Breeding Plan for Common Carp (Cyprinus carpio) in Indonesia: Multiple-Trait Selection

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FOREWORD

To improve and sustain the performance of tropical finfish species farmed in developing countries, the International Network on Genetics in Aquaculture (INGA) has been assisting the member-countries of the network (Bangladesh, Peoples Republic of China, Cote d'Ivoire, Egypt, Fiji, Ghana, India, Indonesia, Malaysia, Malawi, Philippines, Thailand and Vietnam) in developing selective breeding research and development programs.

At the request of the Government of Indonesia, INGA fielded a mission to Indonesia in November 1996, consisting of INGA Research Coordinator Dr. Modadugu V Gupta and Drs. Trygve Gjedrem and Hans Magnus Gjoen of Institute of Aquaculture Research (AKVAFORSK), As, Norway, to assist national scientists in prioritizing aquaculture species and traits for genetic improvement and develop plans for selective breeding of the prioritized species.

Common carp (Cyprinus carpio) is an important species for aquaculture in ponds and cages in Indonesia contributing 135,200 tons to fish production in 1994 (27.4% of total finfish production from fresh and brackishwater aquaculture). The Freshwater Fisheries Research Institute (RIFF), Sukamandi, Indonesia has identified 20 strains of the species from islands of Java and Sumatra, with different colours and body form. In view of the importance of the species in aquaculture and the liking of the Indonesian population for the species, it has been identified as a priority species for genetic improvement through selective breeding.

This report details the plan for selective breeding of common carp using multiple trait selection and has been prepared by Drs. Trygve Gjedrem and Hans Magnus Gjoen of AKVAFORSK and Drs. Atmadja Hardjamulia, Ir. Sudarto, Rudhy Gustiano, Anang Hari Kristanto and Lies Emmawati of the Research Institute for Freshwater Fisheries, Sukamandi and Maman Suparta of the Faculty of Fisheries, University of Pajajaran, Indonesia.

INGA acknowledges the support provided by the Indonesian Network of Fish Genetics Research and Development (INFIGRAD) to the mission and in preparing these plans.

We hope that this document will be useful to researchers in Indonesia for implementing the plans developed and informative to others involved in selective breeding in other countries.

Modadugu V Gupta
INGA Research Coordinator
BREEDING PLAN FOR COMMON CARP (*Cyprinus carpio*) IN INDONESIA: MULTIPLE-TRAIT SELECTION

INTRODUCTION

Total aquaculture production in Indonesia in 1994 was 493,000 tons of finfish, 169,000 tons of shellfish, mainly shrimp and 115,000 tons of seaweed with an estimated value of US$ 2,075 million. In 1994, the production of common carp (*Cyprinus carpio*) was 135,200 tons, from ponds (52,600 tons), cages (28,600 tons) and ricefields (54,000 tons).

The potential for aquaculture in Indonesia is large. The mangrove forest area is estimated to be around 4.29 million ha. It is recommended to use only 20% of the total mangrove area for aquaculture production (830,900 ha). To day only 37% of this area is used for aquaculture. The most suitable species for brackishwater aquaculture are shrimp and milkfish. The area for mariculture is 81 million ha, with potential production of about 47 million tons of fish, shellfish and seaweed. The total area of freshwaters is 55 million ha, consisting of lakes, dams, rivers, swamps and other water bodies. Potential area for freshwater pond fish culture is estimated to be 338,121 ha., with a production potential of 8,900,000 tons per year (DGF, Indonesia, 1994).

Common carp is the most important freshwater fish, consisting of many strains with a variety of colours and body forms. The research Institute of Freshwater Fisheries (RIFF) collected 20 strains from the islands of Java, Sumatra and Bali. The results of the evaluation of the four strains: Rajadanu, Wildan Cianjur, Sutisna Kuningan and Cangkringan Yogyakarta are promising, while Majalaya as the existing populat cultivated strain, has wide distribution.

Common carp can spawn all the year round in Indonesia, and each broodfish can spawn naturally every three months without hormone injection. Simultaneous spawning of common carp can be induced by injecting the females and males with pituitary gland extract or manufactured hormones (Ovaprim from Canada or LRH-A from China). Two injections are administered to the females at an interval of 4-6 hours. More than 75 percent of the females spawn after injection. Males are injected once at the same time as the second injection for the females with a dose of one fifth to one sixth of the dose given to the females.

One of the main problem of common carp culture in Indonesia is early sexual maturation, particularly in males. Inbreeding might be a problem that can effect growth rate since fish farmers in general have
small number of broodstock, sometimes less than 10 fish. Fish diseases, notably *Aeromonas hydrophilla*, are a common problem, especially at fry stage.

