



# DEVELOPMENT OF AQUATIC ANIMAL GENETIC IMPROVEMENT AND DISSEMINATION PROGRAMS:

*Current Status and Action Plans*

Edited by

Raul W. Ponzoni  
Belen O. Acosta  
Alphis G. Ponniah



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WorldFish Center is one of the 16 international research centers of the Consultative Group on International Agricultural Research (CGIAR) that has initiated the public awareness campaign, Future Harvest.

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# PREFACE

The world's food production will have to double to provide food security for the eight billion people projected for 2025. Fish is a vital component of food security, especially in developing countries where it contributes up to 80 per cent of animal protein intake. Aquaculture is expected to bridge the widening gap between fish supply and demand and to contribute to the food and nutritional security of the poor in developing countries. If aquaculture is to fulfill this critical role of supplying the much needed protein in the diet of the poor, it will have to be through an expansion of the area under aquaculture, underpinned by sound management practices and the use of high quality seed from productive strains.

The Norwegian breeding programs conducted in the 1970s for salmonid fish have shown the possibility of increasing growth through selective breeding. However, it was not sure whether these genetic gains could be obtained in the case of tropical finfish, which contribute about 90 per cent of the global aquaculture production. This posed a challenge to the WorldFish Center, to come up with methods for the genetic improvement of tropical finfish species used in aquaculture. The Genetic Improvement of Farmed Tilapia (GIFT) project implemented by the WorldFish Center and its Philippine and Norwegian partners demonstrated the appropriateness of traditional selective breeding for genetic improvement of tropical finfish.

Realizing the opportunities in the selection of tropical finfish as demonstrated by the successful application of breeding programs in crops, livestock, Atlantic salmon and recently in Nile tilapia, the WorldFish Center and developing countries from Asia and Africa established the *International Network on Genetics in Aquaculture (INGA)* as a global forum for applied fish breeding and genetics. With the present membership of 13 countries from Asia-Pacific (Bangladesh, China, Fiji, India, Indonesia, Malaysia, Philippines, Thailand and Vietnam) and Africa (Cote d'Ivoire, Egypt, Ghana and Malawi), with the WorldFish Center as member-coordinator, and 15 advanced scientific institutions, regional, international organizations and the private sector as associate members, the network has been acting as a catalyst for collaboration among member institutions and initiation of national genetic improvement programs for commercially important cultured species.

Developing countries participating in the INGA have achieved progress in their genetic improvement research and breeding programs and some of these countries now have improved fish species that are being disseminated to government and private farms. For instance, the impact evaluation study conducted by the Asian Development Bank in selected Asian countries (Bangladesh, Philippines, Thailand and Vietnam) showed that GIFT and GIFT-derived strains account for 68 per cent of the total tilapia seed produced in 2003. Apart from tilapias, improved species of commercially important carps have also been developed (e.g. silver barb in Bangladesh and Thailand, rohu in India and common carp in China and Vietnam) and are being disseminated. Whereas significant progress has been achieved in this respect, the member countries participating in the network are now at a critical stage of their breeding programs, needing guidance and assistance with regard to their future direction.

Country reports presented at the 2002 INGA Expert Consultation held in Thailand revealed that, in general, the capacity of the member country institutions (both public and private) to maintain and manage the improved strains is weak and overall strategies for the dissemination of these strains are lacking. In view of this, it is considered crucial that these countries, which are at the critical stage in their genetic improvement and dissemination programs, are assisted to address the challenge of ensuring that the gains derived from genetic research and development are sustained and that the improved strains are disseminated effectively so that targeted end-users obtain the maximum benefits. As an initial step to determine the kind of support needed to strengthen the ongoing genetic improvement and dissemination programs and to develop additional ones for new species, an assessment of the specific country needs is essential. Such an assessment must include: (i) defining the status of the ongoing breeding programs and the state of the genetically improved stocks; (ii) critical analyses of bottlenecks that impede the progress of genetic improvement and dissemination; and (iii) formulation of customized solutions to the constraints identified for each country. In response to this, the WorldFish Center in collaboration with the China Academy of Fishery Sciences organized the international "Workshop on Dissemination of Improved Fish Strains: Country-Specific Action Plans" on 21-22 September (2005) in Shanghai, China, to bring together the geneticists, biodiversity and aquaculture experts from developing countries in Asia, Africa and Pacific, and representatives from advanced scientific institutions, regional and international organizations and private sector, to discuss ways to strengthen the ongoing genetic improvement and dissemination programs of member countries, and to develop additional programs for new species.

These proceedings contain the papers presented at the workshop, and a synopsis of small group discussions. The synopsis focuses on the requirements for effective implementation of genetic improvement and dissemination programs, the status of fish breeding programs and genetically improved stocks in the member countries of INGA, constraints to development, effective maintenance and dissemination of improved fish, and country-specific action plans for selected species.

We gratefully acknowledge the Government of China for hosting the meeting of the International Network on Genetics in Aquaculture and for providing the logistical support. Special thanks are also due to workshop participants for their invaluable contributions in the discussions.

**Editors**



# Genetic improvement and effective dissemination: Keys to prosperous and sustainable aquaculture industries

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## Abstract

There is an increasing demand for fish in the world due to a growing population, better economic situation in some sectors, and greater awareness of health issues in relation to food. Since capture fisheries have stagnated, fish farming has become a very fast growing food production system. In this presentation, the author gives an overview of the technologies that are available for genetic improvement of fish, and briefly discuss their merit in the context of a sustainable development. He also discusses the essential pre-requisites for effective dissemination of improved stock to farmers. It is concluded that genetic improvement programs based on selective breeding can substantially contribute to sustainable fish production systems. Furthermore, if such genetic improvement programs are followed up with effective dissemination strategies, they can result in a positive impact on farmers' incomes.

## Introduction

Production systems in developing countries are largely based on the use of unimproved species and strains. As knowledge and experience are accumulated in the management, feeding and animal health issues of such production systems, the availability of genetically more productive stock becomes imperative in order to use the resources more effectively. For instance, there is little point in providing ideal water conditions and optimum feed quality to fish that do not have the potential to grow faster and to be harvested in time to provide a product of the desired quality. Refinements in the production system and improvement of the stock used must progress hand in hand.

In terrestrial animal species (e.g. dairy cattle, pigs, poultry), genetic improvement programs have made a substantial contribution to industry productivity and viability. The gains achieved among plants species have been even more spectacular. There appears to be great potential for improvement in aquatic animal species because comparatively little application of genetic improvement technology has taken place to date. Hence, there is ample justification for the planning, design and implementation of research, development and technology transfer of genetic improvement programs for aquatic species.

Such programs are particularly well suited to contribute to the fulfillment of noble aims, such as increasing the amount of animal protein available to a greater number of the population of developing countries, thus assisting in achieving greater food

security. Furthermore, they can do so in a sustainable manner, and without having undesirable environmental repercussions. In this paper, the author gives an overview of the technologies that are available for genetic improvement of fish, and also discusses the essential pre-requisites for effective dissemination of improved stock to farmers.

## Background against which genetic improvement programs operate

Three factors have resulted in a greater demand for fish in the world; namely, an ever-increasing human population, improved economic situation in some sectors, and greater awareness of the health aspects of food. Since capture fisheries have stagnated, fish farming has become a burgeoning food production system.

## Fish genetic improvement as a means to help achieve sustainable gains

Genetic improvement programs have the following highly desirable attributes:

1. The power to modify the animal to suit a purpose or environment
2. The ability to provide greater food security and poverty alleviation by increasing productivity, reliability and consistency, and probably achieving permanent gain



3. The probability to offer solutions to existing or emerging pathogens, and to environmental challenges
4. The potential to provide a favorable return on investment
5. The capacity to fill the gap between demand and supply without a negative environmental impact
6. The opportunity to assist in managing inbreeding in the production system

Genetic improvement programs for fish can contribute to the production system's output, both in quantitative and qualitative terms, by enhancing traits of major importance, such as:

- Growth rate to harvest weight or time
- Survival
- Stress and disease resistance
- Cold water tolerance
- Sexual maturation
- Product quality
- Feed efficiency

The emphasis placed upon these traits will depend on a number of factors. For instance, the phase of the improvement program, specific circumstances in terms of diseases and environmental challenges, and so on. Typically, a considerable amount of effort is devoted to the improvement of growth rate. This is justified because there are clear advantages in producing larger fish in a given period of time, or fish of a particular size in a shorter grow-out period.

The question then is: how can we improve these traits?

## Steps in the design of a genetic improvement program

The WorldFish Center is attempting to approach work in this area in a logical and systematic manner by addressing, as deemed appropriate in each circumstance, all the activities that the planning, design and conduct of a genetic improvement program entail, namely:

1. Description or development of the production system(s)
2. Choice of the species, strains and breeding systems
3. Formulation of the breeding objective
4. Development of selection criteria
5. Design of a system of genetic evaluation
6. Selection of brood stock and mating systems
7. Design of a system for the expansion and dissemination of the improved stock
8. Monitoring and comparison of alternative programs

Generally, these steps would be taken in the above order, though not always necessarily. There will always be iterations, going back to earlier steps, making modifications, and rectifying courses of action. Attention to all aspects is essential for the conduct and implementation of an effective genetic improvement program. An example of the use of the approach suggested in this paper may be found in Ponzoni (1992), which also provides references on the methodology that may be used. Each one of the above listed steps is briefly treated, with special reference to the improvement of aquatic animal species.

### Brief treatment of each step

#### *Description of the production system(s)*

Before even thinking about genetic improvement, fisheries scientists have to be clear about the range of production systems for which genetic improvement is intended. This step entails specifications such as:

- (i) Nature of the production system (e.g. mono or poly-culture, smallholder, commercial operation, industrial operation)
- (ii) Feeding regime
- (iii) Environmental challenge (disease, temperature, water quality)
- (iv) Sex and age (or size) of harvested individuals
- (v) Social environment

To a large extent, these issues have been addressed in current projects. There could be opportunities, however, in re-examining these range of production systems for which genetic improvement is intended, and, in particular, in anticipating likely developments and possible future production systems.

Identifying major production systems is very important, because there may be no single "genotype" that is "best" in all production environments (i.e. presence of species or strain by environment interaction). If the genotype by environment interactions is suspected (or in fact does exist), treating the expression of the trait(s) in question in different environments as different traits and estimating the genetic correlation between both expressions will be informative.

#### *Choice of the species, strain(s) and breeding systems*

The decisions on the choice of species and strain sometimes are partly made for scientists, as when there are limitations on availability of stock, or well-defined local preferences. However, when possible, making the right choice is important because the gain achieved in this way may be equivalent to several generations of selection.

The choice of species and strains should preferably be

made on the basis of information derived from well-designed experiments of species and strain comparison, and estimation of phenotypic and genetic parameters (heterosis, heritability, correlations among traits, genotype by environment interactions). Such experiments can be complex and costly, but they are very necessary. The Genetic Improvement of Farmed Tilapia (GIFT) approach used for tilapia (and suggested also for carp) is a sound way of addressing the issue. There could be room for refinements of design in some cases, and in-depth analysis of presently available and future data should be conducted. Greater accuracy in the estimates of phenotypic and genetic parameters can result in greater effectiveness of the genetic improvement programs.

Looking for genes that have a relatively large effect on traits of relevance for the production system(s) by statistical procedures in the data collected could yield valuable results. If any were found, they could become candidates for gene mapping and expression studies.

#### *Formulation of the breeding objective*

The formulation of the breeding objective is crucial because it determines “where to go” with the genetic improvement program. The breeding objective is intimately related to the production system. Scientists have to make sure that the trait(s) they improve are those of importance in the actual production system. Generally, these will be the traits that impact upon income or expense in the production system, or those associated with benefits to the user of the improved animals in a non-cash economy, or those that influence sociological preference.

There are two main ways of defining the breeding objective:

- (i) As a statement of intent of desired genetic gain in each trait
- (ii) From a mathematical function describing the production system, deriving an economic value for each trait

The breeding objective usually includes traits such as: growth rate or size, survival rate, age at sexual maturity, disease resistance, tolerance to water temperature or to other water attributes, flesh quality, and feed conversion. Of these, growth rate (or size at a particular age) has been the most popular, partly because its impact is easily perceived and it can be measured. There are risks, however, in over-simplifying the breeding objective to a single trait, as unfavourable correlated responses can occur. Even if not formally included in the breeding objective, traits perceived as being of importance in the production system should be carefully monitored.

The issue of breeding objectives has been addressed

only to a limited extent in some projects. This may be justified by the over-riding importance of size or growth rate. However, there will often be opportunity to refine improvement programs through work on breeding objectives as these evolve. For instance, new traits may have to be formally incorporated as the production system develops, or in response to changing consumer demands. When there is a need for radically different traits, or for very fast improvement beyond what is possible with conventional methods, genetic engineering and the creation of transgenic animals have been proposed as options. However, the costs of implementing such option, and the (often found) lack of acceptability by consumers of the animals thus created, have given rise to considerable controversy, and they should be critically assessed before being proposed as an alternative.

#### *Development of selection criteria*

The selection criteria are characters closely related, but not necessarily identical, to the traits in the breeding objective. The breeding objective is about “where to go” with the genetic improvement program, whereas the selection criteria are about “how to get there”. The selection criteria are the characters the scientists use in the estimation of breeding values and overall genetic merit of the animals.

Selection criteria may be different from the traits in the breeding objective. For instance, the scientists may be interested in increasing market weight, but they may base their selection on weights taken at an earlier age, before reaching market weight, in an attempt to speed up the selection process by choosing breeding animals earlier. Also, there may be cases in which the scientists do not select directly for the trait in the breeding objective, but use an indicator character instead (e.g. length of fish could be used as an indicator of weight).

The characters used as selection criteria are linked to the traits in the breeding objective via genetic variances and covariances. Hence, the need for phenotypic and genetic parameters in the estimation of breeding values for relevant traits.

There may be new developments through gene mapping and marker assisted selection (MAS). There are some traits that can have importance in the breeding objective but they are difficult to measure. Disease resistance and tolerance to some environmental challenges are two examples. For such traits, “conventional” selection procedures based on quantitative genetics sometimes have limitations, and developments in the area of MAS could be valuable.

Even if scientists concluded that crossbreeding was the best alternative as a breeding strategy, they would still have to consider within breed or strain selection, and the above discussion on selection criteria would be appropriate.

The notion of dealing separately with traits in the breeding objective and characters used as selection criteria can be of help in bringing some of the current and likely future work into sharper focus (e.g. placing MAS in the proper context and perspective in relation to genetic improvement work as a whole).

### *Design of a genetic evaluation system*

With an assumption that the production and breeding system, the breeding objective, and the selection criteria have already been established, the environment for selection should be as close as possible to the production environment, unless there is very clear evidence of absence of genotype by environment interactions.

The genetic evaluation system can vary from something very simple, involving just mass selection for one or a few traits, to something much more complex, involving fitting an animal model to the data, or separating sib, or testing progeny for specific traits (e.g. disease after challenge, flesh quality after slaughter). Depending on their ability to identify individuals and to keep track of pedigrees, scientists may use mass selection, family selection, or, best of all, best linear unbiased prediction (BLUP) breeding values combining the available information. With the very high reproductive rate of fish and the relatively low cost per individual, when deemed necessary, it should be possible to set up families for specific purposes, such as evaluating for disease resistance or for flesh quality.

Individual identification (unique and at an early age) of animals and their parents is one area that is likely to impact upon the genetic evaluation system adopted. Developments in DNA technology (DNA fingerprinting) could be of great assistance. This could be an area worthy of consideration in future research and development proposals.

### *Selection of brood stock and mating systems*

Ideally, scientists would only reproduce the “best” individuals. In practice, they need a compromise between selection intensity and effective population size in order to manage risk (inbreeding). The increase in inbreeding is proportional to  $1/2N_e$ , where  $N_e$  is the effective population size. A relatively large  $N_e$  is required to:

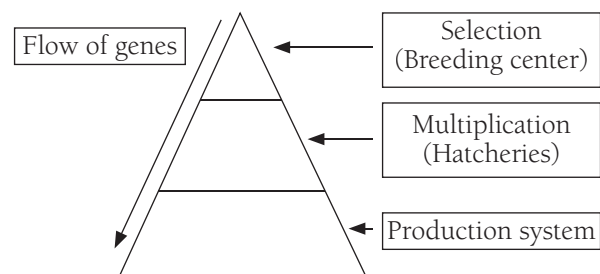
- (i) Sustain long-term genetic variation in the population
- (ii) Manage inbreeding
- (iii) Increase the selection limit
- (iv) Ensure predictable responses to selection

For situations where mass selection is used, Bentsen and Olesen (2002) suggest a minimum of 50 pairs to maintain approximately a 1 per cent increase in inbreeding per generation. With full pedigree information, inbreeding can be managed more effectively, avoiding matings of closely related individuals. When full pedigrees are not an option, sub-dividing the population can help, so that animals can be selected from the various sub-populations.

An aspect that may be worth considering is the establishment of one or more replicates of the selected population for security reasons, in case it was destroyed by disease or some other disaster.

### *Design of a system for expansion*

Genetic improvement typically takes place in a very small fraction of the population. The improvement is achieved in that the “elite” of superior animals is multiplied and disseminated to the production systems. The flow of genes is graphically illustrated in Figure 1.



**Figure 1. Flow of genes from the breeding Center to the production system.**

Fish are very well endowed with their high reproductive efficiency, to develop cost effective structures for the dissemination of genetic gain. The implementation of the genetic improvement program in a relatively small number of animals can be enough to service a very large population involved in production.

Unfortunately, experience shows that when a successful strain is developed and a market for it flourishes, malpractices often proliferate, facilitated by the very high reproductive rate of fish, and stock quality deteriorates as a consequence of inbreeding and small population size. There is no simple way out of this, except perhaps through the creation of a formal structure that is not only technically sound, but also regulates the process and enables the implementation of quality assurance practices. Figure 2 illustrates in a diagrammatic form important considerations that should be made when planning and putting in place a logically based system for the dissemination of improved stock of aquatic species.

*There are options, but we have to make considerations about...*

- ❖ **The resources available**
  - » Staff
  - » Facilities
  - » Capital
  - » Operating location
- ❖ Competence or access to them
- ❖ Size and other characteristics of the industry to be serviced
- ❖ Industry level in terms of technology application and education of members

*Options for multiplication*

- ❖ Through Government stations (often limited in their impact)
- ❖ With participation of private operators
  - » Joint ventures
  - » Licensing of hatcheries
  - » Contracted production
- » Sale of breeders to hatcheries with few conditions
- » Combinations of the above

*Creation of a network of hatcheries*

- ❖ Terms of the agreement
  - » Financial
  - » Operational
- ❖ Training and education of hatchery managers
  - ❖ A brand name for successful marketing
    - ❖ Product standards
    - » Fingerling size and survival
    - » Transport and accounting
    - » Management of inbreeding
    - » Breeders' age (lag)
  - » Lag and options for refreshing
    - ❖ Genetic piracy

**Figure 2. Considerations to be made during the planning and putting in place of a formal scheme for the dissemination of improved stock from breeding centers to fish farmers.**

In designing the system for expansion, the characteristics of the production system have to be taken into consideration again. For instance, if single sex or infertile populations are preferable for production, hormonal treatment in the production system, or chromosome manipulations (e.g. creation of YY males) may be needed in the multiplication phase. The lag created by additional generations before the animals reach the production phase has to be taken into consideration.

The relative sizes of the population sectors involved in selection, multiplication and production should be examined and made consistent with an effective transfer of genetic gain to the production sector.

### *Monitoring and comparison of alternative programs*

Monitoring the genetic improvement program is important to ensure that the anticipated genetic gain is actually achieved. If it is not, action has to be taken to rectify the situation.

Genetic gain can be measured in a number of different ways. The establishment of randomly selected populations is a useful way, particularly when the visual impact created by the comparison of the “selected” vs. “unselected” populations is considered important in increasing the adoption or credibility of results. However, the maintenance of control populations requires funds and effort.

When the visual impact is not a high priority, genetic gain may be estimated using appropriate statistical procedures that rely on the presence of genetic links between generations, instead of establishing control populations. These genetic links enable the estimation of genetic and environmental trends over time. This is an option that could be explored in current and future projects.

There will often be sensible alternatives in the program steps 1 to 7. Generally, testing all of such alternatives in the field will not be possible, but we could conduct theoretical and numerical work to predict likely outcomes. For instance, we may be interested in assessing the consequences of including or ignoring a particular trait in the breeding objective, or in comparing the merit of a single breeding objective in a range of production systems, or in evaluating particular sources of information as selection criteria in the genetic evaluation of animals. At present, there appears to be no work along these lines, but this is an area worthy of consideration in future planning. Sometimes this type of work helps uncover opportunities to increase the effectiveness of the genetic improvement program, or of saving costs and effort.

### **What sort of response can selective breeding achieve?**

Provided there are: 1) abundant genetic variation in the base population, 2) selection for a well-defined, heritable trait(s), and 3) maintenance of genetic variation by controlling inbreeding and avoiding small population sizes, scientists can then expect genetic gains as shown in Table 1. The gain in growth rate experienced in the case of GIFT fish is shown geographically in Figure 3.

