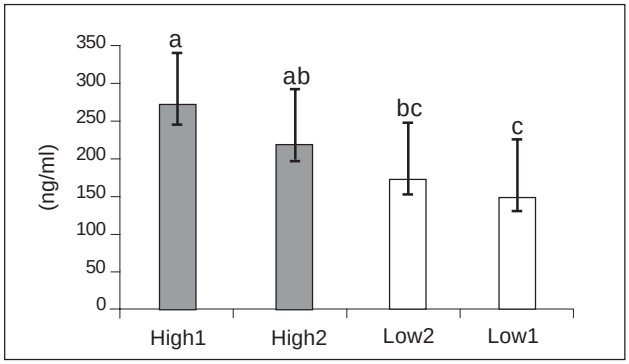

Fig. 3. Plasma cortisol levels in four clones of carp, derived from parents selected for a high or low cortisol response after a cold shock. Means (± SD) with similar superscripts are not significantly different (Tukey's multiple comparison procedure, P<0.05).

see if, and to what extent, a cortisol response of a homozygous clone reflects the phenotype of the sire for this trait (the genetic correlation is 1.) Indeed, high responder clones were significantly different from low ones (Fig. 3).

Clones, correlations and genotype environment interaction

In breeding programs, clone lines (crossbred or pure) can be used to measure different traits on the same genotype, for example, carcass quality at different ages, disease resistance, or gonadal growth (Bongers et al. 1997a). Clone lines might therefore be very useful for estimating genetic correlations and genotype by environment interactions, because performance on the same genotype can be observed in different environments. Since clone lines are not subject to genetic change, they can be used as internal control lines in long-term breeding programs where estimations of genetic progress are based by improvements in husbandry (Vandenputte et al., in press). This is particularly important for fish, where estimates of genotype by environment interactions can vary from negligible to considerable.

Determining genetic correlations between traits in crosses between homozygous clones of common carp

In 1998, a project was started to investigate the impact of various aquaculture practices on stress and health in fish (van Weerd and Komen 1998). Preliminary experiments in which the effect of crowding at different densities (control 25 g l^{-1}; crowded 50 and 100 g l^{-1}) were examined, showed that common carp can adapt to fairly high densities of up to 100 g l^{-1}, provided that water quality conditions (particularly oxygen) are optimal. An additional 1 hour net confinement stressor was applied to both uncrowded and crowded fish as a stress test.

Netting induced a high cortisol response, of a magnitude comparable to a temperature shock of 9°C (compare Figs. 3 and 4). Crowding and netting also induced significant changes in blood glucose, lactate and free fatty acid levels.

The next step in this research will be to investigate the response to crowding and netting in carp strains which have been selected for high and low cortisol response after cold shock and strains selected for a high and low antibody response to a synthetic antigen. The idea is to investigate whether:

- selection for one stressor (e.g., cold shock) conveys resistance to other stressors (positive correlation between stress responses) or
- a negative correlation between antibody response as a trait and cortisol response, as a trait exists.

Sex determination and sex differentiation in common carp

The common carp is a gonochorist species with XX/XY sex determination. Androgenetic progenies usually consist of varying proportions of males, females and sterile fish. The males produce all male offspring when crossed with normal females, and are therefore YY (Bongers et al. 1997b; Bongers et al. 1998). All male progenies are currently used in tests to screen environments on the presence of industrial pollutants with estrogenic activity (Gimeno et al. 1996).