The strategic plan of AARD (Agency for Agricultural Research and Development) for 1995 - 2005 gives high priority to improvement of genetic potential of fish, livestock, crop and micro-organisms (AARD, 1994). In accordance with this, the Indonesian Network of Fish Genetics Research and Development (INFIGRAD) in co-operation with the International Network on Genetics in Aquaculture (INGA) has taken the initiative to establish a breeding program for common carp in Indonesia. In this program, INFIGRAD will have its role as research co-ordinator, and the members may contribute in the research and development under the responsibility of RIFF, Sukamandi, which will serve as the breeding centre where mating, testing and selection takes place.

As RIFF develops and gets experience in running the breeding program, mating, testing and selection will become more and more standard routine work. This activity will also occupy much of the research capacity at RIFF. At that stage, INFIGRAD may assist in reorganising the breeding program in order to reduce the routine breeding work at RIFF and develop other breeding centres.

The effect of a breeding program depends very much on how it is organised to disseminate the obtained genetic improvement rapidly to the farmers. AIAT (Assessment Institute for Agricultural Technology) and FADC (Freshwater Aquaculture Development Centre) can play a central role in the dissemination of the improved fish. It is important that INFIGRAD co-ordinate this process.

**BREEDING GOAL**

The most important economic traits in common carp are:
- Growth rate
- Delayed sexual maturation
- Disease resistance
- Body composition, texture

Body weight should be recorded at the marketed size, which is 250 - 300 g. As the weight is recorded, sex and sexual maturation should be recorded for each fish.

Challenge test procedures to get estimate of disease resistance are developed for *A. hydrophilla* which is considered to be the most serious disease problem in common carp.
Flesh quality is of importance in common carp, and texture is considered to be the most significant quality trait. Instruments are now developed to measure texture in Atlantic salmon, and an investigation should be carried out to apply the method in common carp.

At the start of the breeding program, the breeding goal should therefore be growth rate, disease resistance and reduced frequency of early sexual maturation. As recording methods are developed for flesh texture, it should be included in the breeding goal.

**SELECTION METHOD**

The breeding method should be index selection (see Appendix). This will require tagging of all test fish. Family information is required for improvement of frequency of early sexual maturation and disease resistance. The breeding values of the broodstock will be computed based on:

1. The growth performance of the individual and its full and halfsibs
2. The frequency of early sexual maturation and the survival rate in challenge test with *A. hydrophilla* of the full and halfsibs of the breeding candidates.

The relative weighting of the different traits and sources of information will be determined by the economic importance of the traits for the fish farmers.

The breeding program should be designed to avoid loss of genetic variation and to avoid rapid accumulation of inbreeding (see Appendix). This may be done by securing a large effective population size. The proposed breeding plan ensures a large effective population size, accurate recording of pedigree and evaluation of the inbreeding level after each round of selection. Assuming a heritability of about 0.2 for the traits included in the breeding goal, the design of this breeding program is expected to result in a rate of inbreeding of less than 1% per generation, which will be sufficient to maintain the genetic variation and thus ensure a sustainable genetic progress.

**TEST OF GENOTYPE BY ENVIRONMENT INTERACTION AND FORMING OF BASE POPULATION**

There are many strains of common carp in Indonesia and several experiments have been carried out to compare and describe these strains. The strains considered to be of particular value and therefore should form a synthetic population to be the basis for a selective breeding program are: Majalaya, Rajadanu, Wildan Cianjur and Sutisna Kuningan.
The base population will be formed by crossing these four strains in a complete diallel cross, see Table 1. Eight to ten pairs of broodstock from each strain should be used in the crossbreeding trial.

This diallel cross makes it possible to estimate the magnitude of non-additive genetic effects for growth rate, age at sexual maturation and disease resistance. In the following it is assumed that the heterosis effect is relatively low and, therefore, standard selection for additive genetic effects is chosen to improve common carp. If, however, the heterosis effect is significant and represents a large part of the total genetic variation, the breeding plan should be revised to combine selection and crossbreeding.

Common carp is farmed under very different farming and environmental conditions in Indonesia. It is therefore important to investigate if genotype by environment interaction (correlation between performance under different environmental conditions for each genetic group) is of importance. This should be done by rearing the different groups in the 4 x 4 diallel cross in at least 3 environments (pond, rice-fish pond and cage). The traits in the breeding goal should be recorded at the marketed size (see below). In order to have about 500 kg of bio-mass in each pond at 300 g size (ca. 1650 fish i.e. ca. 100 from each cross), 350 offspring (assuming 30 % survival) from each cross should be tagged at 5 g average size and stocked into each test environment (for three test environments: 1050 from each cross).