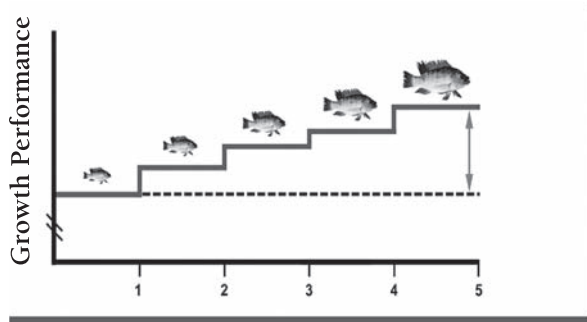
**Table 1. Realized responses to selection in growth rate in three species of fish.**

Species	Gain per generation %	Number of generations
Atlantic salmon	12.0	6
Nile Tilapia (GIFT)	15.0	5
Rohu carp	17.0	3

effects. A number of successful examples exist. Furthermore, if such genetic improvement programs are followed up with effective dissemination strategies, they can result in a highly positive impact on farmers' income.

In the short and medium term, aquaculture genetic improvement programs will be best served by judicious use of proven technology (i.e. based on quantitative genetics), and gradual incorporation of new technologies (e.g. MAS), as evidence on their usefulness becomes available from research, development and validation.

Accumulated selection response



**Figure 3. Genetic gain in GIFT fish over five generations.**

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## Concluding remarks

Selective breeding is a genetic technology that can provide continuous improvement of a fish population. Other technologies (e.g. gynogenesis, hybridization, triploids) should not be looked upon as alternatives, but as supplementary to selective breeding. The genetic improvement procedures recommended and implemented by the WorldFish Center utilize naturally occurring genetic variation. In otherwise sustainable aquaculture systems, selective breeding offers great opportunities without undesirable side



# Lessons from established breeding programs: Terrestrial and aquatic animals

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## Abstract

Some relevant components of selection program theory and implementation are reviewed. This includes pedigree recording, genetic evaluation, balancing genetic gains and genetic diversity and tactical integration of key issues. Lessons learned are briefly described – illustrating how existing method and tools can be useful when launching a program in a novel species, and yet highlighting the importance of proper understanding and custom application according to the biology and environments of that species.

## Introduction

Livestock breeding programs have a long and continuing record of success at making useful genetic change in commercially important traits. However, in many cases there remains a challenge to make genetic change that is relevant to the target production system(s), and to make effective dissemination of the resulting genetic material.

## Where to go?

In its most basic form this is a question about what type of animals we want to generate through genetic change. It is important to specify the environment(s) and production system(s) in which we want the developed stock to perform. Genotype by Environment Interaction can be important. Specifically, the environment in which we conduct breeding programs is often higher quality, more controlled and less stressful than those in which the bulk of production takes place.

We can define breeding directions by specifying rationally derived economic weightings for each trait of importance, or we can use a desired gains approach to explore the range of possible outcomes. A combination of these two is often the best route.

## How to get there?

This is all about how to make genetic change in the desired directions. The main tools for animal breeders have been selection and crossbreeding, but there is a wide range of issues to be accommodated when implementing these. New systems to integrate these issues in an implementation framework are now being used in progressive breeding programs. Molecular genetic technologies are now being

implemented—but they still act as a supplement to more classical methods.

This brief paper will review established and emerging selection systems for making genetic change, with some reference to both terrestrial and aquatic animal industries.

## Genetic evaluation – a refresher

### Background

The phenotype, or trait values, of an animal is influenced by the genes that determine an animal's predisposition to perform within the prevailing environment. The environment itself affects the way in which genes are expressed. A breeding program aims to improve the average phenotype of a population by improving the average genetic merit in successive generations. When evaluating candidate parents for selection, an animal's superiority is therefore measured in terms of its genetic merit, in particular, the component of its own genetic merit that can be transmitted to its offspring. This heritable component is known as an animal's breeding value, and is the value of an animal's genes to its progeny. For a particular trait, the variation between breeding values as a proportion of the phenotypic variation is known as the trait's heritability.

As true breeding values are difficult to measure, predictions of their value are used to rank animals as candidate parents. Different criteria exist for predicting true breeding values, and these differ in their accuracy and associated costs. In some cases, an animal's own phenotypic record is an efficient predictor of its breeding value. Alternatively, criteria can be used which incorporate records from individuals and their relatives. The genetic component of an animal's phenotype includes a fraction of genes that are

identical, by descent, to related individuals. The size of this fraction is proportional to the degree of relationship, e.g. animals with common parents (full-sibs) share, on average, half of their genes. The requirement to record an animal's pedigree increases the cost of selection, but knowledge of the proportion of shared genes between individuals, and records on their phenotype, increases the accuracy of prediction for the individuals involved.

## **Selection methods**

Once a decision is made to initiate a selective breeding program to exploit a population's genetic variation and increase productivity, the type of selection program to best serve the needs of the industry and give the best results must be chosen. In the current context, there are four basic methods of selection:

### *Individual selection (or mass selection)*

Individual selection is based solely on phenotypic records. It is simple and low cost, as this method does not require specialized systems for recording identity and pedigree. Difficulty in recording identity in aquatic species has made individual selection popular, however, the traits for which selection can be applied are limited to those that can be directly measured on the live individual (e.g. not really suitable for carcass quality and disease resistance traits). The accuracy of prediction is determined by the heritability, such that for a highly variable environment, individual phenotype is a poor estimate of breeding value. Thus it is only really effective when the heritability is at least moderately high. The absence of family information in a proper mate selection program increases the risk of deleterious inbreeding - but selecting a large number of parents may offset this.

### *Between-family selection*

Between-family selection predicts the mean breeding value of each family from its phenotypic mean. Families are treated as homogeneous groups so that each family member has the same estimated breeding value. Families are selected as whole groups and so individuals used as parents are chosen at random from the superior families. In general, the rate of response is slow when selecting on family means. However, when the heritability and common environmental variation are low, rates of response are much higher than individual selection. This method also allows for the selection of traits that may only be measured on slaughtered animals, e.g. flesh colour or fat percent. The mean of records taken on slaughtered animals can be used to estimate breeding values for the remaining family members. There are similar advantages for disease resistance traits.

### *Within-family selection*

Within-family selection predicts the breeding value of an individual by the deviation of its phenotype from its family mean. Animals that exceed their family mean by a certain amount would be selected as parents. This method has the greatest value when environmental effects are common to members of a family but different between families, e.g. families kept in separate tanks or pens. Without need to replicate family tanks, this method reduces the size of a facility required to run a breeding program, and with particular mating strategies, can help lower the rate of inbreeding.

### *Combined selection*

Combined selection is a method of evaluation that can incorporate information on an animal's breeding value from several sources. The simplest example is the weighted sum of within- and between-family records, where weights are derived from the heritability and the degree of relationship of individuals within- and between-families. This concept can be extended to include records from more distantly related individuals where each new source of information is appropriately weighted. Increasing the number of records from different relatives increases the accuracy of prediction above that of other methods. The general method used to predict breeding values from the information of many different relatives is known as BLUP (Best Linear Unbiased Prediction). In addition to the use of information from different relatives, the accuracy of prediction is increased by the capacity of BLUP to correct for environmental effects. Therefore, the ranking of candidate parents on EBVs (Estimated Breeding Values) permits selections to be made from a common base across different families, environments and year-classes or cohorts.

BLUP EBVs are the criterion of choice for ranking candidate parents. However, their efficient estimation relies on accurate pedigree records. The cost of keeping such records is marginally different to that of family selection, but the higher accuracy, control of inbreeding and ability to monitor genetic trends makes BLUP selection much better than individual selection. Furthermore, the cost of DNA pedigree recording continues to drop.

## **The importance of pedigree data**

Probably the most fundamental question to ask when designing an aquatic breeding program is whether to record pedigree. This is sufficiently important that it deserves further comment.

Recording the identity of parents of fish in a breeding



operation is neither cheap nor easy. There are costs involved in either maintaining family tanks or in DNA fingerprinting, and fish marking and in data management. There are also potential compromises in production efficiency within the breeding program stock.

Given this, some real benefits from pedigree recording would be expected if it is to be worthwhile. Such benefits include:

#### *Higher selection accuracy*

Knowledge of pedigree, especially sire pedigree, has classically been seen as important information to provide to seedstock buyers in terrestrial species. This has not been for any rigorous technical reason, so much as a feeling that good sires leave good progeny - that like begets like. This was recognised by Charles Darwin in 1852 who stated that:

*“ ... the importance of the principle of selection in regard to Merino sheep is so fully recognised, that men follow it as a trade. The sheep are placed on a table and are studied, like a picture by a connoisseur; this is done three times at intervals of months, and the sheep are each time marked and classed, so that the very best may ultimately be selected for breeding.”*

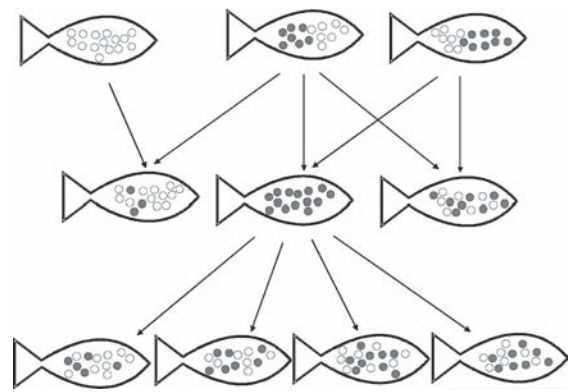
Modern geneticists are luckier than Darwin because they know about genes and how they are transmitted. This means that information from relatives can be used to help evaluate individuals for their breeding value.

It is desirable to select the fish with the best genes because they will leave the best progeny. Relatives share some of their genes – for example full sibs (which share the same father and mother) share half their genes in common (see Figure 1). This means that how well a fish's relatives perform tells us something about the quality of that fish's own genes. Modern genetic evaluation analyses manage to balance the information from relatives to make the best estimates of breeding value (EBV). This results in faster genetic gains, most especially for traits with a low heritability - typical of disease resistance traits.

Pedigree information is also needed to estimate heritabilities and genetic correlations. These parameters can be used to help design more effective breeding programs and give more **accurate EBVs**.

#### *Genetic links between different grow-out sites*

In order to identify the best genes it is necessary to separate the merit due to favourable environment, nutrition and management from the merit due to good genes. Comparing the same genes at different sites can do this. But this does not mean having to raise



**Figure 1. The fish in the middle of this pedigree shares his genes with his relatives (shaded circles represent the genes that this fish carries). So the performance of these relatives helps us to estimate the value of this fish's genes. The more distant the relationship, the lower the proportion of genes shared.**

the same individual fish at different sites. Looking at Figure 1, it can be seen that the same genes exist in fish that are related. So if the performances of two half-brothers (same cock, different hens as parents), one at each of two grow-out sites, are compared, this gives a basis to separate genetic differences from environmental differences.

The tool to do this is BLUP genetic evaluation. It accounts for factors such as the fact that one of the brothers might have had a better quality mother. Of course more linkages than that provided by just two brothers are needed. But given this, BLUP will result in EBV's across farm sites which have taken account of the differences between sites in these confounding effects of environment, nutrition and management schedule.

This connection across sites requires pedigree recording - so that relatedness of fish in the breeding program at different sites is known. However, this gives a rational approach to exploiting breeding stock and lines across the whole industry.

A related benefit is the highly relevant and potentially accurate evaluation of outside stock that may possibly come to be imported. This also requires pedigree recording.

#### *Helps guard against inbreeding*

Development of accurate genetic evaluation systems giving EBV's across sites, could lead to the excessive use of excellent individual fish and their close relatives. This has been seen in domestic land animals - as breeders put more faith in EBV figures. Cases have been seen where most sires in a breeding population are the sons of one top sire - whose semen is also being used widely!

If pedigree recording is not available, inbreeding can only be avoided by reducing selection intensities. However, with pedigree recording, there is considerable power to ensure fast genetic gains while keeping inbreeding levels and rates at a low level, as described later. This also ensures maintenance of the genetic variability to give sustained genetic gains well into the future.

*Enables selection on traits not measurable on live fish*

Pedigree recorded fish that have carcass traits measured or are involved in disease resistance tests provide data to estimate EBVs on their live relatives that are candidates as seedstock.

**Pedigree recording**

There are two main options for recording pedigree:

*“Family tanks”:* The classic option for pedigree recording in aquatic animals that are difficult to tag is to keep full-sib families of fish in separate tanks until they can be tagged. The number of families is limited by facilities, typically between 50 and 500 families are bred per year.

*“DNA pedigreeing”:* The newer option is to use DNA fingerprinting. Over time, costs will reduce. The technology is being used in a number of aquatic breeding programs.

DNA pedigreeing has some technical advantages over family tank designs:

- (i) Fish can be mixed at any time, even as newly fertilized eggs. If possible, it is even permissible to mix sperm (or eggs) from different fish before fertilization, although this leads to some loss of control of selection pressure and design. This early mixing avoids confounding of family genetic merit with tank effects, which can be considerable. The result is more accurate selection, especially for family-based measures such as disease resistance and carcass traits. (Estimated 10 percent to 20 percent gain)
- (ii) Cross-classified mating means that many more families can be generated. With 100 parents of each sex, up to 10,000 families can be generated. This gives a richer pedigree design (individuals have maternal half-sibs as well as paternal half-sibs), leading to more accurate EBVs and more gains. It also gives more information on non-additive (or ‘nicking’) effects, and more power to estimate parameters such as heritability from data on the resulting progeny. (Estimated 5 percent to 10 percent gain)

- (iii) It may also allow a more commercially-typical rearing environment for fish in the breeding program, making the measurements taken more relevant and useful. (Estimated 10 percent gain)

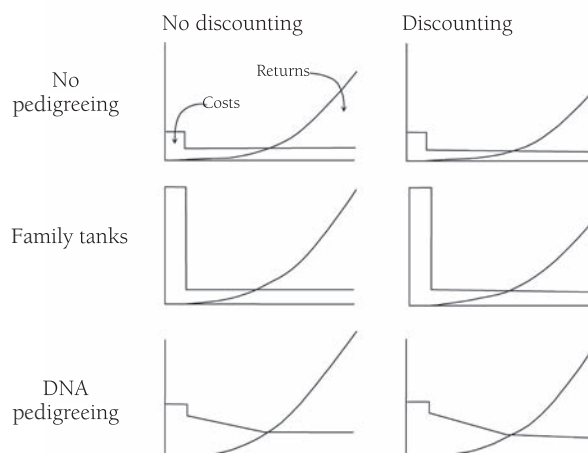
Considering the cost of tissue sampling and genotyping progeny, family sizes will be reduced and this will constitute a component disadvantage (estimated 5 percent). However, there are some clever designs that help to manage costs while achieving a good response.

On balance, considerably more response to selection is likely to be achieved using the DNA pedigreeing approach.

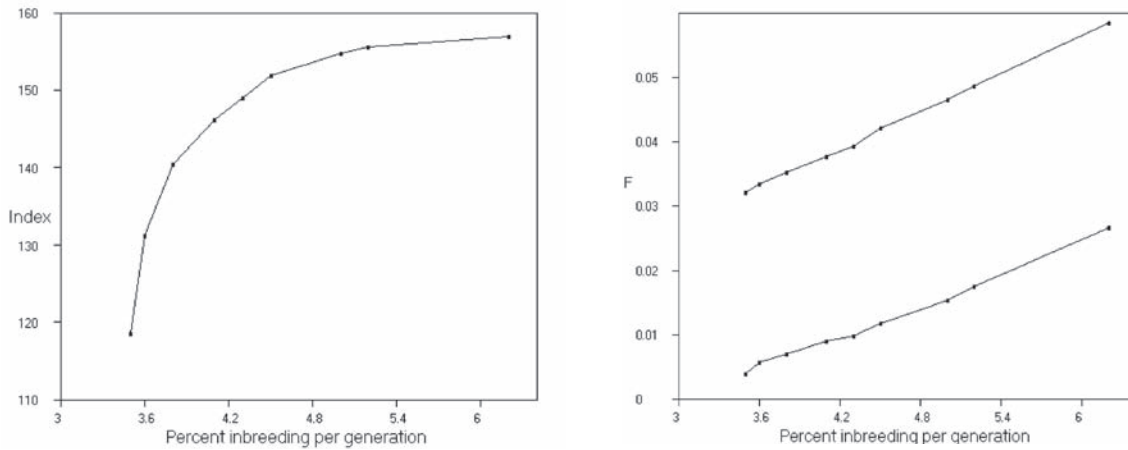
For simple illustration, three options for a breeding program design are to be considered:

- Individual or mass selection (no pedigree)
- Family tanks
- DNA pedigree

The three options have different cost profiles, as shown in Figure 2.. These diagrams are not drawn to scale - it is the pattern that is important here. The time scale is probably about 4 or 5 generations, and returns will likely be generally much higher than indicated when integrated across an industry. Capital costs are incurred early and returns come late, which makes the profiles less favourable when discounting future dollars is undertaken. Ongoing costs for DNA pedigreeing are assumed to decrease in real terms over the next several years.



**Figure 2. Three options for a breeding program design.**



**Figure 3. An example of the balance among genetic gain (Index), inbreeding rate per generation, and inbreeding level in progeny (F).**

### Maintaining diversity

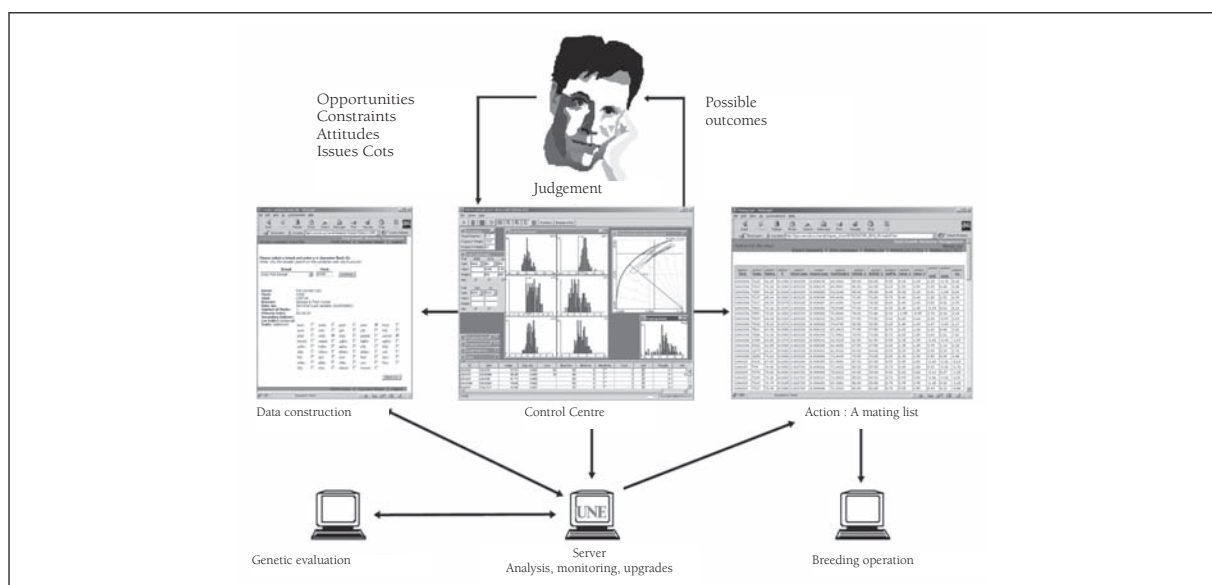
The left pane of Figure 3 shows the range of options for a breeding program at the stage of making selection and mating decisions. ‘Index’ is a single score covering all traits. The connected points in the figure are possible outcomes predicted for progeny generated from the selections and matings made. Maximum gain reflects emphasis on selection of fewer parents chosen from the best few families – and this gives the highest long-term inbreeding risk (on the horizontal scale).

On the other hand, avoiding inbreeding by selecting across families also leads to lower gains. We want high index values but low inbreeding values, and the curve in the left pane of the figure is the frontier of optimal outcomes, given differing emphasis on genetic gain and long-term inbreeding. A key task is

to decide where on this frontier we want to.

Long-term inbreeding is effectively the same issue as genetic diversity. Using more parents and/or less related parents gives more diversity and less inbreeding in the longer term. We can also avoid inbreeding in the short-term by minimizing the relationship between fish that are mated to each other. The right pane of the figure shows how the level of inbreeding in progeny conceived (F) can be reduced from the upper line (random mate allocation) to the lower line (minimum relationship mating, using full pedigree information). Results are lower to the left of the graph, as more sires are used giving more opportunity to avoid mating relatives.

This approach gives power to monitor and control the balance between genetic gain and genetic diversity.



**Figure 4. A mate selection system that allows dynamic viewing and choice of outcomes – Total Genetic Resource Management (see [www.xprime.com.au](http://www.xprime.com.au)).**

**Table 1. Some Animal Breeding issues.**

<p>Selection on EBV Genetic diversity Optimal contributions Progeny inbreeding Limits on reproduction Logistical constraints Marker Assisted Selection Multi-stage selection Breeding population size Crossbreeding</p>	<p>Connection between sub-pops. Corrective mating / trait distribution Multiple objectives / line splitting Scheduling to meet demand Seedstock dissemination Quarantine barriers Other health management issues Use of reproductive technologies Costs Funding limits</p>
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### Mate selection - an integrating approach

Breeding program design can be pre-determined and implemented through sets of rules, or it can emerge as a consequence of decisions made at the level of individual matings. This latter is the tactical approach, with decisions made tactically in the face of prevailing animals and other resources. It has recently been taken up in the running of progressive breeding programs in sheep, beef, dairy, pig, poultry and some aquatic breeding programs (TGRM, illustrated in the Figure 4).

Tactical implementation of breeding programs provides a practical means to integrate technical, logistical and cost issues facing animal breeders. Moreover, tactical implementation benefits from opportunistically optimal use of prevailing animals and other resources, resulting in better outcomes.