In gynogenetic progenies, males are often detected at

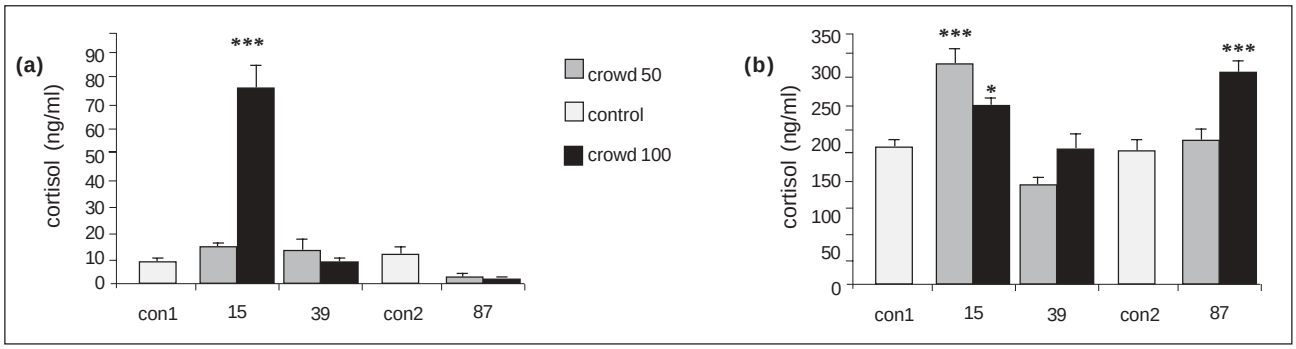

Fig. 4(a). Changes in plasma cortisol levels during crowding in common carp over an 87-hour period. (b) Plasma cortisol levels in control and crowded common carp after a 1-hour net confinement, during the crowding period. *(p<0.05); *(p<0.001) denote significant differences to the controls.**

high frequencies. The analysis of one such case showed that XX animals, which are homozygous for a recessive mutation in a putative sex determining gene *mas*(-culinization), undergo female to male sex reversal (Komen et al. 1992b; Komen et al. 1992c).

In order to identify the function of *mas* in sex determination, the sex reversal of XX (*mas/mas*) common carp was investigated by comparing clones of XY males, XX(*mas/mas*) males and XX(*mas*/+) females. Fish in each of these clones are genetically uniform and of the same sex. From this study, it was concluded that sex reversal in XX (*mas/mas*) animals might becaused by a precocious production of 11-oxygenated androgens, which gradually overrule female sex differentiation by male sex reversal of female steroid producing cells. Based on these findings, a new female to male sex reversal protocol for common carp was designed, using 11 keto-androstenedion (Box 2).

Box 2:
Methyltestosteron (MT) is the most widely used hormone to induce sex reversal in cyprinids. However, its efficiency is variable, and high incidences of sterility have been reported (Komen et al. 1989; Komen and Richter 1993). In common carp, 11-keto androstenedion (KA) is the most important steroid in male sex differentiation. The efficiency of KA to induce functional sex reversal in common carp was compared with 17a-MT. Two homozygous clones and four F_1 hybrids were treated with either 50 ppm 11 KA or 50 ppm MT, incorporated in the pellet, for a period of 5 weeks, starting from 6 weeks post-hatch. While results with MT were typically variable, with high numbers of intersexes in some clones and of sterile fish in others, KA induced a 100% female to male sex reversal in all strains. The protocol for sex reversal with KA is currently being tested in field conditions in cooperation with AIT, Thailand.

The Future: Microsatellite Markers and the Search for QTL

Between 1996 and 1998, some 83 highly polymorphic poly(CA) type microsatellite markers have been developed in common carp (Crooijmans et al. 1997). Clones containing a (CA) repeat were isolated from a common carp genomic library and sequenced. The number of repeats found was high compared to other mammals, but comparable with other teleost fishes. Most microsatellites are highly polymorph (average of 4.7 alleles) with a few markers giving additional polymorphic amplification products. It is suspected that these loci are tetraploid. Of the 83 markers, 11 are currently used as a panel to screen homozygous clones and to perform pedigree analyses. The developed microsatellites appear to be species-specific. Cross hybridization with DNA of Indian carp *Catla catla* gave no amplification.

One of the centre's main activities for the near future will be the search for markers in common carp which are linked to QTL. For this, an F_1 hybrid cross was produced between an ornamental (koi) carp and a domesticated common carp, as these strains appear to be genetically most distant. Koi and edible common carps differ in many traits, due to their domestication history. Androgenetic and gynogenetic progenies will be produced for direct AFLP mapping. The progenies are expected to differ in a large number of single and multilocus traits, i.e., scalation, color, sex determination, onset of maturity, fecundity, growth, etc., and it is expected that markers will segregate with major genes affecting these traits. In the end, such markers will be very valuable for selective breeding programs with common carp anywhere in the world.

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SPECIES INDEX

D

E

F

G

H

I

K

L

R

S

T

V

W

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