If substantial genotype by environment interaction is found between important farming environments, it should be considered to split the breeding program in two or more programs with different breeding goals. For example, separate breeding programs for cage culture and pond culture.

START OF THE BREEDING PROGRAM

In the first round of selection, one should assure that a majority of the best crosses are represented in the forming of the first generation. This means that only the lowest performing crosses should be discarded as broodstock. This is important in order to ensure broad genetic variation in the future breeding population. Still, selection within the best crosses should be performed.

PRODUCTION OF FAMILIES

The production of families will follow the design described in Figure 1. One testing round is performed in 6 months. The population should be divided in two, and the first half of the population (family 1-100) will be tested the first 6 months, and the other half (family 101-200) will be tested the proceeding 6 months.
Selected and sexually mature broodstock of about one year of age should be used. In due time, hormone treatment should be used to synchronise the spawning. Milt from one male should be used to fertilise eggs from two females to produce both full and halfsib groups. A total of 100 halfsib groups and 200 fullsib groups should be produced.

From each spawning at least 1000 eggs should be transferred into a rearing hapa. Newly hatched larvae from each family should be transferred into one hapa, placed in an earthen pond, and reared there until they attain an average body weight of 5 g. The fingerlings will then be individually tagged, following the method developed by the GIFT project. A total of 160 fingerlings should be tagged per fullsib family, amounting to 32 000 tagged fingerlings per generation. Of these, 100 fingerlings per fullsib family should be communally stocked in a pond at RIFF, Sukamandi, 60 fingerlings per fullsib family should be sent for separate testing in; cage culture (15), pond culture (15) and disease challenge test (30) (Fig. 2).

**SELECTION OF BROODSTOCK**

Selection of broodstock for the next generation should be based on breeding values estimated on all available data from Sukamandi and the test stations for cage and pond culture together with the results from challenge test against *A. hydrophila*. Records of fish from the test-stations should be taken prior to those at Sukamandi. Based on records of body weight and frequency of early sexual maturation of the fish at the test-stations and on records from the challenge test, a preliminary ranking of families should be computed.

As fish are recorded at Sukamandi, a first selection of broodstock should be done taking this preliminary ranking of families into account. A sufficient number of top ranked broodstock should be conditioned for mating, while unselected fish may be slaughtered.

Final selection of broodstock to produce the next generation in the breeding program should be based on a selection index including the following traits (Figures 2 and 3):

- Survival from challenge test of full and halfsibs
- Body weight and frequency of early sexual maturation of full and halfsibs at the test stations

=> First family-index (growth rate, early sexual maturation and disease resistance)

- Body weight of all individuals and frequency of early sexual maturation recorded at RIFF, Sukamandi
A final combined family (early sexual maturation and disease resistance) and individual index (growth rate).

Assortative mating should be avoided, i.e. the mating among selected breeders should be random. Further, there should be a restriction on number of selected breeders from each of the best families. The 2nd best breeders should be kept for mass production of seed for dissemination.

The 5 males with highest breeding values should be stocked and used in the next round of testing. The purpose of this repeated mating is to build genetic bridges or ties between each round of testing and to estimate genetic gain. Selection of offspring from these 2nd time breeders should be restricted.

The routines for production of the next generation of 200 fullsib families will then be repeated as described earlier (Figure 1).

**DISSEMINATION OF IMPROVED SEED TO THE FISH FARMERS**

In order to maximise the benefit from the breeding program, the genetic improvement should reach the fish farmers without delay. From RIFF, Sukamandi, there are mainly three routes for dissemination, namely trough AIAT, FADC and directly to private hatcheries. In this process of transferring improved stock, both seed and broodstock may be used.

After the production of fullsib families for the breeding program have been completed, the selected parents should be used for mass production of seed for dissemination. The progeny of the selected parents will be top genetic quality seed, followed by the progeny of the second best selected breeders from the final selection (see above). Furthermore, fish from the best 50% of the fullsib families may be stocked in breeding ponds for mass production of fingerlings that may be disseminated as seed to collaborating operators. The top selected broodstock may also be transferred to other collaborating operators for repeated spawning.

It is very important that the collaborating breeding centres are informed and educated in how to use the new broodstock. The new breed should not be used as the old pure strains since offspring of the selected broodstock are not to be re-entered into breeding. It is also important that the hatcheries understand that it is important to use top quality seed from the breeding system.
ESTIMATION OF GENETIC GAIN

The genetic gain of the breeding program may be estimated by the recurrent use of 5 males with the highest breeding value in each round of testing. At every second testing round, this repeated mating will facilitate evaluation of the genetic gain that is obtained in the previous generation since the males then will be mated to females of different generations.