In any breeding operation, there is an almost infinite range of actions – selections and matings, or “mate selection sets” - that can be made, involving decisions on issues such as those shown in Table 1.

Each mate selection set is predicted to have a given utility to the breeder - based on outcomes for these various issues. The tactical approach works by searching across these possible routes ahead, and finding one that is predicted to suit the breeder's needs, either the very best solution, or something sufficiently close to it. This has only recently become possible because of the development of efficient computing algorithms that mimic evolutionary processes to approach the best solution.

### Lessons learned

Genus has a long history in many countries of running progressive breeding programs in pigs (through the Pig Improvement Company, PIC). The systems and know-how built up have formed a basis to develop both classical and novel approaches to breeding programs in shrimp (through SyAqua, in Hawaii, Mexico, Brasil, Thailand and recently in Kentucky). Genus also has R&D programs for genetic improvement in other species.

### What lessons have been learned?

- Breeding programs have many elements in common across species, including:
  - Every animal has a father and a mother, with few exceptions.
  - The genetic information systems outlined in the appendix can be the same or similar across species.
  - All breeding programs need to define target directions/outcomes for genetic change, and achieve an optimal balance of fast genetic gains and conserved genetic diversity.
  - Many aspects of optimizing breeding program design are common across species. The best designs can be very different, but the underlying design methods and tools can be the same or similar.
  - The task of discovering genetic markers and mutations that are useful in breeding programs is very similar across species. The building of a “gene discovery pipeline” has given dramatic improvements in speed and cost-effectiveness across four key species, to date.
  - Designs for testing carcass and disease resistance traits have many aspects in common.
- Breeding programs have many key elements that differ across species, including:
  - Reproductive systems, levels, and behaviour can differ considerably across species, affecting ability to control and synchronize matings, manage mating ratios, preserve gametes, boost reproduction, generate polyploidy etc.
  - Other aspects of life-cycle differ, affecting the optimal timing and pattern of mating events and the optimal population structure.
  - Ease of tagging individuals can differ considerably, affecting the optimal balance, in both pattern and extent, of use of DNA pedigreeing, family containment and other pedigreeing/family evaluation strategies used.

- o Production environments can differ dramatically, especially for aquatic species, so that we must pay specific attention to development of performance under a range of conditions.

When launching breeding programs in novel species we have the double task of exploiting existing tools and know-how, while having a good understanding of those factors that make this species require custom systems and breeding management. With the ongoing emergence of aquaculture, a number of organizations are gaining experience in this double task. It can be achieved in a sufficiently large organization with an R&D chain that comprises connected teams in groupings such as:

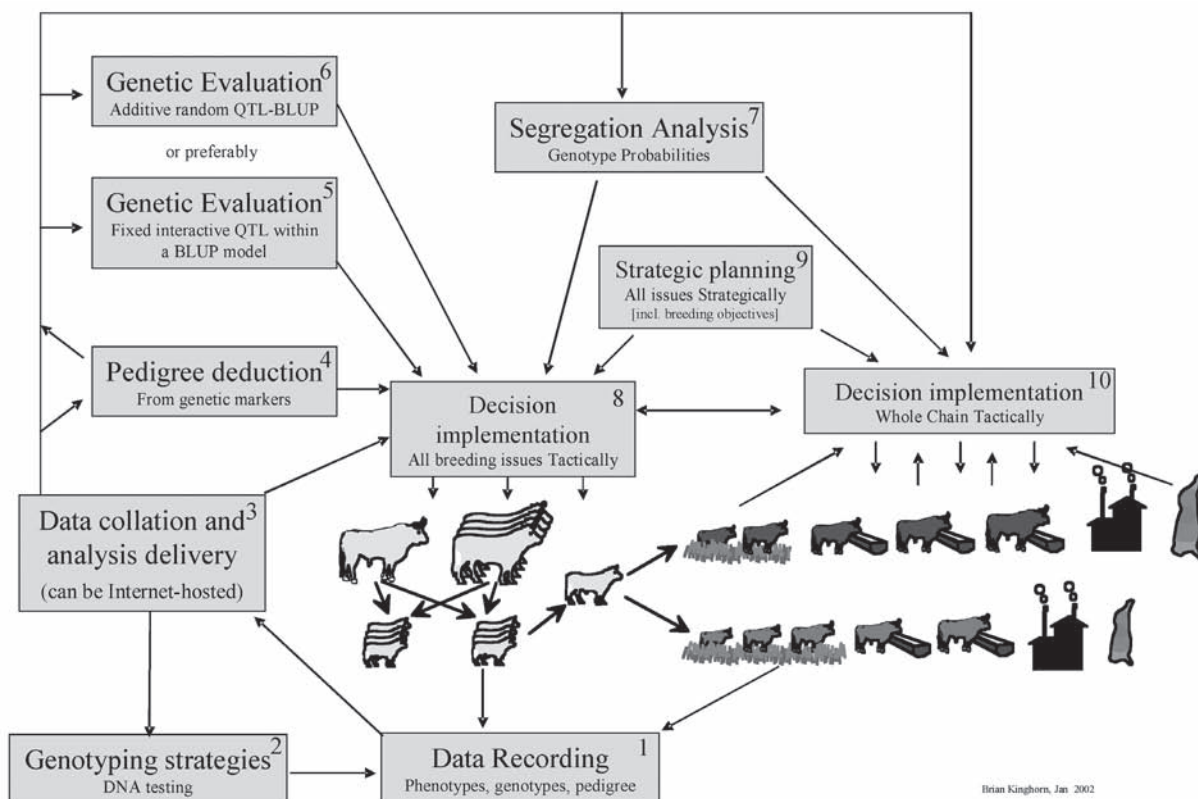
1. Development of fundamental quantitative and molecular methods and core tools across all species
2. Development of applicable components that are as species-specific as required: databases, genetic evaluation, mate selection, etc. (see Appendix).
3. Implementation of breeding programs in teams dedicated to one or a few species, using tools and support from all parts of the chain.

The closer to practical application, the more species-specific activities become.

The question can be asked: Should we just run simple programs, such as mass selection, in developing countries where resource and skill levels may be limiting? This is a valid point for many terrestrial species such as cattle, but the high fecundity of most aquatic species means that we can concentrate high-quality breeding effort in relatively small and contained breeding programs, at a relatively low cost. The biggest challenges remaining may then be data recording at field test sites in different environments and dissemination of genetically improved stock to industry.

### Appendix : Information system

1. Data Recording. This is a key component. In some cases, special tools and methods are required to make measurements, especially for traits related to carcass quality and disease resistance. Robust and accessible databases are critical to exploitation of progressing approaches such as mate selection.



Some Key information systems in animal breeding.



2. Genotyping Strategies. Genotyping is becoming increasingly widely practiced, with applications using both genetic marker loci and known gene loci. Inferring genotype from the known genotypes of relatives and/or linked loci has the potential to play a useful role in reducing costs of tissue sampling and genotyping. Segregation analysis, described below, can be used for calculating genotype probabilities. These in turn can be used in an iterative genotyping strategy – they are used to help choose which individuals and loci to genotype in each iteration.
3. Data collation and delivery. Our experience is that the Internet facilitates very effective distributed deployment of services using operators located close to end-users/customers. Internet hosting also provides opportunities for technical support, and a simpler path to scaling up operations.
4. Pedigree deduction. Good method and software can be used to solve complex parent-allocation problems – such as to deduce the parents of progeny out of a syndicate mating of tens or hundreds of parents.
5. Genetic Evaluation – Fixed interactive QTL within a BLUP model. Direct or ‘diagnostic’ markers are simplest to use here, as we can treat them as fixed but interacting effects. For linked markers, we can modify transmission probabilities in segregation analysis to calculate QTL genotype probabilities.
6. Genetic Evaluation – Additive random QTL BLUP. This is increasingly being used for genetic evaluation where genetic marker information is available. It is a relatively simple extension of classical method. However, it aims to more accurately evaluate the average genetic merit of individuals for given traits, and misses the added opportunities to exploit the known mode of action of discovered genes, and the interactions among them that we increasingly find to be important.
7. Segregation analysis. This type of analysis is key to a number of genetic information systems, including items 2, 4, 5 and 8 in this list.
8. Tactical Decision Implementation for breeding. As described above. This integrates technical, logistical and cost issues affecting breeding decisions into a single framework.
9. Strategic Planning tools. Integration of a range of design evaluation and planning tools into a single project-planning framework.
10. Decision Implementation for whole supply chain. Design in animal breeding and production programs is classically implemented through sets of rules to follow. However, a tactical approach uses all prevailing information to develop an action report that dictates management decisions directly.

# Potential Applications of Reproductive and Molecular Genetic Technologies in the Selective Breeding of Aquaculture Species

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## Abstract

The use of reproductive and genetic technologies can increase the efficiency of selective breeding programs for aquaculture species. Four technologies are considered, namely: marker-assisted selection, DNA fingerprinting, in-vitro fertilization, and cryopreservation. Marker-assisted selection can result in greater genetic gain, particularly for traits difficult or expensive to measure, than conventional selection methods, but its application is currently limited by lack of high density linkage maps and by the high cost of genotyping. DNA fingerprinting is most useful for genetic tagging and parentage verification. Both in-vitro fertilization and cryopreservation techniques can increase the accuracy of selection while controlling accumulation of inbreeding in long-term selection programs. Currently, the cost associated with the utilization of reproductive and genetic techniques is possibly the most important factor limiting their use in genetic improvement programs for aquatic species.

## Introduction

Selective breeding in aquaculture species has been very successful, averaging a genetic gain of 10 to 20 per cent per generation (Ponzoni et al. 2005). Such progress has been achieved through the application of quantitative genetics and statistical methods, whereby genetically superior animals are identified, based on their own performance or that of their relatives. Recently, the advent of molecular genetics has opened possibilities for direct selection of animals on genotype or, alternatively, selection based on linkage associations between markers and quantitative trait loci (QTL). During the last decade, efforts have been made to enable the incorporation of molecular genetic information in practical genetic improvement of both plants and animals. However, the benefits from the use of these technologies will not be fully realized unless the cost of genotyping is reduced (Dekkers and Hospital 2002). By contrast, reproductive technologies, especially artificial insemination and in-vitro fertilization (IVF), have significantly increased the rate of genetic improvement and have had a large impact on the breeding structure of livestock species (e.g. Nicholas 1996; Kinghorn et al. 1999; van Arendonk and Bijma 2003). For aquaculture species, these areas of research have been barely touched upon, and their application to selective breeding programs has been very limited. The objective of this paper is to present some thoughts on four technologies that are considered to have potential for current breeding programs in carps and tilapia, namely: marker-assisted selection, DNA fingerprinting, in-vitro fertilization, and cryopreservation.

## Application of molecular information

### Marker-assisted selection

The usefulness of molecular information in genetic improvement programs depends on advances made in four main areas of research: molecular genetics (genetic markers and linkage maps), genes and quantitative trait loci (QTL) detection, genetic evaluation systems, and marker-assisted selection. So far, genetic maps have been constructed for tilapia (Kocher et al., 1998; Lee et al. 2005), common carps (Sun and Liang 2004), rainbow trout (Young et al. 1998; Sakamoto et al. 1999; Nichols et al. 2003), Atlantic salmon (Moen et al. 2004a), kumara prawn (Moore et al. 1999; Li et al. 2003), *Peneaus monodon* (Wilson et al. 2002) and catfish (Lui et al. 2003). However, only a limited number of studies have found QTL affecting cold tolerance (Cnaani et al. 2003) and salinity tolerance in tilapia (Lee 2003), cold tolerance in common carps (Sun and Liang 2004), infectious pancreatic necrosis in rainbow trout (Ozaki et al. 2000), infectious salmon anemia in Atlantic salmon (Moen et al. 2004b), thermal tolerance (Perry et al. 2001), development rate (Sundin et al. 2005) and pyloric caeca number (Zimmerman et al. 2005) in rainbow trout. To the best of our knowledge, there have not been any causative mutations or candidate genes controlling performance and production traits reported in aquatic species. Hence, the potential for direct genotype-assisted selection (GAS) or introgression-assisted selection (IAS) cannot be realized at this stage, although in theory the IAS method could be carried out with informative markers. By contrast,

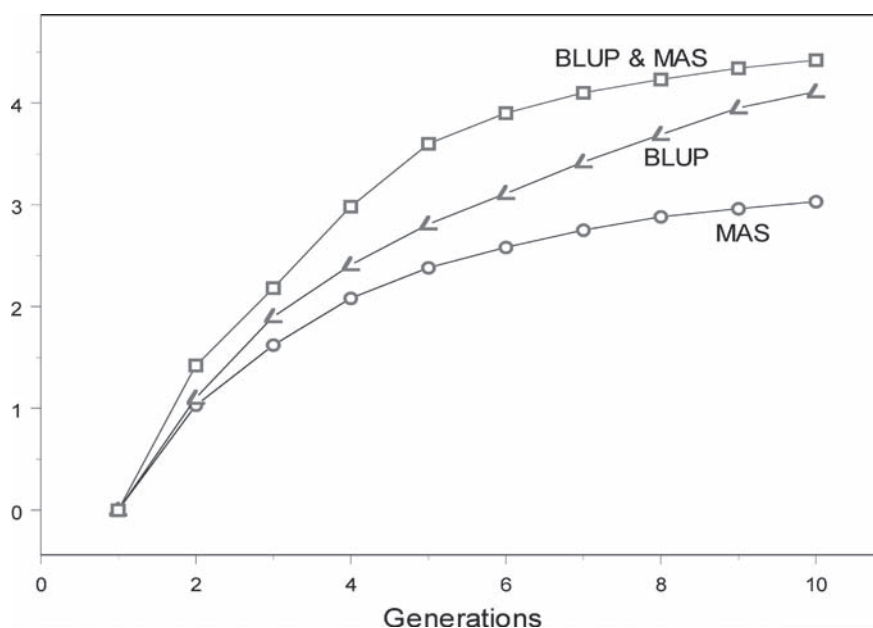


several direct DNA tests have been developed in plants and animals; in both cases, the application has focused on direct genetic markers.

Based on linked markers published for aquaculture species in the literature, there are two possible uses of marker-assisted selection (MAS): in cross populations between inbred lines, and within strains (Dekkers 2004). For each of these methods, three strategies can be employed, namely: 1) selection on estimated breeding values (EBV) derived from markers alone (MAS), 2) selection on markers-based EBV first and then on polygenic EBV, and 3) index selection combining both QTL-EBV and polygenic-EBV (COMB).

**MAS for crosses between inbred lines:** As firstly proposed by Lande and Thompson (1990), Zhang and Smith (1992) compared three strategies: selection on marker score alone (MAS), BLUP selection (only polygenic EBV) and index selection combining both markers-based EBV and polygenic EBV (COMB) in an F2 generation population, with 100 markers in a 2000 cM genome. Genetic gain was the highest with combined selection on both QTL-EBV and polygenic-EBV (COMB), followed by BLUP, and the lowest with MAS (Figure 1). The rate of response to MAS decreased over generations because recombination (crossing-over during meiosis) caused an erosion of the association between markers and QTL in the low density map used. The MAS strategy has potential for selection of traits that are difficult to measure (e.g. flesh quality) because it does not require extensive phenotypic recording. For aquaculture species, inbred lines are seldom available and when they are, they have very low fitness. Hence, at present, this approach is not of practical value in aquatic animal improvement programs.

**MAS within strains:** This method has potential of selection for traits that are measured on slaughtered animals (flesh quality) or traits that are recorded in only one sex (sexual maturity in female) (Table 1). The efficiency of MAS within strain is largely dependent on heritability of the interested traits, size of QTL effects and recombination rate, increasing for lowly heritable traits and with the proportion of the variance explained by the QTL (Meuwissen and Goddard 1996). The advantage of MAS selection, however, decreases over generations due to fixation of QTL and loss in polygenic response. Despite high efficiency expected from theoretical prediction (2 to 60%), this method of selection requires extensive recording of both phenotypic and genotypic data for several generations prior to selection in order to accurately estimate QTL effects. In addition, prior knowledge of QTL regions that segregate within the population limits its application to the currently existing breeding programs in tilapia or carps because QTL mapping studies need to be conducted prior to implementation of MAS. In freshwater finfish, flesh quality is not a trait of a primary emphasis and the fish are priced on live body weight in all countries participating in International Network on Genetics in Aquaculture (INGA). Hence, breeding objectives for farmed finfish have mainly focused on improvement of body weight at harvest or growth-related traits. For disease resistance, most of the species, especially tilapia, are generally disease free if well managed, and highly adapted to local conditions. Nevertheless, epidemics occasionally occur in these species. Improvement of survival rate can be achieved by modification of environmental factors, such as better management, feeding or water quality. The benefits of including these hard-to-measure traits (flesh quality or disease resistance) by either conventional selection or MAS depend largely on their economic values.



**Figure 1. Genetic gain from three different selection strategies in an F2 population created by crossing between two inbred lines for a trait with heritability of 0.25 (Data plotted were extracted from Zhang and Smith 1992).**

**Table 1. Percentage of genetic gain from index selection combining EBVs for both QTL and polygenes relative to conventional BLUP selection (Derived from Meuwissen and Goddard 1996).**

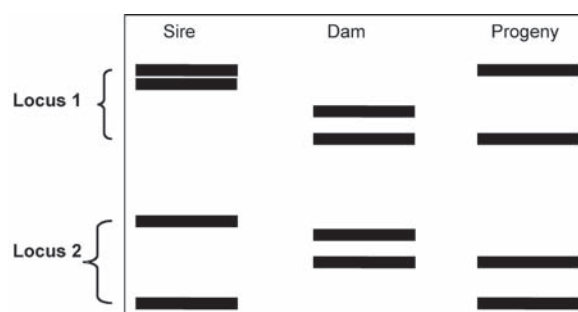
Generations	Types of traits measured				Heritability for BS and AS				% variance explained by QTL			
	CT	SL	BS	AS	0.1	0.27	0.1	0.27	6.7	13.3	26.7	46.7
1	64	38	38	9	21	9	45	38	5	13	29	47
2	62	37	30	5	17	5	36	30	5	12	23	40
3	55	31	25	4	13	4	34	25	4	10	19	33
4	39	21	15	2	6	2	23	15	4	7	12	25

CT = carcass traits, SL = sex limited traits, BS = traits measured before selection and AS = traits measured after selection

## DNA fingerprinting

DNA fingerprinting (Figure 2) can be used for genetic tagging and parentage verification, control of inbreeding, elimination of deleterious recessive genes, and prediction of heterosis. Genetic tagging and control of inbreeding are of practical significance in aquaculture breeding programs. The posterior assignment of parents and tracking origins of family allow pooling of all families from incubation, thus enabling communal testing very soon after hatching. This overcomes two major problems encountered in aquaculture species. First, both maternal genetic and common environmental effects (caused by separate rearing of full-sibs until they reach the size at which they can be physically tagged) can be avoided. Second, the number of tested families can be increased without the need for increasing facilities (e.g. tanks, ponds). As a consequence, the use of DNA markers is expected to increase genetic gain without a rapid accumulation of inbreeding. In breeding programs carried out under field conditions, tag losses may be as high as 5 to 35 per cent. An example of this is on GIFT tilapia (Ponzoni, pers. com). Although loss in genetic gain is yet to be quantified in aquaculture breeding programs, results in dairy cattle indicate that pedigree errors may reduce genetic gain by 3 to 10 per cent (Spelman et al. 2002). The loss in genetic gain is greater for lowly heritable traits than for highly heritable ones because the accuracy of EBV for traits with low heritability relies more on information from relatives' performance than an individual's own performance.

Pedigree analysis using microsatellite markers in general has a very high degree of accuracy. The use of between 8 and 14 microsatellites gives a 90 to 95 per cent chance of correct assignment of offspring to pairs of parents in mating designs involving 92 to 240 parental pairs (Estoup et al. 1998; Fishback et al. 2002 and Vandeputte et al. 2004). This result is supported by Villanueva et al. (2002) in a deterministic simulation study where four highly polymorphic loci developed for salmon are sufficient



**Figure 2. Analysis of DNA fingerprint for 2 loci.**

to assign 99 per cent of the offspring to the correct pair of parents with 100 crosses involving 100 males and 100 females. An additional marker is required for correctly assigning 99 per cent of the offspring when 100 crosses are produced from 10 males and 10 females. Both these experimental and theoretical results indicate that parentage identification is possible with the DNA markers currently available in several fish species.

However, the technology is still expensive for aquaculture species (cost of genotyping at 15 to 30 Euro per sample, Vandeputte 2003). Hence, cost-benefit analysis should be carried out to assess the economic desirability of incorporating this technology into breeding programs. For cattle and sheep in Australia, the acceptable price for a wide adoption of the DNA test by the industry is from 10 to 15 AUD<sup>a</sup> while the current rate is about 35 AUD (Kinghorn et al. 1999). It should be noted also that genetic tagging does not completely replace the need for physical tags, because the individuals need to be associated with both the parentage and the performance information for genetic evaluation and selection purposes. This would add extra costs to selection programs. The cost of genetic tag is expected to go down with the development of DNA chips, by which many SNP (single nucleotide polymorphisms) markers can be genotyped simultaneously and cheaply.

<sup>a</sup>1 USD = 1.39 AUD

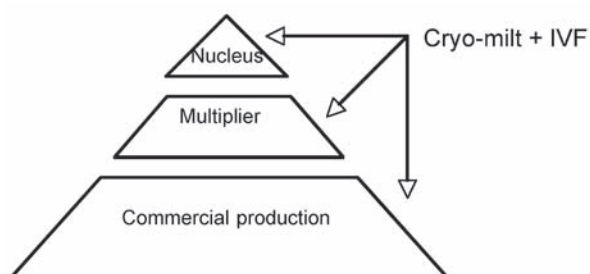
## Genetic characterization of strains

The identification of populations or strains with superior characteristics is one of the most critical steps before the commencement of selective breeding programs, especially of new aquaculture species. DNA markers can be used to identify genetically distinct populations. Characterization of genetic variation among populations in this way aims to group and to help determine strains to be included in strain evaluation trials. A mixed (synthetic) base population may then be established from the best performing individuals involving all strains in a diallel cross design. However, results with electrophoresis analysis in fish and terrestrial animals have shown that, despite the high level of homogeneity at molecular levels that are typical of many crustaceans, there are still markedly differences in production characteristics between the strains (e.g. Jones et al. 2000). A typical example is the large genetic variability in a performance trait such as live weight in the GIFT fish population undergoing several generations of selection (Ponzoni, unpublished results), although the observed heterozygosity ranged only from 0.026 to 0.071 in the founder strains (Macaranas et al. 1995). It is, therefore, concluded that genetic characterization of strains before assembling breeding population may be of some use in establishing that two or more populations are likely to be closely related, but it is of no value in terms of ascertaining performance or genetic variation for performance traits.

## Reproductive technologies

### In-vitro fertilization (IVF)

IVF has been successful in several aquaculture species and the technique is well developed and can be applied in the current breeding programs in carps and tilapia. The main advantage of IVF is that it allows design of different mating schemes in selection programs. In particular, the use of a factorial (complete, incomplete, by set, rectangular) design



**Figure 3. Roles of cryopreserved milt and IVF in a pyramid breeding structure.**

enables separation of additive, dominance, and maternal components of variance. This would give unbiased prediction of breeding values and would result in greater accuracy of selection. Compared with hierarchical mating, the factorial design results in greater genetic gain at the same level of inbreeding (Dupont-Nivet et al., in press). It should be noted that the increase in genetic gain with IVF is a result of an increase in the accuracy of selection and a decrease in the level of inbreeding, but not generation interval because in-vitro maturation of oocytes (immature eggs) has not yet been developed. Hence, the generation interval for females cannot be lower than the age at which the female fish reach sexual maturity. In practice, the cost of setting up an artificial incubator system to implement IVF in selective breeding is generally reasonable (Danting, personal communication). It is, therefore, recommended that the IVF technique could be incorporated in current genetic improvement programs for both carps and tilapia.

### Cryopreservation of milt

To date, preservation of eggs and embryos has not yet been possible in aquatic species (except for oysters and seabream). The species in which successful cryopreservation of milt has been achieved are listed in Table 2. The roles of cryopreserved milt in a traditional pyramid breeding structure are illustrated in Figure 3. In selection programs at nucleus level, cryopreserved milt and embryos may be used as a control to measure genetic gain with minimum bias. This is mainly because the frozen material can present a wider genetic base than a random unselected control of limited size, and there is no accumulative genetic drift over time. The improved genes of superior sires proven from the selection programs are then transferred to either hatcheries (multiplier) or producers (commercial grow-out production). In a number of species, e.g. Atlantic salmon (Salte et al. 2004) or oysters (Adams et al. 2004), cryopreserved sperm can be applied in practice. It is a safe way to disseminate the improved genes between herds or populations. As some species spawn once during their life (e.g. salmonids), or in the case of pink salmon that all spawn at two years old, cryopreserved milts can be introduced between populations to reduce the risk of inbreeding. In the future, once large-scale genetic evaluation is underway, cryopreserved sperm can be used to create genetic connectedness through a "reference sire" scheme. In this way, the genetic merit of all animals across herds (country) or years can be directly compared, ranked and selected as parents; thus, genetically superior broodstock can be identified and widely used. This approach has significantly increased the genetic gain in performance traits of farm animals.

**Table 2. Successful milt cryopreservation in aquatic species.**

Freshwater Finfish	Marine Finfish	Crustaceans	Mollusc	Invertebrate
Carp	Atlantic cod	Prawn	Oyster*	Sea urchin
Tilapia	Herring			
Catfish	Grouper			
Pike	Mullet			
Salmonids	Plaice			
	Turbot			
	Striped bass			
	Red snapper			
	Sea perch			
	Pacific bluefin tuna			
	Gilthead seabream*			

**\*Embryo cryopreservation successful**

In the GIFT project, the gene bank for cryopreserved milt was established and is still being maintained. Given availability of facilities and resources, a similar program should be initiated for the carps. Despite the potential benefits of the technique, commercial cryopreservation protocols for practical production are still uncommon in farmed aquaculture species, except for Atlantic salmon (Salte et al. 2004) and oysters (Adams et al. 2004).

### Limitations to the application of genetic and reproductive technologies in genetic improvement programs

At this stage of development in molecular genetics, two major issues that limit application of genetic markers are as follows:

**Technical issues:** There has been a lack of high resolution linkage maps in most of the aquaculture species. The efficiency of MAS is low if markers are located far from the target gene. Even when molecular markers are closely mapped, false-positive detection of marker and gene association also results in low efficiency of MAS. In our current existing breeding programs for tilapia and carps, MAS should be used only when there is a tight linkage between markers and the gene of interest. Experiences in both plant and animals indicate that MAS is successful with traits controlled by single gene with major effects, but little progress has been made with traits controlled by multiple genes. This creates a need to develop new generation markers (e.g. SNP), physical and comparative maps, and to integrate them into linkage maps to increase the ability to identify functional mutations or candidate genes in aquaculture species.

**Costs of MAS:** Currently, the cost of utilizing markers is possibly the most important factor that limits implementation of MAS in genetic improvement programs. At various stages of MAS development, areas that represent large costs include laboratory equipment, consumables, infrastructure, marker development, genotyping, data recording and labor. The question of whether these costs can be compensated by economic returns from genetic gain using MAS still remains open. In addition, several constraints and limitations for the application of molecular genetic information include intellectual property rights, joint research collaborations among international institutions, the lack of manpower, and research funding.

### Concluding remarks

Based on the currently available knowledge in aquatic species and the lessons from plants and animals, one possibility of utilizing molecular markers in aquaculture practical breeding programs is genetic tagging. The feasibility of cryopreserving spermatozoa and the conduct of in-vitro fertilization offer opportunities for increasing the rate of genetic improvement while constraining level of inbreeding.



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# Breeding Programs On Atlantic Salmon In Norway – Lessons learned

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## Abstract

An early establishment of selective breeding programs on Atlantic salmon has been crucial for the success of developing efficient and sustainable salmon farming in Norway. A national selective breeding program was initiated by AKVAFORSK at the beginning of the 1970s, by collecting fertilized eggs from more than 40 Norwegian river populations. Several private selective breeding programs were also initiated in the 1970s and 1980s. While these private programs were initiated using individual selection (i.e. mass-selection) to genetically improve growth, the national program was designed to gradually include all economically important traits in the breeding objective (i.e. growth, age at sexual maturation, disease resistance and quality traits) using a combined family and within-family selection strategy. Independent of which selection strategy and program design used, it is important to secure and maintain a broad genetic variation in the breeding populations to maximize selection response. It has been documented that genetically improved salmon from the national selective breeding program grow twice as fast as wild Atlantic salmon and require 25 per cent less feed, while salmon representing the private breeding programs all show an intermediate growth performance. As a result of efficient dissemination of genetically improved Atlantic salmon, the Norwegian salmon farming industry has reduced its feed costs by more than US\$ 230 million per year! The national selective breeding program on Atlantic salmon was commercialized into a breeding company (AquaGen) in 1992. Five years later, several private companies and the AKVAFORSK Genetics Center (AFGC) established a second breeding company (SalmoBreed) using breeding candidates from one of the private breeding programs. These two breeding companies have similar products, but different strategies on how to organize the breeding program and to disseminate the genetically improved seed to the Norwegian salmon industry. Greater competition has increased the necessity to document the genetic gain obtained from the different programs and to market the economic benefits of farming the genetically improved breeds. Both breeding companies have organized their dissemination to get a sufficient share of the economic benefits in order to sustain and improve their breeding programs.

## Introduction

Norway is producing about 540,000 t of Atlantic salmon per year. More than 95 per cent of the production is exported and Norwegian salmon supply 45 per cent of the world market. An early establishment of selective breeding programs on Atlantic salmon has been critical for the success of salmon farming in Norway, and now the farming industry is depending on genetically improved seed from commercial breeding programs to remain competitive. In the following paper, we will give a brief summary of lessons learned from developing selective breeding programs on Atlantic salmon in Norway. A special attention will be given to the situation of today; how different breeding companies have organized their breeding programs and dissemination of genetically improved seed.

## A national breeding program

A national selective breeding program on Atlantic salmon was initiated by AKVAFORSK at the beginning of the 1970s. Since Atlantic salmon has a four-year generation interval with a high death rate after spawning, four base populations (“year-classes”) were established to supply genetically improved seed each year.

## Base populations

Fertilized eggs representing a total of 400 full-sib families (FS-families) were collected from more than 40 Norwegian river populations to secure a broad genetic variation in all base populations. While different river populations were equally represented in the base populations, this changed dramatically



after two to three generations of selection. Genetic material from the Namsen River dominated the first year-class (72%), together with five other river populations, and genetic material from a mixed population representing several river populations dominated the second year-class (55%), together with genetic material from seven pure river populations. Genetic material from a mixed population from the Nidelv River and Gaula River populations dominated the third year-class (90%), together with genetic material from two other populations, and, finally, all genetic material in the last year-class originated from a mixed hatchery population (the Mowi breed). The genetic variation has been maintained in the year-classes, however, because the genetic variation among individuals within the same population is found to be much larger than the genetic variation between populations.

### **Breeding objective**

Initially, the breeding candidates were selected based on their growth performance of breeding candidates and the test fish were kept in cages from the time of stocking as smolt until harvesting. The breeding objective has become more complex by gradually including more economically important traits; age at sexual maturation (1981: 1st generation), resistance to Furunculosis (1993: 4th generation), resistance to Infectious Salmon Anemia, ISA (1994: 4th generation), filet color (1994: 4th generation), fat content (1995: 4th generation), fat distribution (1995: 4th generation), growth in freshwater (2001: 7th generation), body shape (2001: 7th generation) and, finally, resistance to Infectious Pancreatic Necrosis, IPN (2001: 7th generation).

### **Selection strategy**

Only body weight at harvest and body shape can be recorded for the breeding candidates since these need to be kept alive until production of the next generation of families. Other traits, such as disease resistance and quality traits, require that relatives of the breeding candidates (e.g. full-sibs and half-sibs) be sacrificed to obtain the data. The national selective breeding program on Atlantic salmon has, therefore, used a “combined family and within-family selection” strategy to simultaneously improve all traits included in the breeding objective.

### **Accurate breeding values**

The breeding candidates are ranked and selected based on their total (aggregated) breeding value, which combines breeding values for each trait defined in the breeding objective according to their economic importance (i.e. index selection). Breeding

values are estimated using advanced statistical programs that combine all sources of information (i.e. about the breeding candidates themselves and their brothers, sisters, and cousins). This requires an optimal breeding design, in which both full-sib and half-sib families are produced, and where the pedigree of all breeding candidates and test fish are recorded and stored in a database.

### **Genetic gain**

Genetically improved Atlantic salmon of the 7th generation are now being disseminated to Norwegian farmers. Results from studies with offspring of the 5th generation suggest a selection response of 14 per cent per generation for growth and a correlated response of 4-5 per cent per generation for feed utilization. It follows that the farmed Atlantic salmon in Norway today grow twice as fast as their wild counterparts and require 25 per cent less feed. As a result of the national selective breeding program, the Norwegian salmon farming industry has reduced its feed costs by 1.5 billion NOK (more than US\$ 230 million) per year! The increased growth rate has also shortened the production time (reduced from 40 months in 1975 to only 20 months today), which increases the turnover rate, increases the survival rate and reduces the need for expensive medication. The frequency of early sexually maturing fish has been reduced by 12.5 units, equal to a selection response of 8 per cent for each generation. It has been more difficult to estimate selection responses in resistance to different diseases due to the testing regime used. However, a high correlation between family ranking in challenge-tests and a natural outbreak of Furunculosis (a bacterial disease) in cages suggest that the testing regime is effective to study the genetic variation of disease resistance in Atlantic salmon. A recent study, in which extreme groups (high and low resistance) and a wild control group were challenged with ISA-virus, confirmed that selective breeding is an effective strategy to improve the disease resistance of Atlantic salmon. In this experiment, where the extreme groups had been produced using breeding candidates with very high (HR) or very low (LR) breeding values for ISA-resistance, the wild control group had a survival rate of 58 per cent while the HR and LR groups had a survival rate of 71 and 23 per cent, respectively.

### **Private breeding programs**

Several private breeding programs on Atlantic salmon were initiated in the 1970s and 1980s, such as Bolaks, Mowi, Jakta and Rauma. Owing to a lack of resources (know-how and research facilities), these programs were initiated using individual selection (mass-selection) as the strategy to genetically improve Atlantic salmon. Individual selection, which utilizes information about the breeding candidates, is only

effective to improve traits that can be recorded on live animals and have a medium to high heritability. Therefore, the breeding objective of these private programs has only included growth (recorded as harvest weight) and, in some cases, age at sexual maturation.

## Comparison of improved breeds

A study was conducted by one of the largest feed companies in Norway to evaluate the growth performance of different wild and domesticated breeds of Atlantic salmon. Twelve full-sib families were produced to represent each of four domesticated breeds: the national breeding program, Bolaks, Mowi and Jakta, and two major river populations in Norway, namely the Namsen River and Alta River. The study confirmed that salmon of the national breeding program (offspring of the 5th generation) grew twice as fast as offspring of wild Atlantic salmon. Test fish representing the private breeding programs all showed an intermediate growth performance, confirming that mass-selection (with a restricted accumulation of inbreeding) can also be effective to genetically improve the growth performance of aquaculture species.

## Breeding companies

The maintenance of selective breeding programs not only requires a lot of resources (both human and financial resources), but also has the potential to generate considerable profit for the owners. Since most aquaculture species have a high reproduction capacity, the costs of maintaining a breeding program can be divided according to the large number of seed produced. In breeding programs for farm animals, the cost/benefit ratio has been estimated to vary from 1:5 and up to 1:50. This ratio is much more favorable for Atlantic salmon and other aquatic species since the production cost of brood stock is considerably lower than that of farm animals.

The national breeding program on Atlantic salmon in Norway was commercialized in 1992. Private companies have also become more active in recent years to develop alternative breeds of Atlantic salmon in Norway. More competition has put strong pressure on the breeding companies to reduce their maintenance costs and improve the quality of their products – service and genetically improved seed.

At present, there are two major breeding companies providing genetically improved seed of Atlantic salmon in Norway – AquaGen and SalmoBreed. These companies have similar products, but different strategies on how to organize the breeding program and dissemination of genetically improved seed to the Norwegian salmon industry. AquaGen and SalmoBreed have similar market shares in Norway.

Companies controlling other breeds (Marine Harvest and Rauma Group) have marginal market shares.

AquaGen was established in 1992 when the national breeding program on Atlantic salmon was commercialized. Initially, the company maintained its four year-classes by producing 300 FS-families each year. The company has continued to include more traits in the breeding objective, which today accounts for ten economically important traits. The importance of different traits in the breeding objective has changed over time and is now ranked as follows: quality traits (40%), disease resistance (30%), growth (25%), and age at sexual maturation (5%). To face increasing competition from SalmoBreed, efforts have been made to reduce costs and improve the genetic quality of the seed. First, the generation interval has been reduced from four to three years to speed up the genetic gain. Secondly, the four year-classes are combined in one breeding population of 400 FS-families to standardize the genetic quality of the seed (and perhaps to reduce the costs of family production). Subsequent to these changes, FS-families will be produced every three-year. The company will use freeze-stored milt to produce commercial seed other years.

Several private companies (Bolaks, Jakta, Erfjord Stamfisk and Osland Havbruk) joined forces in 1999 to establish a new breeding company – SalmoBreed. The company operates a cost-efficient breeding program on Atlantic salmon by using existing facilities provided by the cooperating companies and services provided by the AKVAFORSK Genetics Center (AFGC). The breeding program WAS initiated by using breeding candidates originating from the Bolaks-breed. The company, however, is operating an “open” breeding population, which means that superior genetic material from other sources can be tested and included in the breeding populations in the future. The breeding program is maintaining four year-classes by producing 300 FS-families each year. The breeding objective is similar to that of AquaGen.

## Dissemination

The strong competition between breeding companies ensures that any genetic gain obtained in the selective breeding programs on Atlantic salmon is rapidly disseminated to the farms. The salmon farming industry in Norway is divided into specialized producers of brood stock/eggs, hatcheries that produce fingerlings/smolts, and large farms that feed the salmon in sea cages until they reach a suitable harvest weight. The breeding companies usually control several brood stock stations that are used as multipliers to increase the quantity of genetically superior seed. Two alternative organization models can be used to disseminate the genetically improved seed to the farms, either a centralized or decentralized organization.

## Centralized organization

In a centralized organization (Figure 1), the breeding company keeps strict control of the breeding candidates and decides what kind of products are disseminated to the customers – commercial hatcheries or large production companies. Usually, the breeding candidates are kept at two locations in case of a disease outbreak. The genetically superior breeding candidates are used to produce the next generation of families in the breeding program, from which some individuals are again used as breeding candidates and others as test fish. The test fish are transferred to test stations (e.g. bio-secure facilities, commercial farms) to be tested for different traits (e.g. disease resistance, quality traits, growth performance in different test environments) as specified in the breeding objective. All test fish are killed after testing and only data are collected to estimate the breeding value of the breeding candidates. The breeding candidates are selected and ranked according to their total (aggregated) breeding worth. The available breeding candidates produced each year will usually be too few to produce enough commercial seed (i.e. fertilized “eyed-eggs”) for dissemination. Therefore, the breeding company will make a special arrangement with other brood stock stations to function as multipliers. These brood stock stations will receive fertilized “eye-eggs” or smolts from the breeding company and use these for three or four years later to produce commercial seed (“eyed-eggs”) for dissemination to the salmon farming industry.

## Decentralized organization

In a decentralized organization (Figure 2), the breeding candidates are kept at several cooperating broodstock stations. The genetically superior breeding candidates at one or several brood stock stations are used to produce the next generation of families in the breeding program. These families are transferred to the breeding station as fertilized “eye-eggs”. A random sample of individuals from each family produced will be used as either breeding candidates or test fish. The breeding candidates are distributed to the cooperating brood stock stations, while the test fish are transferred to test stations (e.g. bio-secure facilities, commercial farms) to be tested for different traits (e.g. disease resistance, quality traits, growth performance in different test environments) as specified in the breeding objective. The test fish are killed after testing and all breeding candidates are selected and ranked according to their total (aggregated) breeding values, based on collected data from the brood stock stations and test stations. However, the available breeding candidates that have been distributed to the cooperating brood stock stations might be too few to produce enough commercial seed (i.e. fertilized “eyed-eggs”) for dissemination. Special female lines are, therefore, produced at each brood stock station

to increase their capacity for the production of commercial seed (“eyed-eggs”).

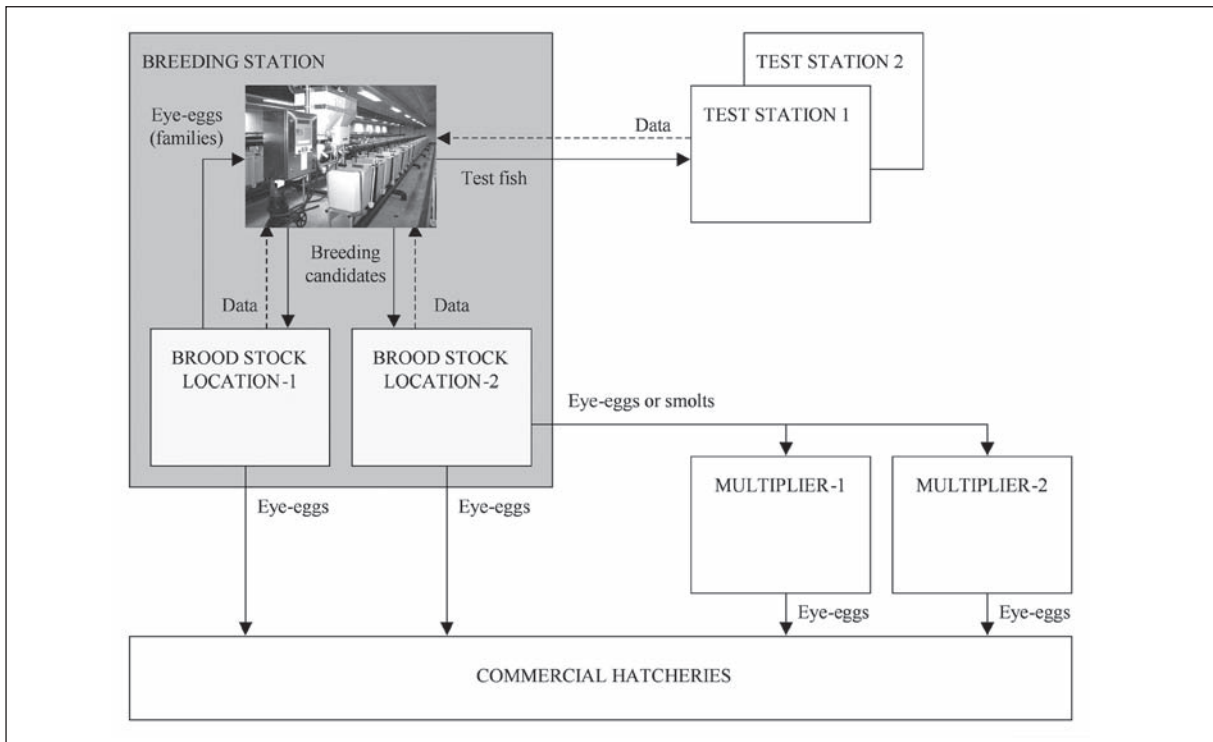
## Customized seed

The next generation of families in the breeding program is produced by superior breeding candidates that have been selected based on their total (aggregated) breeding value, which combines the breeding value for each trait in the breeding objective according to their economic importance to the entire farming industry in Norway. Some salmon producers might, however, place a different importance to the traits included in the breeding objective. Therefore, the breeding companies are now also disseminating “customized seed” produced by breeding candidates that have been selected according to a different combination of recorded traits from those used in the breeding program.