EXPECTED BENEFIT FROM BREEDING PROGRAMS

When additive genetic variation is present in a trait, there will always be response to selection if efficient selection methods are applied. In the literature there are several estimates of response to selection for increased growth rate in fish, both in large scale breeding experiments and in breeding programs. The following estimates should be mentioned (given as genetic gain in percentage per generation): For coho (Pacific) salmon, 10.1; for rainbow trout, 13; for Atlantic salmon, 10.6-14.2; for channel catfish, 12-20; and for Nile tilapia, 17%. An average figure of these estimates are ca 15% genetic gain per generation for growth rate. This means that it should be possible to double the growth rate in less than 7 generations. This is a larger genetic gain than usually obtained in farm animals, because fish and shellfish have larger genetic variation in growth rate and have higher fecundity; consequently, it is possible to apply a much higher selection intensity.

The benefits of genetic improvement in growth rate are reduction in both fixed costs and production costs, the latter due to lower energy requirement for maintenance for the entire life span, and often a correlated response can be observed as an improved feed conversion rate.

The Norwegian breeding program, which today supply genetically improved eggs of Atlantic salmon and rainbow trout to more than 70% of the farming industry, has a cost/benefit ratio of 1/15. Similar estimates are also found from breeding programs in farm animals. This ratio will depend largely on the total production output that benefit from the genetic improvement. In view of the high production of common carp in Indonesia, an even better ratio may be expected for the proposed breeding program.
Table 1. Scheme for crossing of the four strains of common carp

<table>
<thead>
<tr>
<th>Females from strain No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Majalaya)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2 (Rajadano)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3 (Wildan Cianjur)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>4 (Cangkringan Yogyakarta)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

X: Mating of 8-10 pairs for each reciprocal cross
FIGURE 1. MATING AND REARING DESIGN FOR ONE TESTING ROUND
FIGURE 2. TESTING, RECORDING AND COMPUTING OF BREEDING VALUES
FIGURE 3. SELECTION OF BREEDERS AND DISSEMINATION OF GENETICALLY IMPROVED COMMON CARP TO FARMERS, IN EACH ROUND OF TESTING
Appendix

Some definitions of common expressions from quantitative genetics and selective breeding plans:

**Base population**: The initial random-mating population that form the base for the selection experiment or selection program. It is customary to assume that the inbreeding coefficient is zero in the base population, and this is therefore the reference for estimation of inbreeding in later generations. This requirement may not always be met, but all efforts should be made to establish a base population of unrelated individuals.

**Family selection**: Selection based on information from fullsibs and/or halfsibs to estimate the breeding value (may also include information from other relatives). The selection is among families and not within family since no information is available to distinguish between family members (it can, however, be combined with other information in a multiple-trait index which enables us to select the best overall breeding candidate within family). The method requires that information of relatives are recorded, which means that fullsib groups must be reared separately until the fish have reached a size for which a marking system can be applied.

**Fullsibs**: Offspring from the same sire and dam, i.e. same pair of parents.

**Dam**: Female parent.

**Generation interval**: The average age of the parents at the birth of their selected offspring.

**Halfsibs**: Offspring from one sire (i.e. paternal halfsibs) or one dam (i.e. maternal halfsibs). This means that within a halfsib family some of the fish may also be fullsibs, e.g. when one sire is mated to two dams.

**Heritability** expresses the extent to which phenotypes, i.e. the observed value of that trait, are determined by the genes transmitted from the parents. It is given by the ratio: (additive genetic variance) / (phenotypic variance)

**Hierarchical mating structure**: A mating structure where for instance one male is mated with two females. Simulation studies have shown that this structure gives maximised genetic gain.

**Inbreeding** means the mating together of individuals that are related to each other by ancestry. This is unfavourable for two reasons: 1) it leads to inbreeding depression (low performance) in many traits, especially fitness traits (e.g. survival and fertility), and 2) it leads to decreased genetic variation.

**Index selection**: Selection based on a combination of sources of information to estimate the breeding value, from the individual itself, and from relatives, especially fullsib or halfsib-information. The method requires that genetic relationship among the individuals must be recorded, which means that fullsib groups must be reared separately until the fish have reached a size for which a marking system can be applied.

**Individual selection**: Selection based on the performance of the individual itself only, to be distinguished from Family and Index selection. The method do not require a marking system, but the number of offspring from each family that are allowed to contribute their genes to the next generation must be restricted in order to control inbreeding.

**Sire**: Male parent.