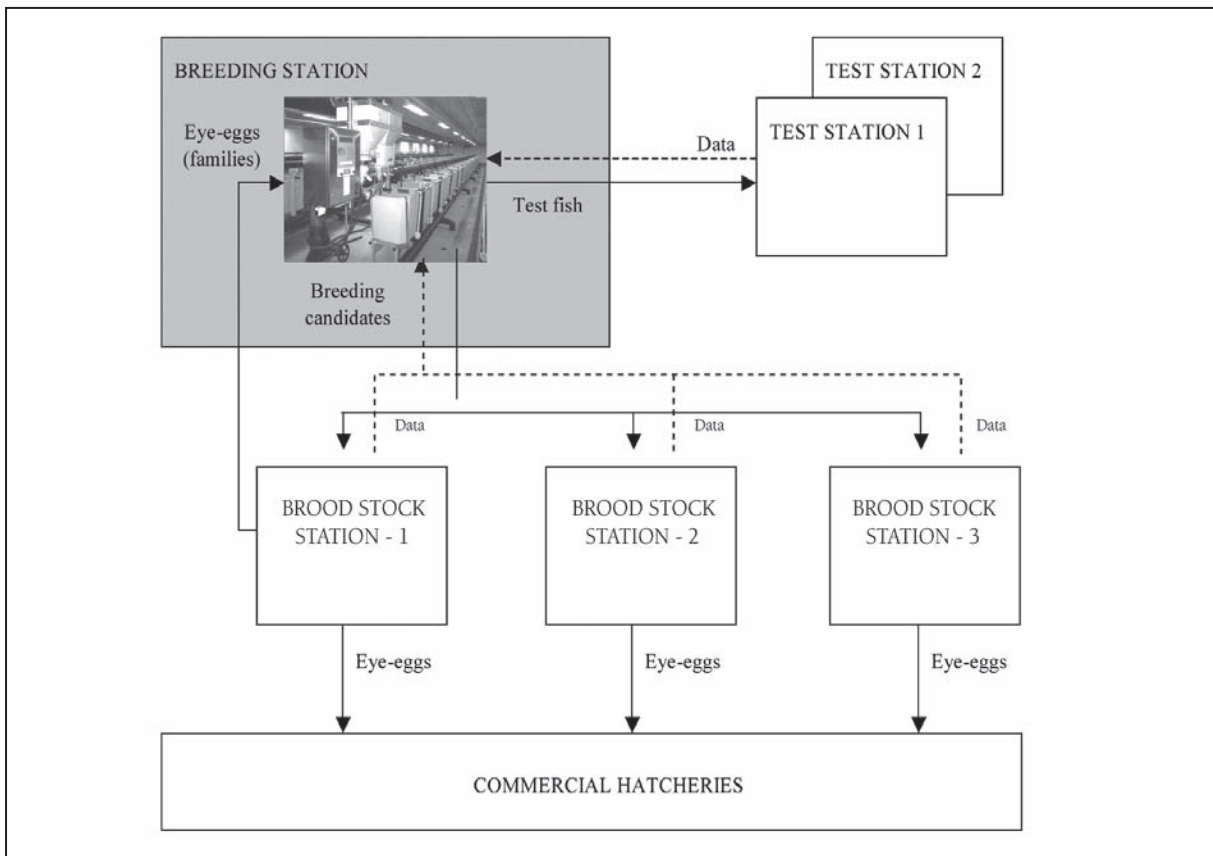
## Conclusions

Based on the experience of developing breeding programs on Atlantic salmon in Norway, the following general conclusions can be made:

- The early establishment of selective breeding programs is crucial for the success of developing an efficient and sustainable aquaculture production.
- The selective breeding programs can become more advanced as more traits are included in the breeding objective (i.e. it is possible to develop simple breeding programs into advanced multi-trait selection programs).
- It is important to secure and maintain a broad genetic variation in the breeding populations by restricting inbreeding.
- The genetic gain obtained in breeding programs should be effectively disseminated to the farming industry without much delay to maximize the benefits of the programs (i.e. to secure a low cost-benefit ratio).
- Commercialization of national breeding programs into breeding companies might be necessary to limit their dependency on international and/or governmental financing/support.
- Breeding companies need to document the genetic gain obtained in the programs and market the economic benefits of farming genetically improved aquaculture breeds.
- Breeding companies should organize their dissemination to obtain a sufficient share of the economic benefits in order to sustain and improve their breeding programs.



**Figure 1. Centralized organizations used to disseminate genetically improved seed of Atlantic salmon to commercial hatcheries.**



**Figure 2. Decentralized organizations used to disseminate genetically improved seed of Atlantic salmon to commercial hatcheries.**

# Lessons from the Breeding Program on Common Carp in Hungary

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## Abstract

Common carp is one of the most important cultured freshwater fish species in the world. Its production in freshwater areas is the second largest in Europe after rainbow trout. Common carp production in Europe was 146,845 t in 2004 (FAO Fishstat Plus 2006). Common carp production is concentrated mainly in Central and Eastern Europe. In Hungary, common carp has been traditionally cultured in earthen ponds since the late 19th century, following the sharp drop in catches from natural waters, due to the regulation of main river systems. Different production technologies and unintentional selection methods resulted in a wide variety of this species. Just before the intensification of rearing technology and the exchange of stocking materials among fish farms (early sixties), "landraces" of carp were collected from practically all Hungarian fish farms into a live gene bank at the Research Institute for Fisheries, Aquaculture and Irrigation (HAKI) at Szarvas (Bakos and Gorda 1995; Bakos and Gorda 2001). In order to provide highly productive hybrids for production purposes starting from 1964, different strains and crosses between Hungarian landraces were created and tested. During the last 40 years, approximately 150 two-, three-, and four-line hybrids were produced. While developing parental lines, methods of individual selection, inbreeding, backcrossing of lines, gynogenesis and sex reversal were used. This breeding program resulted in three outstanding hybrids: "Szarvas 215 mirror" and "Szarvas P31 scaly" for pond production, and "Szarvas P34 scaly" for angling waters. Besides satisfying the needs of industry, the live gene bank helped to conserve the biological diversity of Hungarian carp landraces. Fifteen Hungarian carp landraces are still maintained today in the gene bank. Through exchange programs fifteen foreign carp strains were added to the collection from Central and Eastern Europe, as well as Southeast Asia (Bakos and Gorda 2001).

Besides developing the methodology to maintain live specimens in the gene bank, the National Carp Breeding Program has been initiated in cooperation with all the key stakeholders in Hungary, namely the National Association of Fish Producers (HOSZ), the National Institute for Agricultural Quality Control (OMMI), and the Research Institute for Fisheries, Aquaculture and Irrigation (HAKI). In addition, methodologies or technologies for broodstock management and carp performance testing have been developed. This National Carp Breeding Program is being implemented successfully since the mid-1990s.

## Introduction

Common carp is one of the oldest and most cultured fish in the world. Its farming and breeding has a long history that started over 4000 years ago in China and several hundred years ago in Europe. During the last decades, India, Indonesia and Vietnam started to culture common carp, as a result of purposeful fish introductions.

To plan and develop a carp breeding program, it is important to have basic information about the existing carp populations, to know the applied production technologies and to be aware of consumers' demand and market requirements.

Local populations and landraces of common carp were developed within the cultivated species, as a result of various environmental conditions, and particular breeding efforts of fish farmers. The landraces differed in their genotypes, as well as in their qualitative and quantitative characters. These differences were inherited by their progenies. Systematic breeding and selection work of common carp started 45 years ago in Hungary, when the gradually intensifying farms required highly productive carp populations.

As a first step of the genetic improvement program, the most significant common carp strains with good performance were collected from Hungarian fish farms. Since 1962, fifteen Hungarian strains and an



equal number of foreign strains represented the basis of the future breeding program and genetic research activities; they comprised a live gene bank at the Research Institute for Fisheries, Aquaculture and Irrigation, Szarvas, Hungary.

The main direction of selection was to improve those quantitative and qualitative traits, which directly or indirectly influenced productivity of carp under the given production-environmental conditions.

Viability, growth rate, food conversion, dressing yield and fat content were found to be the most important traits for both producers and consumers.

Productivity of 5-8 common carp strains were compared under similar environmental conditions every year by the following methods:

- Artificial propagation in the same hatchery;
- Nursing and fingerling rearing during the first year in the same farm;
- Group marking at the end of the first year;
- Wintering in a common pond in the same farm;
- Producing market-size fish during the second year in grow-out ponds in three different fish farms.

### **Individual or mass selection**

During the process of selection, the specifically chosen individuals played a significant role in the development of new lines of broodstock. It was important to identify outstanding "line-founder" individuals, based on the knowledge of biological characters and productivity features of locally cultivated species. Individual selection was the basis for sorting young brood fish candidates in the breeding program. While accomplishing this breeding process, special attention was given to:

- the origin of the population;
- the performance of the given strain; and
- the typical external characteristics of the strains.

During the planning of the selection program, priority was given to the so-called parallel selection, meaning the selection for several important traits at the same time. In order to assist simultaneous evaluation of several characters, a so-called "selection index" has been developed. Ranking of the five most economically important quantitative traits is expressed by their "weight" in a 100-point valuation system: weight gain – 30 points; survival – 25 points; feed conversion – 20 points; dressing yield – 15 points; and fat content – 10 points. Results from a comparative test of five crosses and/or hybrids (in 1991) are shown as an example of applying the "selection index" (Table 1).

Every year several different strains were tested under similar conditions. To be able to compare their performance among themselves and during different years, a standard control group was used and a "group average" was calculated for each given year. Tested varieties were compared on one hand to the performance of the standard control group and on the other hand to the group average of the testing year.

Based on the results of the initial comparative performance tests, it is concluded:

1. There were measurable differences among the performance of the tested populations;
2. Traditional methods of individual and positive mass selection showed slow progress in some quantitative characters, because:
  - the variability of the tested traits was limited;
  - the probability of their heritability was low; and
  - the interval between consecutive generations was long.

### **Intraspecific hybridization**

Based on the experiences mentioned above, priority was given to intraspecific hybridization from the mid-sixties. By that time, the method of artificial propagation of common carp made it possible to produce more crossing combinations annually. Starting from 1964, several landraces were crossed with one another with the expectation of a positive heterosis effect in the F1 generation of progenies. While developing the parental lines, various methods were used including individual selection, inbreeding, backcrossing of lines, gynogenesis and sex reversal.

However, not all the crosses resulted in positive heterosis. The F1 progenies sometimes have poorer performance than the parental lines. To obtain highly productive heterosis hybrids, not only is a high-level breeding program required, but also luck is sometimes needed. During the last 40 years, more than 150 crossing combinations of common carp strains were created and the performance of their progenies was tested in the Research Institute for Fisheries, Aquaculture and Irrigation. "Only" three outstanding hybrids with a high positive heterosis effect were established as being suitable for commercial-fish production. The "Szarvas 215" is a three-line mirror hybrid, the "Szarvas P31" is a three-line heterozygote scaly hybrid, and the "Szarvas P34" is a two-line homozygote scaly hybrid. Their breeding schemes are shown in Figures 1, 2 and 3).

The superiority of some hybrids is apparent mostly in their survival rate and adaptability. Differences between the parental and hybrid performances are

not so noticeable if the production conditions are optimal; however, under poor conditions hybrids actually perform better than the parental lines (Bakos and Gorda 1995).

The heterosis effect can be increased by:

- crossing several genotypes with higher level of inbreeding, and
- creating three- or four-line hybrids.

By mating hybridized individuals among themselves, the heterosis effect can be decreased in the second and further generations (Hulata 2001; Kirpichnikov 1981). As an example, results of the performance of experimental crossings of hybrid "5x1" in Generations I and II are presented in Table 2. An important task during the breeding program is to avoid inbreeding depression. To maintain a commercially viable fish population, two lines "A" and "B" would be ideal to keep simultaneously in closed groups with strict selection in every generation. For example, females can be selected from line "A" and males from line "B" to ensure that fish farms with controlled quality brood fish produce high quality seed as shown in Figure 4 (Hulata 1995; Wohlfart 1993).

As a result of crossing experiments, three outstanding hybrids were produced:

- Szarvasi 215 three-line mirror;
- Szarvasi P31 three-line heterozygote scaled; and
- Szarvasi P34 two-line homozygote scaled.

All of them "obtained" the so-called "state-recognized hybrid" status because their performance (e.g. 15-20 per cent higher productivity) significantly exceeded the performance of other strains.

Distribution of highly productive hybrid populations was implemented by selling adults, ready for the propagation of maternal and paternal lines to fish producing farms.

During 1972-94, approximately 12,000 hybrid spawners were sold to commercial fish farms. During the eighties, about 80 per cent of the carp production in Hungary originated from the hybrids of the Research Institute for Fisheries, Aquaculture and Irrigation (HAKI), Szarvas.

## Brood stock management

Broodstock management is an essential part of a well-designed breeding program. The main elements of brood stock management are as follows:

- Initial knowledge about the origin, domestication and breeding process of cultivated strains;
- Rearing and maintenance of brood fish;
- Selection of broodstock;

- Preparation of spawners for reproduction;
- Tagging and marking all the selected brood fish individually;
- Ensuring optimal environmental conditions of all populations;
- Keeping good accounts of the hatchery registration book;
- Separating males and females until the appropriate time for propagation;
- Feeding females and males with protein-rich food, complemented with vitamin A and E;
- Knowledge about the affinity of females and males, to avoid harmful inbreeding depression of the next generation. (See Table 3 for the results of a special crossing and back-crossing experiment of common carp full-sib and daughter mating, which caused deterioration in F<sub>1</sub> generation); and
- Elimination of anatomically deformed individuals when young brood stock candidates are selected.

Brood stock management is the basis for successful reproduction at hatcheries and fingerling production at specialized fish farms. Lessons in this area are published elsewhere (Varadi et al. 2002).

## Breeding programs

The aim of a breeding program is to develop the most suitable fish population for satisfying specific needs (by farmers, anglers, conservationists, consumers, etc.). The breeding program includes all the genetic and selection methods that are suitable for improving productivity of a given fish population (Wohlfarth et al. 1987).

In a short-term breeding program, the fish breeder chooses the young brood stock that will be the parents of the next generation.

The criteria of brood fish selection include:

- Detailed information about the morphological and production characteristics of the population;
- Suitable market size fish of the same age within a population;
- Preferable average size and plus variants;
- Desirable sex ratio;
- Avoidance of inbreeding; and
- Marking of selected young brood fish candidates either individually or by group.

To fulfill a well-designed long-term breeding program, special personal, technical and biological conditions are needed, including several lines or varieties of cultivated species.

The consciously designed breeding programs should be directed to certain production or breeding purposes, such as:



- To increase the productivity of one or more quantitative traits;
- To improve the quality of the final product;
- To develop more disease-resistant strains;
- To achieve better adaptability to the environment of intensive production;
- To develop monosex female or male populations; and
- To maintain a live gene bank for the preservation of the basic genetic diversity.

Such a breeding program demands continuous and strict cooperation among the fish producers, researchers and the state officials controlling the breeding program. In the case of the Hungarian carp breeding program, 80 per cent of the Hungarian carp production was based on the hybrids of HAKI, developed during the 1980s. One of the lessons learned is that production and environmental conditions have strongly determined the breeding quality and spawning potential of the breeders. Production technology should be suitable to the biological and environmental requirements of common carp.

The national carp breeding program in Hungary is based on the following:

- A legal framework for animal production (Animal Breeding Act);
- Availability of carp populations kept in the state-owned live gene bank, or maintained at private farms;
- The National Institute for Agricultural Quality Control (OMMI) organizing performance tests, collecting data, and providing backstopping administration;
- The Hungarian "National Association of Fish Producers", serving as coordinator;
- The Research Institute for Fisheries, Aquaculture and Irrigation supervising the program;
- Financial support from the Ministry of Agriculture and Regional Development; and
- Meeting consumers' demands.

Fourteen Hungarian carp breeding farms, representing 14 per cent of the total number of fish farms, are members of the registered breeding units. They maintain, select and improve their own breeding material. Only these breeding farms with registered hatcheries can sell seed of common carp to other farms. If they do not register, fish farms can use their stocking material only in their own farms.

The breeding work is controlled by the OMMI. All the registered carp strains should be tested every five years. Production data are collected and registered in a computerized database.

The productivity of five different strains is tested annually in order to determine the genetic progress on the basis of the achieved quantitative traits.

In Hungary, a register book has been elaborated so that the following data are recorded:

- Origin of the given common carp strain;
- Morphological characteristics of the population; and
- Results of productivity under given environmental and production conditions.

Distribution of genetically improved carp seed is promoted by a state subsidy, of which the value is around 17 per cent of the price of fingerlings at the moment.

As a result of the existing carp breeding program, and efforts to improve the quality of the seed production in Hungary in 2002, about 80 per cent of the total fingerlings sold originated from certified breeders.

## Conclusions

The breeding program of common carp has been successfully carried out in Hungary for more than forty years and has resulted in the following:

- The establishment of a live gene bank of common carp;
- The methodology to maintain live gene banks;
- The development of three productive hybrids for different conditions of fish farms and natural waters; and
- The establishment of the National Breeding Program for carp.

The proper implementation of the National Carp Breeding Program made it necessary for the development of these methodologies:

- Methodology of progeny performance testing;
- Methodology of licensing and controlling fish farms and hatcheries; and
- Methodology of controlled fish seed distribution.

Close cooperation among major stakeholders (National Association of Fish Producers, National Research Institute for Fisheries, Aquaculture and Irrigation (HAKI), and National Institute for Agricultural Quality Control (OMMI)) was the basis for implementing a successful breeding program of common carp in Hungary.

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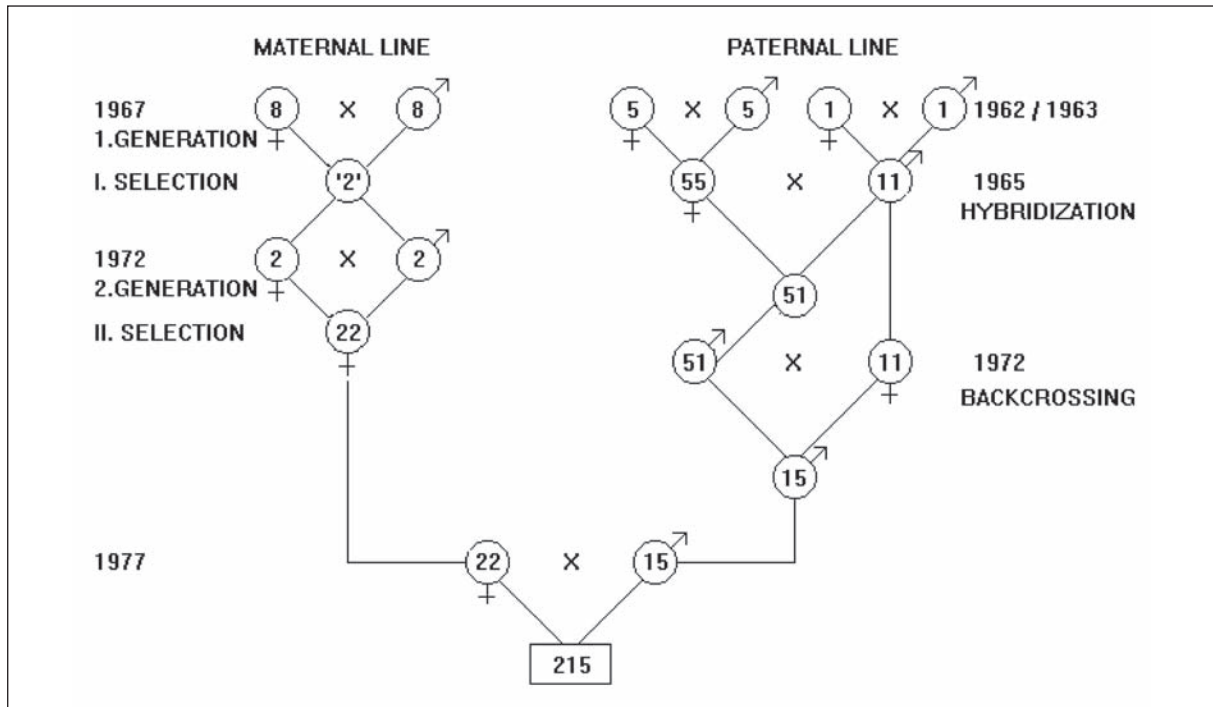


Figure 1. Breeding scheme of the Szarvas 215 hybrid.

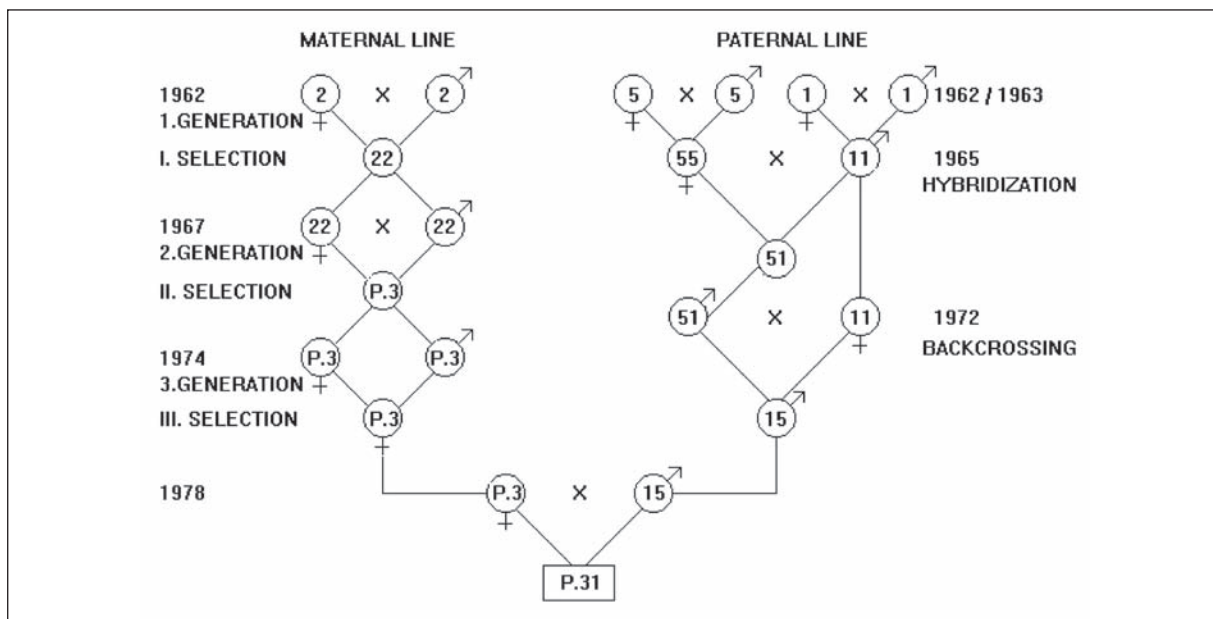
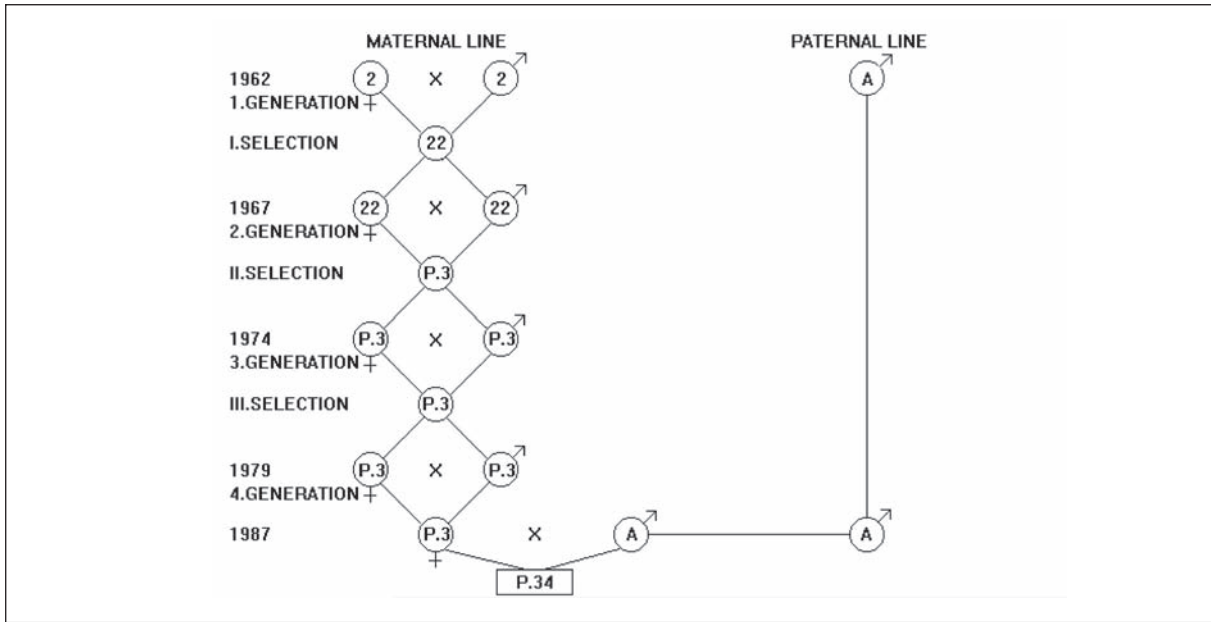


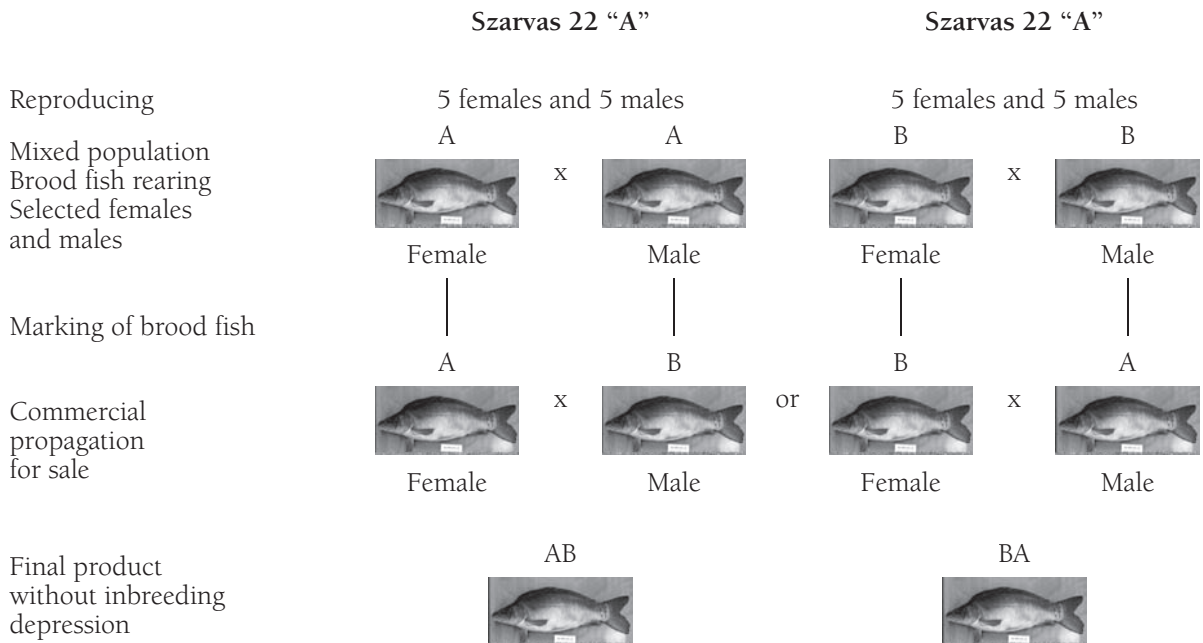
Figure 2. Breeding scheme of the Szarvas P31 hybrid.



**Figure 3. Breeding scheme of the Szarvas P34 hybrid.**

Example of Szarvas 22 and Szarvas 5  
 Select two groups of spawners with a different origin

### Common carp



Renew the parental stocks "A" and "B" every 5 years and select new young brood fish  
 Group marking system: fin clipping  
 Individual marking system: PIT-tag

**Figure 4. Scheme for avoiding inbreeding by using two independent lines.**

Table 1. Selection index (SI) as a practical way to compare productivity of hybrids on the basis of 5 economically important quantitative traits (tested in 1991, pond surface 2 ha).

Traits Groups	Weight gain		Survival		Feed conversion		Dressing yield		Fat content		SI Summation of scores
		Max. score		Max. score		Max. score		Max. score		Max. score	
	g	30	%	25	kg/kg	20	%	15	%	10	
Sz. 215	842	19.8	72.5	18.9	3.21	16.6	63.3	14.9	15.5	7.6	78.8
15 x D	752	17.7	48.0	12.5	4.53	11.7	62.8	14.8	11.9	10.0	66.1
N x D	830	19.5	52.0	13.5	4.32	12.3	63.5	15.0	12.9	9.1	69.7
Sz. P31	1176	27.7	187.5	22.8	3.23	16.5	61.0	14.4	14.5	8.2	89.7
Sz. P34	1272	30.0	95.6	25.0	2.96	19.8	60.0	14.1	12.2	9.7	98.7
Sz. P36	1232	29.0	82.0	21.4	2.67	20.0	61.0	14.3	14.2	8.3	93.2

Table 2. Decreasing of the heterosis effect in the F<sub>2</sub> generation of 5 x 1 hybrids compared to the 77 standard control population.

Characteristics		I. Generation			II. Generation		
		5 x 1	77	Deviation, %	51 x 51	77	Deviation, %
Survival	%	64.2	51.0	+13.2	46.0	40.1	+6.9
Growth	g	1446	1314	+10.0	1213	1174	+3.3
FQ	kg	1.53	2.15	-28.1	1.58	1.81	-12.9
Dressing yield	%	65.06	64.40	+0.66	63.89	64.43	-0.54
Fat content	%	14.77	16.91	-2.14	12.26	12.72	-0.46
Evaluation by the 100 point system		94.2	82.1	+14.7	82.7	76.8	+7.6

Table 3. Inbreeding depression of common carp by different inbreeding levels: decreasing the main quantitative traits.

Parent species Female x male	Initial weight g	Survival rate* %	Weight gain g	Dressing yield %	Fat content %	Body deformations %
A: 44 x 72	47	65.6	1040	62.2	13.8	0.0
B: 77 x 4	53	46.2	896	62.4	14.9	5.6
C: 54 x 4	44	46.5	728	62.2	13.1	10.6
D: 44 x 4	43	45.8	687	61.3	11.9	8.0
E: 44 x 44	47	41.6	695	62.8	9.7	5.7

\* Survival rate is given for two growing seasons

A - Three-line hybrid

B - Two-line hybrid

C - Two-line hybrid, backcrossed with male 4

D - Inbreed line, father x daughter pairing

E - Inbreed line, full-sib pairing

The females of A, D and E are the same individuals of the inbred line 44

The males of B, C and D are the same individuals

# Lessons from the Breeding Program of Rohu

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## Abstract

For the first time in India, selective breeding work has been initiated at the Central Institute of Freshwater Aquaculture, Bhubaneswar, India in collaboration with the Institute of Aquaculture Research (AKVAFORSK), Norway. Rohu has been chosen as the model species because it enjoys the highest consumer preference among Indian major carps (IMC) although its performance was observed to be slower than other IMC. As this was the first ever selection work on any Indian major carp, many procedures and techniques for successful implementation of the programs were standardized (i.e. production of full-sib groups, establishment of model hatchery for selective breeding of carps, rearing of full-sib groups in partitioned nursery ponds, individual tagging with the Passive Integrated Transponder (PIT) tag, communal rearing, sampling, data analysis, field testing and dissemination of improved rohu). After four generations of selection, an average of 17 per cent higher growth per generation was observed in improved rohu.

## Introduction

Indian major carps such as Catla (*Catla catla*), Rohu (*Labeo rohita*), and Mrigal (*Cirrhinus mrigala*) are relatively fast-growing fish in India. They are mutually compatible and their food habits are also complementary to each other so these fish gained popularity not only in India, but also in neighboring countries such as Bangladesh, Pakistan, Myanmar, Thailand, and Vietnam. After the success of induced breeding technology, a large number of hatcheries have been established in the country. Since seed is the basic input in the culture system, its production has been accorded the highest priority. While India has attained self-sufficiency in carp seed production, most hatcheries do not follow any genetic norms while producing carp seed. As a result, the Indian carp hatcheries are experiencing deterioration of the quality of carp seed because of inbreeding (Eknath and Doyle 1990).

Among the Indian major carps, rohu is one of the most preferred species in the country and commands a higher price in the market. The species is also an excellent game fish owing to its easy acceptance of anglers' bait. Andhra Pradesh, West Bengal, Assam and Orissa are the most important states for aquaculture production and rohu is most preferred species in these states. In view of its fast growth and high demand, it is possible that in the future, monoculture of the species might be undertaken by farmers instead of the present polyculture. However, its performance in terms of growth is slower when compared to other species in the multi-species culture system. Besides, rohu is highly susceptible to diseases. Thus, in India there was an urgent need

for better procedures for seed production and genetic improvement in terms of growth, survival and other traits of economic importance to rohu through selective breeding.

Taking all these factors into consideration, a project on the genetic improvement of rohu, particularly for better growth performance through selective breeding, was initiated for the first time in India in 1992 at the Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, in collaboration with the Institute of Aquaculture Research (AKVAFORSK), Norway. The funds required for carrying out the research were provided by the NORAD Agency, and the infrastructure (pond and laboratory) and manpower to execute the project activities were provided by the CIFA under the Indian Council of Agriculture Research (ICAR), New Delhi. The scientists of AKVAFORSK, Norway provided consultation services. The main aim of the project was to develop a national selective breeding plan for rohu and disseminate improved rohu to fish farmers of India for quality seed production.

## Selective breeding of rohu

The main objectives of the selective breeding of rohu project were: (i) to obtain information about the magnitude of the genetic variation for growth and survival in rohu, (ii) to develop manpower at the CIFA with strong knowledge on quantitative genetics and selective breeding, (iii) to develop a breeding program of rohu based on the results obtained, and (iv) to disseminate improved rohu to fish farmers through a number of multiplier units.



## The base population

The selective breeding project of rohu was initiated with six stocks as the base population. Rohu stocks were procured from five different river systems of India (i.e. Ganga, Yamuna, Brahmaputra, Sutlej and Gomati) (Table 1). The fry or fingerlings collected were quarantined for about two weeks during which time they were kept in cement cisterns. To these five riverine stocks, the CIFA hatchery stock was added as sixth stock. After quarantine, the fish were marked by fin clipping or M-procaine blue dye marking or a combination of both. They were stocked in communal ponds for rearing until sexual maturity.

The base population is very important in a selective breeding program. Genetic variability is essential to start any genetic improvement program. Genetic characterization of these six stocks indicated a wide variation within each Stock. The variations within stocks were much more significant than between-stock variations.

**Table 1. Base population and year of procurement.**

Stock	Base population year-classes	
	1993	1994
Local (CIFA) hatchery stock	X	
Ganga	X	X
Gomati		X
Yamuna		X
Sutlej		X
Brahmaputra		X



**Figure 1. Base population from different rivers of India.**

## Brood fish raising and management

Fin clipped or dye marked fish were reared in monoculture ponds to raise brood fishes. Vitamin enriched feed was provided to the brood fish at 2-3 per cent of body weight. The ponds were also regularly fertilized with organic manure. Health monitoring was done frequently by checking the fish monthly. Other management practices were followed according to the general pond environment prevalent in India.

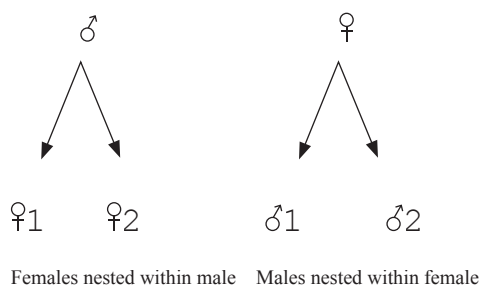
## Fish breeding and mating design

The Ovaprim hormone was used for the breeding of rohu. Five hours after the hormone injection, male milt was collected separately in small vials, which were serially numbered. The milt was then refrigerated until fertilization time. After stripping of females (generally 5-6 hours after hormone injection), fertilization was carried out with the pre-determined male milt according to the breeding plan.

To date, twelve year-classes of full-sib and half-sib families have been produced with five complete generations of selection. Parent-offspring genetic ties were established between the two populations found from the 1993 and 1994 base population year-classes (Table 2). The choice of mating design, males nested within female or vice versa, was dependent on the number and body size of the female breeders available in the actual year-class (Figure 2).

**Table 2. Production of different generations.**

Generation	Population	
	1	2
0	1993	1994
1	1995	1996
2	1997	
3	1999	2000
4	2001	2002
5	2003	2004
6	2005	



**Figure 2. Nested mating design.**

### Production of diallele crosses

To estimate the magnitude of heterosis for harvest body weight and survival crossing, different rohu stocks produced using 3X3 diallele cross. One included Ganga, Yamuna and Local (Diallele I), and another included Local, Brahmaputra and Sutlej. This was performed for the 1995 year-class where a total of 18 crosses were produced. The local stock was common for both crosses. Thus, 17 different stock combinations were produced (Table 3).

Data analysis indicated that the total heterosis for each of the six stock crosses was low or negative and the average heterosis was also low and in most cases not significantly different from zero. In terms of survival, the heterosis was negligible and not significantly different from zero. Hence, it was concluded that genetic improvement through cross-breeding of rohu has little practical significance (Gjerde et al. 2002).

### Incubation of fertilized eggs

The incubation of fertilized eggs, hatching and further rearing until fingerling size are critical stages in a selective breeding program. A combined selection method (utilization of own, full-sib and half-sib records in the selection decisions) was adopted in the rohu selective breeding program. Hence, rearing was done separately until the fish attained taggable size. During the initial phase of the project (i.e. 1993-99), the incubation of fertilized eggs was carried out in hatching hapas fixed in a pond. High rates of mortality occurred in the hatching hapas due to unavoidable environmental hazards such as a sudden change in the temperature, strong winds, and predatory fish.

Consequently, it was decided that a specialized hatchery had to be constructed for the production of full-sib family groups. The hatchery was constructed specifically to cater to the needs of the selective breeding program. The hatchery proved to be very effective with almost 100 per cent recovery of families for the last five years. This hatchery can be a model for any selective breeding of carp species (Das Mahapatra and Sahoo 2003).

**Table 3. Diallele cross.**

Sire strain	Dam strain				
	Ganga	Yamuna	Local	Brahmaputra	Sutlej
Ganga	X	X	X		
Yamuna	X	X	X		
Local	X	X	X	X	X
Brahmaputra			X	X	X
Sutlej			X	X	X

### Rearing of full-sib groups

On the fifth day after breeding and 72 hours after completion of the hatching process, the spawn are transferred to nursery ponds at 5000 / 100m<sup>2</sup>. The spawn are reared separately in nursery ponds until they attain taggable size. Selection through full-sib families requires a good number of nursery ponds. Each nursery pond (200 m<sup>2</sup>) is partitioned into two nursing areas by partition cloth. A rich plankton crop is always ensured before stocking the spawn and also during the rearing phase. Supplementary feed is also provided regularly (Saha et al. 2003)

For any selective breeding program, the number of full-sib groups is very important. A small number of full sib groups in a breeding program of rohu create a lot of problems in the later generations. The fish did not attain taggable size in small indoor tanks, so earthen ponds were utilized in the rearing program. In the estimation, a high common environmental effect on the full-sib groups for tagging was observed.

In a recent experiment, it was also observed that rohu spawn could reach taggable size in a cement cistern of larger size (10x5x1.5 m) and large common environment effect could be avoided.

### Tagging

Tagging of individual fish is essential in a selective breeding program involving family selection or a combined selection. Initially, different indigenous tags including surgical suture with plastic chips, and vinyl thread with plastic chips were tried, but they all proved to be unsuitable for rohu. Since the fish is an active swimmer, retention of the external tags was very poor and secondary infection was observed in most cases. Therefore, Passive Integrated Transponder (PIT) tags were used instead when the spawn reached a size of 10-15 g. PIT tags were implanted on their abdomen of the fingerlings with the help of a tag implanter. The individual growth status was also recorded before tagging. After implantation the fish were kept in separate tanks overnight in case of any possible mortality. After that, they were stocked in communal ponds for further growth experiments. An equal number of fingerlings were stocked from each full-sib group in the communal ponds.

PIT tags were found to be very suitable for tagging of rohu. The size range of 10-15 g was also observed to be the most suitable for the tagging of this fish (Das Mahapatra, et al. 2001).

### Test environments at the CIFA

At the CIFA, tagged fish were stocked in three well-prepared communal ponds of 0.1 ha each under monoculture, and two ponds of 0.4 ha each under polyculture. In polyculture, rohu was stocked along with catla and mrigal in the ratio of 1.2:1:1, respectively; this practice continued until 1997. After 1997, monoculture of rohu was practiced in the selective breeding program.

An analysis of the data indicated that for growth and survival, the pure stock as well as crosses rank similarly ( $r = 0.74 \pm 0.27$ ) in monoculture as well as polyculture, and that development of specialized varieties for each of the two production systems is not required (Reddy et al. 2002)

### Estimation of genetic parameters

The body weights recorded at tagging, sampling (6 months after tagging) and harvesting were considered. The harvest body weights of fish raised in monoculture, polyculture, and in different agroclimatic zones were considered as the same trait.

For the tagging body weight, the heritability estimate was very low while the effect common to full sibs other than additive genetics was very high. For the sampling and harvest body weights, the heritability was a medium magnitude (Table 4). Thus, the prospect for the genetic improvement of growth in rohu is promising.

**Table 4. Estimation of heritability of body weight.**

Body weight	H <sup>2</sup> ± se
Tagging	0.05 ± 0.07
Sampling	0.23 ± 0.09
Harvesting	0.23 ± 0.06

A different, less risky procedure for rearing the newly hatched larvae from different full-sib groups until tagging size needs to be developed.

### Realized response to selection and genetic gain

Each year, a control group was produced using 10 male and 10 female parents with an average breeding

value for the harvest body weight. The control group was reared in replicated nursery ponds. They were tagged individually and reared in the growout ponds together with the tagged fish from all the selected families. In the field tests, local hatchery stocks were also used along with the control and selected families in the rearing program.

Harvest body weight data recorded for the control animals and the selected animals were used to obtain estimates of the realized selection response per generation for the harvest body weight (i.e. as the difference in the mean performance of all the selected families and the control group).

One of the main objectives in a breeding program is to maximize the genetic gain per generation of selection. This is obtained by selecting individuals with higher genetic merit or breeding value. Different methods are available to estimate breeding values. In the rohu breeding program, the selection index procedure is used to estimate the breeding value. Information from full sibs, half sibs and individuals is considered for breeding value estimation. This procedure efficiently combines all the available information about one as well as several traits recorded on the breeding candidate and its relatives into an index of genetic merit. In the rohu project, an average genetic gain of 17 per cent per generation was observed after five generations of selection in the research center. However, a much higher response was observed in different field testing centers.

### Statistical analysis

Editing of the data and basic statistical analysis were performed using the SAS statistical package.

For each year-class, breeding values for the harvest body weight (for the trait selected in each year-class) were calculated for all the breeding candidates (using own, full- and half-sib body weight records), and for an SAS program developed in the project.

### Field testing of improved rohu

Field testing was initiated and carried out from 1999 to 2001 at different centers listed below (Table 5).

1. Kausalyaganga State Fish Farm
2. Rahara, West Bengal
3. Vijayawada, Andhra Pradesh
4. Ludhiana, Punjab

In all these centers, improved rohu showed significantly higher growth than the control and local hatchery stocks.

**Table 5. Year-class and locality where selected families and control groups were tested.**

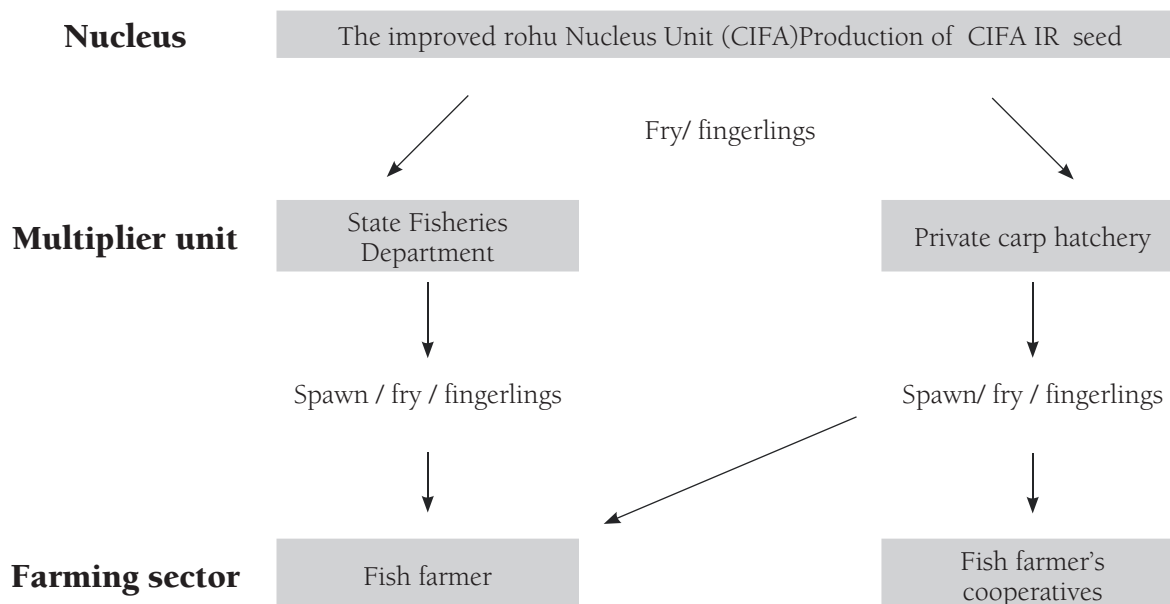
Year class	Locality	Selected families	Selected control	Local control
1995	CIFA (Orissa)	X	X	
1996	CIFA (Orissa)	X	X	
1997	CIFA (Orissa)	X	X	
1999	CIFA (Orissa) Andhra Pradesh State Dept (Orissa)	X X X	X X X	X
2000	CIFA (Orissa) Andhra Pradesh Punjab West Bengal	X X X X	X X X X	X X X
2001	CIFA, Orissa West Bengal Andhra Pradesh	X X X	X X X	X X
2002	CIFA, Orissa	X	X	

### Dissemination of improved rohu

Initially, the dissemination of improved rohu is planned for the following three states:

1. Orissa (through the State Fisheries Department)
2. West Bengal (CIFA, Regional Center)
3. Andhra Pradesh (Private Hatchery, Sairam hatchery)

The improved rohu, popularly called “Jayanti”, as it was named in 1997 (i.e. the 50th anniversary of Indian independence - Swarna Jayanti), has been released to several hatchery owners so that they can provide better quality seed to the fish farmers.



**Figure 3. Dissemination plan for improved Rohu.**

## Dissemination plan for the Jayanti rohu

Through an effective dissemination mechanism, it is possible that research products will reach the ultimate users (i.e. fish farmers). At present, rohu dissemination has been planned through different meetings and suggestions from collaborative institutes, international institutes and peer groups. The scope of the dissemination plan outlined below has the means for modifications with change in situations of nucleus and multiplier units.

Dissemination at present will be done with a single nucleus (i.e. CIFA).

The basic elements of a multiplication and dissemination program (Figure 3) according to the production and distribution process, are as follows:

1. Distribution of *Jayanti* brood stock: Research Institute (i.e. CIFA) provides broodstock to selected hatcheries.
2. Multiplication: hatcheries (Government, private farmers), using brood stock from the Research Institute, produce seed (fry or fingerlings) for distribution to grow-out farmers.
3. Nursing: seed from the hatcheries are reared prior to stocking in grow-out ponds. The rearing may be conducted by the end-user farmers themselves or by multiplier units that, in turn, sell or distribute the reared seed (fry, fingerlings, yearlings) to grow-out farmers.

### Addressing farmers' needs

While designing and implementing a multiplication and dissemination program, the focus of the institute is often on the improved seed and the mechanisms required to make the improved seed available to the farmers. Unfortunately, such a focus on the seed may cause the institute and its multipliers to lose sight of the farmers and their needs.

The institute will ensure that farmers are able to obtain the maximum benefit offered by genetically improved seed. This will require that the farmers be provided with adequate training, education and technical support. Although government extension programs exist, the institute and its multipliers will carefully evaluate the extension services provided to the farmers by the Government.

The dissemination plan for improved rohu will be state-specific. In Andhra Pradesh, since the private hatchery is doing a very good job, the improved rohu may be disseminated through it. Regional Research Centers (RRC) of CIFA at Andhra Pradesh are monitoring the program. Selection of an additional

hatchery in Andhra Pradesh other than the SaiRam hatchery as a multiplier unit needs further study and suggestions.

In Orissa, since Government hatcheries are selected as multiplier units they will breed and supply seed to the farmers. At present, six hatcheries have been stocked with improved rohu. Additional Government hatcheries can be stocked with improved Rohu for proper distribution.

In West Bengal, the present Regional Research Center of CIFA is acting as a multiplier unit. The West Bengal State Fisheries Department and some private hatcheries may be contacted, and dissemination can be planned under the supervision of the RRC, Rahara.

Apart from these states, some other states such as Chattisgarh and Tripura, or any other suitable states or organizations may be considered for multiplier units in future.

It is also the responsibility of the multiplier units to collect feedback from the farmers. Jayanti rohu grow-out farmers are required to adhere to the conditions that are described below.

- a. Improved rohu seed received will only be used for table size fish production.
- b. The Jayanti rohu will not be utilized or retained in the farm for breeding and propagation purposes.
- c. Fish farmers should follow the complete package including feeding and sampling procedures provided by the nucleus through multiplier units.
- d. The farmers will provide feedback regarding production and sale to the multiplier unit or nucleus from time to time.
- e. Any violation of the above conditions will result in legal actions by the multiplier or nucleus (CIFA).

### Addition of new trait to growth in rohu

Recently another trait (i.e disease resistance) against *Aeromonas hydrophila* was initiated at the CIFA, India in collaboration with the AKVAFORSK, Norway. Standardization of the mass challenge test for rohu was completed. A wide variation in the survival percentage was observed in different full-sib groups. The project is in progress.



## Conclusions

Improved Jayanti rohu- is the first genetically improved fish of India. In order to capitalize on the efforts made for the development of Jayanti rohu, its dissemination to farmers must be effective. The notion of hatcheries engaging in the production of their own broodstock may be discouraged. Experience shows that this is likely to result in inbreeding and impaired performance, and this will damage the reputation of Jayanti rohu. Therefore, vigilant effort is required for effective dissemination of improved rohu.

The experiences and lessons learned from the selective breeding of rohu are plenty, such as the production of full-sib groups in rohu carp, individual tagging methodology, selective breeding hatchery management, and data analysis. These experiences can be utilized by other carp selective breeding programs to avoid initial failures.

A lesson to be learned from other (terrestrial) species is that the processes of multiplication and dissemination occur in a more systematic and effective manner when special resources are assigned to the task.

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# Policies on Release of Improved Fish Strains in China

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## Abstract

Since 1991, the certification, release and maintenance of new species for aquaculture have become part of the national policy in China. During the past 15 years, this policy has been conducted and improved and has begun to show its significant role in Chinese fisheries. This paper describes the updated system of certification, release and maintenance of new species for aquaculture in China.

## Present certification system

### Challenges of aquaculture in the 21st century

The history of aquaculture in China dates back at least 2500 years; however, it has developed rapidly since the 1950s. The breakthrough of artificial propagation of Chinese carps in 1960s changed the traditional practice of collecting the wild fry from the rivers, and formed a strong seed basis that gave an essential support to the rapid development of aquaculture during the 1960s and 1970s. Further, China's aquaculture has entered a new era since 1978, when the open-policy and economic reform was adopted. The total output of aquaculture in 2004 reached 32.08 million tonnes, representing 65.46 per cent of the total aquatic production.

In the freshwater aquaculture section, the most commonly farmed species are the native carps, mandarin fish, and river crab. The exotic species that are cultured broadly are tilapia, rainbow trout, channel catfish, and largemouth bass. In marine aquaculture section, the representative principal species shifted to seaweeds (kelp and porphyra) in the 1960s, to shellfish (mussel and clam) in the 1970s, to scallop in the 1980s, to shrimps in the 1980s and 1990s, and to fish from 1990 onward.

At present, there are about 60 species of fish, over 10 species of crustacean, over 20 species of shellfish, and more than 10 species of seaweeds that are cultured in different farming systems.

The challenge of aquaculture development in the new millennium is high. The Chinese population is predicted to rise from the present 1.2 billion to 1.6 billion by 2026. This situation and the increase in

living standards have presented the Chinese with several challenges as well as opportunities to meet the rising demand for low- and high-quality animal products, in particular the aquatic products. Apart from this, marine fish stocks from the wild are decreasing; hence, the Chinese fishery development policies have focused on expanding aquaculture as a key strategy to meet the changing national demand and consumer patterns. However, to get to this level, there are three key issues that need to be considered properly: (1) environmental carrying capacity, (2) genetic improvement, and (3) disease control. First, it is urgently needed to develop the environmentally sustainable production systems, namely water saving, land saving, feed saving and low waste culture systems, within the carrying capacity. Second, since most of the species cultured are still from wild stock and without genetic improvement, new genetically improved strains or varieties are needed to pour new energy into development of aquaculture. Finally, the outbreak of new disease must be prevented by effective disease control measures to strengthen the industry.

In view of the national strategies for the development of both inland and marine aquaculture, it has been recognized that increasing the input to production alone is not enough. Good quality seed and its diversity are necessary prerequisites.

In 1991, the Government of China approved the establishment of the National Certification Committee of Aquatic Wild and Bred Varieties (NCCA-WBV) under the Ministry of Agriculture (MOA).

After the establishment of the NCCA, the certification system has been extended into provincial level in some major provinces. But only the NCCA has been authorized by the central government to certify the improved fish strain and the Ministry of Agriculture (MOA) has been authorized to release the improved fish strain.

### *Organization of the NCCA*

The NCCA consists of geneticists, aquaculturists, and administrators from research institutes, universities, as well as officials of the Bureau of Fisheries, Ministry of Agriculture. The secretariat of the NCCA is under the umbrella of the National Fisheries Technology Extension Center in Beijing. The Committee holds its meeting annually to discuss (i) the certification of good aquatic species, and (ii) the evaluation of the national aquatic seed farms.

### *Mandate of the NCCA*

*Certification of good species for aquaculture.* In Article 16 of the “Fisheries Law”(2000), it is stated that “any new aquaculture species”, can only be extended after first being certified by the National Certification Committee of Good Aquatic Species, and then approved by the Fisheries Authority of the National Council, Ministry of Agriculture of China.

The term “good aquatic species” includes four groups: economically important wild stocks, genetically improved varieties, good hybrids, and good exotic species.

*Evaluation and examination of the National Aquatic Wild and Bred Seed Farm* This assesses the major institutions that maintain the popular nature of genetically improved varieties, hybrids and exotic species as well as the wild stocks used in aquaculture.

*Establishment of the certification regulations and policies.* The following regulations and policies have been made and released to the public: (i) the National Certification Standard for Good Aquatic Species; (ii) the Management Standard for the Quality Control of Products of Aquatic Seed Farms; (iii) the Approaches to the Evaluation and Certification of the National Aquatic Seed Farm, (iv) the Standard for Production

Management of the Aquatic Seed Farm; (v) the Key Points for Construction of the National Aquatic Seed farm, (vi) the Operative Technology Standard for the Production of Major Cultured Fish, and (vii) the Management of Aquatic Brooders and Fry.

*Certification procedures.* In order to process the certification of good seeds, two types of documents should be submitted: (i) principal document, and (2) attached documents. The principal document is an application report whose major contents include the original sources of seeds, breeding process, major characteristics, extension and evaluation. The attached documents include a research report, a technical report (covering reproduction, seed production, genetic characterization, inspection of genetic characteristics by an authority appointed by the NCCA), an identification report on disease resistance by an authority appointed by the NCCA (if required), and an on-farm testing report made during the last two years.

### **Release**

In Article 16 of the “Fisheries Law” (2000), it is stated that “any new aquaculture species can only be extended after first being certified by the National Certification Committee of Good Aquatic Species and then approved by the Fisheries Authority of the National Council”. The Fisheries Authority of National Council is the Ministry of Agriculture of China.

All certified and released species/strains are listed in Appendix I.

### **Maintenance**

In order to maintain the nature of certified and released species, the Government has established 36 national seed farms (Appendix II)

## List of certified and released species

	Common name	Scientific (Latin) name	Certification number
1	Xingguo red common carp	<i>Cyprinus carpio singuonensis</i>	GS01 □ 001 □ 1996
2	Purse red common carp	<i>Cyprinus carpio wuyuanensis</i>	GS01 □ 001 □ 1996
3	Pengze crucian carp	<i>Carassius auratus var pengzenensis</i>	GS01 □ 001 □ 1996
4	Jin common carp	<i>Cyprinus carpio var.jian</i>	GS01 □ 004 □ 1996
5	Songpu crucian carp	<i>Carassius auratus gibelio var.songpu</i>	GS01 □ 005 □ 1996
6	Cold tolerant strain of purse red common carp	<i>Cyprinus carpio wuyuanensis</i>	GS01 □ 006 □ 1996
7	Selected strain of German mirror common carp	scattered <i>Cyprinus carpio</i> mirror	GS01 □ 007 □ 1996
8	Hybrid of Nile tilapia × blue tilapia		GS02 □ 001 □ 1996
9	Fushou tilapia ( <i>Oreochromis mossambicus</i> × <i>O. niloticus</i> )		GS02 □ 002 □ 1996
10	Yin common carp {scattered mirror common carp × [nucleo-transfer purse red common carp (nuclear donor) × crucian carp]}	<i>Cyprinus carpio</i>	GS02 □ 003 □ 1996
11	Fen common carp (Xingguo red common carp × Scattered-scale mirror common carp)	<i>Cyprinus carpio</i>	GS02 □ 004 □ 1996
12	Heyuan common carp (Purse red common carp × Yuangjiang river common carp)	<i>Cyprinus carpio</i>	GS02 □ 005 □ 1996
13	Yue common carp (Purse red common carp × Xiangjiang river common carp)	<i>Cyprinus carpio</i>	GS02 □ 006 □ 1996
14	Trecrossed common carp (Heyuan common carp × scattered scale common carp)	<i>Cyprinus carpio</i>	(GS02 □ 007 □ 1996)
15	Furong common carp (Scattered scale mirror common carp × Xingguo red common carp)	<i>Cyprinus carpio</i>	GS02 □ 008 □ 1996
16	Allogynogenesis crucian carp (Fangzheng silver crucian carp × Xingguo red common carp)	<i>Carassius auratus gibelio</i>	GS02 □ 009 □ 1996
17	Nile tilapia	<i>Oreochromis niloticus</i>	GS03 □ 001 □ 1996
18	Blue tilapia	<i>Oreochromis aureus</i>	GS03 □ 002 □ 1996
19	Large mouth bass	<i>Micropterus salmoides</i>	GS03 □ 003 □ 1996
20	Colossoma	<i>Colossoma brachypomum</i>	GS03 □ 004 □ 1996
21	Channel catfish	<i>Ictalurus punctatus</i>	GS03 □ 005 □ 1996
22	Rainbow trout	<i>Salmo gairdnerii</i>	GS03 □ 006 □ 1996
23	Donalson strain of rainbow trout	<i>Salmo gairdnerii</i>	GS03 □ 007 □ 1996
24	Leather catfish	<i>Clarias lazier</i>	GS03 □ 008 □ 1996
25	German mirror common carp	Scattered <i>Cyprinus carpio</i> mirror	GS03 □ 009 □ 1996
26	German mirror common carp	Scattered <i>Cyprinus carpio</i> mirror	GS03 □ 009 □ 1996
27	Rohu	<i>Labeo rohita</i>	GS03 □ 011 □ 1996
28	Giant prawn	<i>Macrobrachium</i> sp	GS03 □ 012 □ 1996
29	Cuba frog	<i>Rana catesbiana</i>	GS03 □ 013 □ 1996
30	American frog	<i>Rana</i> sp	GS03 □ 014 □ 1996
31	Bay scallop	<i>Argopecten irradians</i>	GS03 □ 015 □ 1996
32	Xiayi scallop	<i>Patinopecten yessoensis</i>	GS03 □ 016 □ 1996
33	Pacific oyster	<i>Crassostrea gigas</i>	GS03 □ 017 □ 1996
34	“901” kelps	Kelp	GS01 □ 001 □ 1997
35	Songpu common carp	<i>Cyprinus carpio</i>	GS01 □ 002 □ 1997
36	GIFT Nile tilapia	<i>Oreochromis niloticus</i>	GS03 □ 001 □ 1007
37	No.1 Pujiang blunt-snout bream	<i>Megalobrama amblycephala</i>	GS01 □ 001 □ 2000
38	Glass red common carp	<i>Cyprinus carpio wanan</i>	GS01 □ 002 □ 2000
39	Turburt	<i>Scophthalmus maximus</i>	GS03 □ 001 □ 2000
40	Buffalo fish	<i>Ictiobus cyprinellus</i>	GS03 □ 002 □ 2000
41	Xiangyun common carp		GS02 □ 001 □ 2001
42	Xiangyun crucian carp		GS02 □ 002 □ 2001
43	Red-white long-tail goldfish	<i>Carassius auratus</i>	GS02 □ 001 □ 2002
44	Blue long-tail goldfish	<i>Carassius auratus</i>	GS02 □ 002 □ 2002
45	SPF white shrimp	<i>Penaeus vannamei</i>	GS03 □ 001 □ 2002
46	No 1 Yellow Sea Chinese shrimp	<i>Penaeus orientalis</i>	GS01 □ 001 □ 2003
47	Songhe common carp	<i>Cyprinus carpio</i>	GS01 □ 002 □ 2003

	Common name	Scientific (Latin) name	Certification number
48	???	<i>Xiphophorus helleri</i>	GS01 □ 003 □ 2003
49	Black dragon common carp	<i>Cyprinus carpio</i>	GS01 □ 004 □ 2003
50	Yuxuan yellow river common carp	<i>Cyprinus carpio</i>	GS01 □ 001 □ 2004
51	Dongfan 2 hybrid kelp	<i>Laminaria japonica</i>	GS02 □ 001 □ 2004
52	Rongfu hybrid kelp	<i>Laminaria japonica</i>	GS02 □ 002 □ 2004
53	Dalian 1 hybrid abalone	<i>Haliotis discus</i>	GS02 □ 003 □ 2004
54	???	<i>Chelydra serpentina</i>	GS03 □ 001 □ 2004
55	???	<i>Pangasius sutchi</i>	GS03 □ 002 □ 2004
56	???	<i>Hyriopsis schlegli</i>	GS03 □ 003 □ 2004

Notes:

GS□G: National level, S: Certificated; 01□Group of genetic improved variety; 02□ Group of hybrid; 03□ Group of exotic; 04□ Group of wild; 001□ Order of certification, by year and by group; 1996□ Year certified

## Appendix II

### List of certified and national farms for maintenance of the genetic property of improved fish

	Farm name	Target species/strains	Notes
1	Hangjiang Yangtze River "four Chinese carps" wild stock farm, Jiangsu	Four Chinese carps	
2	Ruichang Yangtze River "four Chinese carps" wild stock farm, Jiangxi	Four Chinese carps	
3	Laohe Yangtze River "four Chinese carps" wild stock farm, Hubei	Four Chinese carps	
4	Laohekou "four Chinese carps" ecological store, Hubei	Four Chinese carps	
5	Wild fish farm, Hunan	Four Chinese carps	
6	Jiaxin "four Chinese carps" wild stock farm, Zhejiang	Four Chinese carps	
7	Jiujiang Penzhe crucian carp farm, Jiangxi	Penzhe crucian carp	
8	Fangzhen silver crucian carp farm, Helongjiang	Fangzhen silver crucian carp	
9	Tilapia seed farm, Guangdong	Tilapia	
10	Nanjing tilapia seed farm, Jiangsu	Tilapia	Closed
11	Qingdao tilapia seed farm	Tilapia	
12	Shangdong tilapia seed farm	Tilapia	
13	Xinguo red common carp farm, Jiangxi	Xinguo red common carp	
14	Wuyuan Purse red common carp, Jianmgxi	Purse red common carp	
15	Fanchang mitten crab farm, Anhui	Mitten crab	
16	Changsha soft-shelled turtle farm, Hunan	Soft-shelled turtle	
17	Shaoxin soft-shelled turtle farm, Zhejiang	Soft-shelled turtle	
18	Nanton zhichai farm, Jiangsu	<i>Porphyra</i>	
19	Yantai kelp farm, Shangdong	<i>Laminaria</i>	
20	Qinghai wild fish farm	<i>Gymnocypris przewalskii</i>	
21	Rizhao Chinese shrimp farm, Shandong	<i>Penaeus orientalis</i>	
22	Weihai flatfish farm, Shandong	<i>Paralichthys olivaceus</i>	
23	Penlai turbot farm, Shangdong	Turbot	
24	Hangzhou black bream wild stock farm, Zejiang	<i>Megalobrama terminalis</i>	
25	Dongguan Luca soft-shelled turtle farm, Guangdong	Soft shelled turtle	
26	Songjiang fish farm, Shanghai	Pujiang 1 bream (blunt snout bream)	
27	Geihu bream farm, Jiangsu	Pujiang 1 bream (blunt snout bream)	
28	Rainbow trout farm, Jinling	Rainbow trout	
29	Gaoshun Yangtze reive mitten crab farm, Jiangsu	Mitten crab	
30	Huanxin aquatic farm, Tianjing	Freshwater fish	
31	Duofu turtles farm, Hubei	Turtles	
32	Tropical fish farm, Hainan	Tropical fish	
33	Liangzhihu bream farm, Hubei	Blunt-snout bream	
34	Ninde yellow croakle fish farm, Fijian	<i>Pseudosciaena crocea</i>	
35	Renque "four Chinese fish" farm, Hebei	Four Chinese carps	
36	Zhongjie tilapia farm, Hebei	Tilapia	
37	Luye tilapia farm, Fujian	Tilapia	
38	Catfish farm, Sichuan	<i>Silurods meridionalis</i>	



# Maintaining the “Public Good” Nature of Improved Fish Strains: Dissemination of knowledge and materials

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## Abstract

Many sources of information that discuss current problems of food security point to the importance of farmed fish as an ideal food source that can be grown by poor farmers, (Asian Development Bank 2004). Furthermore, the development of improved strains of fish suitable for low-input aquaculture such as Tilapia, has demonstrated the feasibility of an approach that combines “cutting edge science” with accessible technology, as a means for improving the nutrition and livelihoods of both the urban poor and poor farmers in developing countries (Mair et al. 2002). However, the use of improved strains of fish as a means of reducing hunger and improving livelihoods has proved to be difficult to sustain, especially as a public good, when external (development) funding sources devoted to this area are minimal<sup>1</sup>. In addition, the more complicated problem of delivery of an aquaculture system, not just improved fish strains and the technology, can present difficulties and may go explicitly unrecognized (from Sissel Rogne, as cited by Silje Rem 2002). Thus, the involvement of private partners has featured prominently in the strategy for transferring to the public technology related to improved Tilapia strains. Partnering with the private sector in delivery schemes to the poor should take into account both the public goods aspect and the requirement that the traits selected for breeding “improved” strains meet the actual needs of the resource poor farmer. Other dissemination approaches involving the public sector may require a large investment in capacity building. However, the use of public sector institutions as delivery agents encourages the maintaining of the “public good” nature of the products.

## What is a “Public Good” Nature?

The term “public good” is derived from a concept formulated by economists that allows us to differentiate between those goods that are “non-rivalrous” and “available for use by all simultaneously”, from those that are not<sup>2</sup>. For organizations that are primarily concerned with transferring know-how, technical information and materials to the poor farmer, a “working” definition of public goods, though technically flawed, may prove to be a more practical alternative definition. For example, such a “working definition” would include as public goods the products/knowledge with the attributes of being:

- Useful (beneficial)
- Accessible to all
- Distributed/disseminated
- Amenable to simultaneous use with no exclusivity

Such a definition allows those of us that work “close to the ground” to have a more definite understanding of what are called public goods. It should be noted that such a working definition does not refer to ownership, intellectual property rights (IPRs), or other legal/regulatory issues. Nor is there a concern about benefit-sharing *per se*.

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<sup>1</sup> As an example of the relative paucity of support, a report issued by the U.S. Department of Commerce, the U.S. National Oceanic and Atmospheric Administration, and the U.S. National Marine Fisheries Service (The Rationale for a New Initiative in Marine Aquaculture 2002), indicated that the support for aquaculture from USAID was only US\$3 million, out of the total (2001) budget of roughly US\$7,587,278,000 (Source of 2001 budget figures : <http://www2.usaid.gov/pubs/cbj2002/request.html>)

<sup>2</sup> For a current discussion of the formal definition of the term “public good”, the information at the Wikipedia site: [http://en.wikipedia.org/wiki/public\\_good](http://en.wikipedia.org/wiki/public_good), may be of interest to readers. For a more classical formal definition, see [http://www.dur.ac.uk/t.i.renstrom/teaching/PUBECON/Lect\\_12.pdf](http://www.dur.ac.uk/t.i.renstrom/teaching/PUBECON/Lect_12.pdf) or [http://www.who.int/trade/distance\\_learning/gpgh/gpgh1/en/index1.html](http://www.who.int/trade/distance_learning/gpgh/gpgh1/en/index1.html). Another interesting discussion can be found in the reference, “A Theory of the Theory of Public Goods” by Randall G. Holcombe, which can be viewed at the URL: [http://www.mises.org/journals/rae/pdf/rae10\\_1\\_1.pdf](http://www.mises.org/journals/rae/pdf/rae10_1_1.pdf).

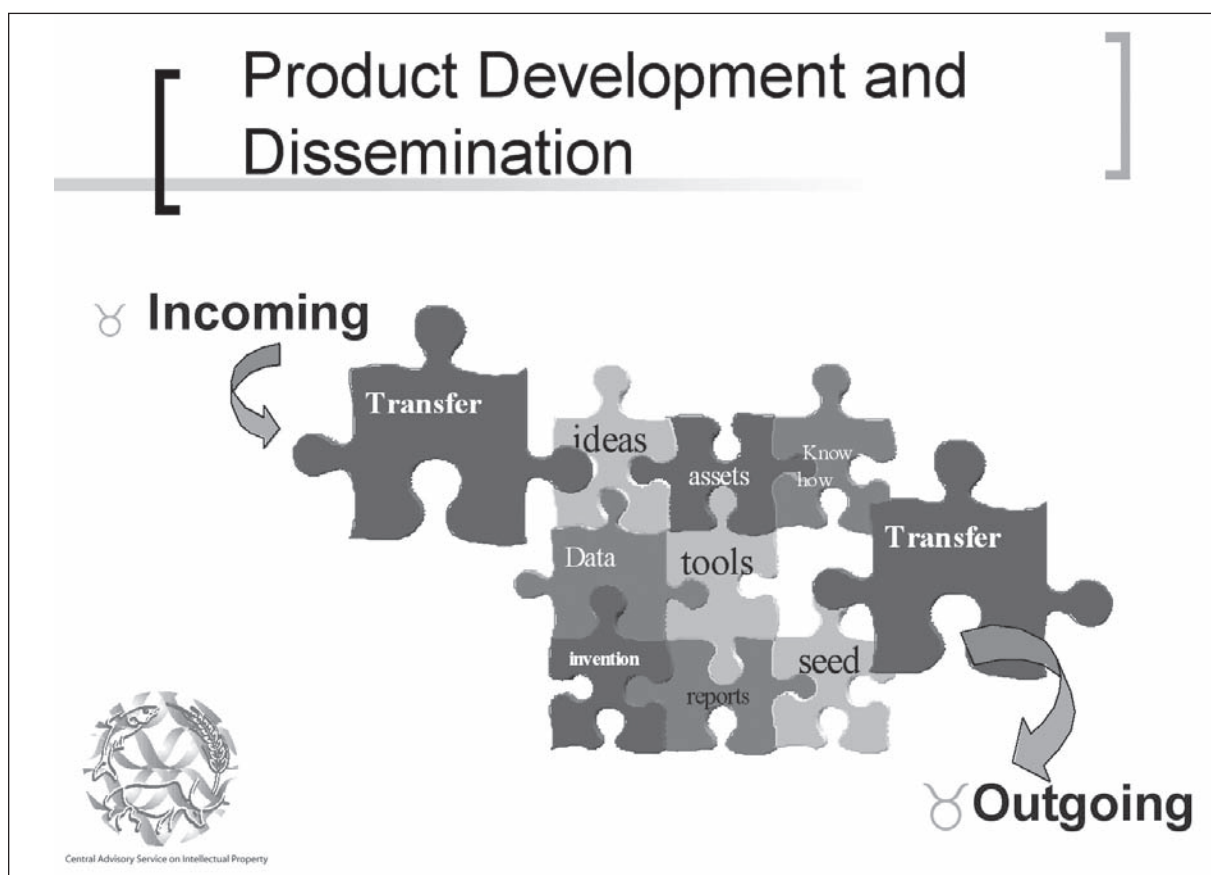
## Why should we be concerned about maintaining the “public good” nature of improved fish strains?

### Some possible reasons include:

- The most straightforward way of ensuring access and distribution to the poor farmer
- Traditional/historical way to catalyze small entrepreneurs in developing countries
- Burden of limiting potential legal problems of the originator

Distribution of products that are public goods is an effective and equitable means by which those of us that work in the public arena can attempt to live up to our mandate for addressing poverty and

malnutrition. Public funding of research that produces products that address the needs of poor farmers is the fundamental mode for improvement of livelihoods through agriculture. In addition, public investment in the plant breeding sector with the subsequent uptake of these varieties by entrepreneurs is one approach to a “sustainable” way to address poverty and malnutrition in developing countries; this has been highlighted as the classical way in which seed companies are established, thus taking on the role of supplying improved seed to the farmer, (D. Duvick, as cited in Fernandez-Cornejo 2004)<sup>3</sup>. The emphasis for the last bullet point is brought home by the fact that product development usually includes the improvements that one institution has made, combined with inputs made by or belonging to others, as illustrated in Figure 1.



**Figure 1. Product development and dissemination.**

<sup>3</sup> For a discussion of this point from an “entrepreneurial” view, see “International Agricultural Development: Role of Private Industry,” by Bruce Maunder, at the URL: <http://cropandsoil.oregonstate.edu/News/Publicat/Kronstad/38.html>. For a view from the opposite side, see, “Stolen Seeds: The Privatization of Canada’s Agricultural Biodiversity”, by Devlin Kuyek, at the URL: [http://www.interpares.ca/en/publications/pdf/stolen\\_seeds.pdf](http://www.interpares.ca/en/publications/pdf/stolen_seeds.pdf).

In the product development pathway, the rights and responsibilities that are associated with all the inputs need to be well defined and noted to ensure that none of the inputs used imposes restrictions that preclude the use or distribution of the end product to the poor. Even though there is no explicit mention of IPRs or legal and regulatory concerns when we are describing or defining a public good, it is easily seen that there is a need to make sure that no ownership/rights/regulatory issues interfere with the distribution of knowledge and/or products. By using a particular input, it is possible that we would undo the “public good nature of our product”. For example, if a proprietary marker for a particular gene allele was obtained from the owner under a material transfer agreement (MTA) that stated the marker was only to be used for “research purposes and not to select for fish that would be distributed for consumption or breeding”, great care would need to be taken to make sure that the terms of this MTA were not violated. It should be noted that in this example the marker, as a separate entity, would not end up in the selected fish. However, the use of the marker would still be prohibited if the resultant fish were going to be distributed outside of the research setting. If we have a product that cannot be distributed, then we no longer have a product that, in a practical sense, still has a public good character.

Presented in Table 1 are proposed categories of restrictions that can affect the “public good” nature of a product; this emphasizes the legal/IPRs constraints that should be identified with the use of inputs or resources in the production of any public good. While perhaps not all would share the view that the originator of the public good product should shoulder the responsibility of clearing legal and regulatory hurdles for its distribution, this author believes this is a key requirement for an equitable mode of access to public goods.

Access to improved fish strains is the most direct way to assist poor farmers attempting to utilize aquaculture as a means of improving their nutrition and livelihoods. Farmers may not have the money, resources, knowledge, energy, or necessary negotiation skills to obtain fish from a commercial source or to breed improved fish from seed stocks supplied to them. It is incumbent on those in institutions financed by public monies to provide knowledge and materials to stakeholders and clients as public goods, i.e. free of restrictions that prevent their use and distribution by the poor. In the Consultative Group on International Agricultural Research (CGIAR), it is our core business to provide public goods for poor farmers in developing countries<sup>4</sup>.

**Table 1. Potential constraints to distribution of products.**

Potential constraints to distribution of products:	
<ul style="list-style-type: none"> <li>■ Legal/IPR</li> </ul>	<ul style="list-style-type: none"> <li>■ What are the inputs to this product?</li> <li>■ Are there agreements, contracts, etc., associated with these inputs?</li> <li>■ Are there any IPRs over these inputs?</li> <li>■ Any provisions in funding agreements that might inhibit distribution of products?</li> <li>■ Compliance with national, regional, international commitments/treaties in users' areas?</li> </ul>
<ul style="list-style-type: none"> <li>■ Biosafety</li> <li>■ Food safety</li> <li>■ Other regulatory constraints</li> </ul>	

<sup>4</sup> The mission of the CGIAR is: “To achieve sustainable food security and reduce poverty in developing countries through scientific research and research-related activities in the fields of agriculture, forestry, fisheries, policy, and environment.” For more information about the CGIAR, please see the webpage at the URL: <http://www.cgiar.org>.

## How can we maintain the “public goods” nature of improved fish strains?

A process for accomplishing this can be carried out by a practice called “**Asset Identification**”. For the products already developed, this can be initiated by adequately describing them. Such a description might include the selection criteria and/or other phenotypic/genotypic information associated with an improved strain. It is important to document what has been done by staff of the institution and what ideas staff might have for carrying out further work on the materials that have been developed. All relevant documents such as funding contracts, material transfer contracts/agreements, agreements signed by visiting scientists working on projects associated with each project, etc. need to be organized and examined for language that might restrict potential distribution.

For products at a planning stage, a recommendation should be drafted concerning a technology transfer plan, or a product development and distribution plan. Such a plan would seek to answer questions such as:

- What are the needs of potential users?
- What is the plan for developing the strain, growing out seed stock, and disseminating fingerlings, from the start of the project?
- What capacity building/resource procurement activities need to be initiated to prepare farmers for utilizing the improved strains?
- What are the inputs and investment burdens on users (resource poor farmers)?
- Will partners be needed for product development and dissemination?
- How will monitoring, evaluation, and impact analysis be incorporated into the research plan?

Such a plan would lead to the development of a dissemination/business plan, and allow for the identification of skills, knowledge, and experience needed for each step in the plan. It would also allow for strategic decisions regarding capacity building or the use of existing expertise and capacity.

It is of course obvious that funding will need to be obtained under contracts that do not restrict our ability to distribute materials as public goods. Therefore, it is most likely that money will need to be public funding or some type of governmental intervention, or from philanthropic institutions. If the money is from private entities, these firms should be provided with a motivation such as tax incentives, etc. given to business entities that provide funding to public agencies/organizations/institutions, (National Academy Press 1999). (In addition, with less money allocated to fund research than in the past, others have proposed various schemes such as the awarding

of monetary “prizes” to those that produce the most useful public goods, (Love and Hubbard 2005).)

What about partnerships? Collaborative arrangements between institutions with complementary skills and/or assets have long been a means of producing public goods. In the past, particularly in agriculture research and product development aimed at producing public goods for the poor farmer in developing countries, these partnerships were between public partners (For example, see Mensah and Bie 1999). Public-private-partnerships (PPPs) are being promoted by a variety of organizations as a means of providing resources, know-how, and technology to public sector organizations (Spielman and von Grebmer 2004). Regardless of the type of partnership that is established, it will be necessary to go through a process of coming to an agreement regarding skills and assets to be contributed by each partner, roles and responsibilities that each partner will have, and means of resolving conflicts in the partnership ( For example, see Henson-Apollonio 2005). There will need to be a “coming to an agreement” over roles, responsibilities, and expectations in dealing with conflicts. Even the selection of traits, e.g. high growth rate, disease resistance, maturation rate, tolerance to high salinity water, may need to have a formal agreement among all partners. In the end, sometimes it may not be possible to reach an agreement, as indicated by the case of the company Icy Waters, a charr aquaculture company in northwestern Canada and the Nunavut Tribe (NWMB Meeting Minutes 1997). However, all would agree that it is important to establish this beforehand, if possible.

It is very important to develop the legal structure to support the development and dissemination plan, in language that is clear to staff from each institution. In addition, it is likely that different types of partnerships arrangements and agreements for different types of partners will be needed in order to ensure the “public good” nature of the improved fish strains, knowledge and/or know-how. Understandings among partners should place emphasis on “well-defined” roles in PPPs. In any research project, especially in putting together PPPs, time should be spent on defining the purpose of the research, i. e. what the expected “public good” products will be. This allows for the understanding of several important things such as:

- What partners will be needed, with what skills or resources and what steps to be taken to engage in a positive and “public good” framework?
- How to choose partners wisely?
- How to build in M&E and impact assessment methods, and to choose the partners to do this?
- What resources (financial and human) will need to be invested in transactions?
- What might be the negative aspects of the proposed research (e.g. introduction of alien species) and how will these negative aspects be overcome, or can they be overcome with the

technology that is available in the context in which the products will be utilized?

- What problems are likely to arise?
- What is a communications strategy?

It might even be necessary to develop and adopt an Intellectual Property Policy Statement to ensure that there are no misunderstandings regarding the “public good” nature of the proposed research products. If at all possible, agreements should be reached, wherein the text can be made available, at least to researchers, if not always to the public at large. (An example from another biological field is the US Public Health Service/DuPont Pharmaceutical MOU for access to “Cre-Lox” mice. See, URL: <http://www.ott.nih.gov/pdfs/cre-lox.pdf>)

The need to have local partners involved in producing public goods cannot be overstated, especially in aquaculture projects. Governmental agencies/ministries should be identified, including those that deal with quality control of food fish, water resources, and biodiversity/environmental regulators. In addition, with aquaculture projects, some public goods may take the form of advocacy/building capacity in advocacy. It is quite likely that, in order to gain acceptance for improved fish strains, the projects will involve some degree of advocacy that may include public consultations/discussions regarding the following aspects:

- Whether governments are only concerned with “technical problems” solvable by experts
- Whether regulation is the “state’s responsibility”
- Whether “industry” should self-regulate in their own interests when state capacity is insufficient
- Whether the contribution for improving the sustainability of the industry is from small scale farmers; hatcheries and traders; local communities where farms and factories are sited; and consumers and broader civil society including the international research community
- Whether the poor can have access to wetlands

In line with the above, consider this excerpt from a review article on the GIFT project:

“As poor farm practices or other environmental problems can inhibit the effective use of the improved GIFT strains, the Foundation has begun providing technical support to Philippine farmers of the GIFT strain.”

Greer and Harvey (2004)

Another area that should be considered is the publications that are associated with research and experience in aquaculture. Authors should be encouraged to publish their results, stories, and recommendations in “Open Access” journals and/or to make copies of their manuscripts available on publicly accessible websites. (See: <http://www.doaj.org/articles/about#definitions>, <http://www.eprints.org/documentation/handbook/overview.php>, and <http://creativecommons.org/>. for more information.) Authors should also ensure that their publications serve as defensive publications, i. e. serving as prior art for the purposes of preventing the patenting of their ideas and/or innovations. (For guidance, see Adams and Henson-Apollonio 2002.)

In conclusion, thoughtful planning and attention to detail in project management are necessary for assuring the “public good” nature of improved fish strains, knowledge and know-how associated with improved strains, including even those that are produced by public institutions with public funds and resources.



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