BREEDING PLAN FOR MRIGAL *(CIRRHINUS MRIGALA)* IN VIETNAM: INDIVIDUAL (MASS) SELECTION TO IMPROVE GROWTH RATE

Report No. 2

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INTERNATIONAL NETWORK ON GENETICS
IN AQUACULTURE
July 1996

FOREWORD

The International Network on Genetics in Aquaculture (INGA) was established in 1993 and is being coordinated by the International Center for Living Aquatic Resources Management (ICLARM), with the objective to contribute through collaborative research, to the domestication and sustainable performance of tropical finfish species farmed in developing countries and to strengthen national capabilities for genetic enhancement of farmed fish through exchange of germplasm, methodologies and through training and interactive forums.

Studies undertaken in recent years for improving breeds of salmon in Norway and tilapia in Philippines by ICLARM and collaborating Philippine and Norwegian institutions, have led to increased awareness among researchers the need for undertaking programs for improvement of breeds of species that are of aquaculture importance in their countries. INGA has been assisting the member countries in developing national breeding programs. This report on **Breeding Plan for Mrigal *(Cirrhinus mrigala)* in Vietnam: Individual (Mass) Selection to Improve Growth Rate** is an outcome of such an effort and has been prepared by Drs. Hans Bernhard Bentsen and Trygve Gjedrem of the Institute of Aquaculture Research (AKVAFORSK), Norway and Dr. Tran Mai Thien and Mr. Nguyen Cong Dan of Research Institute for Aquaculture No.1 of Vietnam. Dr. A.E. Eknath of ICLARM has assisted INGA and the authors in planning and development of this breeding program, which is greatfully acknowledged.

We hope that this document will be useful to other researchers and planners in developing breeding programs in their countries.

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INTRODUCTION

Total freshwater aquaculture production in Vietnam in 1995 was about 300 000 tons and from brackish water aquaculture about 115 000 tons. Of the freshwater aquaculture, about 200 000 tons were produced in North Vietnam. Mrigal production is mainly located in North Vietnam. Total production of mrigal was about 45 000 tons, mainly from pond culture in the lowland region. However, mrigal is also a suitable species in the midland and highland area, even in the cold season. The species is a mid prized product (11 000 Dong per kg, as compared to 14 000-16 000 Dong for tilapia and 15 000-20 000 Dong for common carp). Production of mrigal is mainly for local consumption. All carp hatcheries will produce mrigal seed. The broodstock in the hatcheries in North Vietnam all originates from RIA1.

In North Vietnam, spawning season is in April/May until late August. Sexual maturity occurs at 2 years of age. Broodstock will normally be used for 3 breeding seasons, and breeding may be repeated three times in one season. The third breeding in the season is considered to give poorer quality seed than the first and second breeding. Because young and small breeders normally mature and spawn earlier in the season, and early produced fry are better paid, seed producing farmers tend to prefer to use this early maturing and small sized broodstock as much as possible. This may have caused a selection for small size and early maturation.

Spawning is 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. About 75 percent of the females will spawn after injection. Males are injected once at the same time as the second injection of the females with a dose of 1/5-1/6 of the dose given to the females. All males will normally respond to the injection. The females and males are then stocked together in a spawning tank at a ratio of 1:1 according to body weight. Natural spawning occurs after 4-6 hours depending on the water temperature. Fertilised eggs are collected and stored for hatching in incubation tanks with high water flow, and will hatch after 12-15 hours depending on the water temperature (31-27 °C). The hatchlings are kept in the circular incubation tanks for 3-4 days until the yolk sac has been absorbed. At the end of this period, the larvae is start-fed with powder from boiled chicken egg yolk. At a total age of 5-6 days after fertilisation, the larvae is transferred to nursery ponds at a density of 150-200 per square meter, where they are fed for one week with a mixture of cooked rice bran, rice powder, soya been powder, and sometimes with fish meal mixed with water. The mixture is sprayed on the water surface. The same mixture without cooking are then fed for another 3 weeks until they reach fry stage (2-3 cm). The fry may be sold or transferred to rearing ponds at a density of 15-20 per square meter until fingerling size (6-8 cm) at an age of 8 weeks

or large fingerlings (10-12 cm) at an age of 12 weeks, still with feeding and additional natural food by fertilising the pond. Fingerlings are then stocked in grow-out ponds at a density of 1.5-2 fish per square meter in mono culture. Harvest may start in January at an age of 8-9 months and a size of 300-500 grams.

BASE POPULATION

About 1 000 fingerlings of mrigal carp were imported in 1984 from the Thangon State Fish Farm in Laos to Thu Duc Fish Farm of the Research Institute for Aquaculture No. 2, Ho Chi Minh City with the support of the Interim Mekong Committee. After quarantine and acclimatisation, the fish was transferred to the Cai Be Fish Farm of RIA 2 in Tien Giang Province. Another import of 10 pairs of breeders were imported from the same source in 1986. Mrigal is not considered an important species in South Vietnam. In June 1986, 6 males and 6 females of the first import were transported from RIA 2 to RIA 1 at a size of 700-1000 grams, where they were spawned two weeks after transportation. Since then, about 4 to 5 generations have been produced at RIA 1. Broodstock has been randomly chosen. Observations indicate that since the introduction, growth rate has declined year by year, and the fish has become mature at lower age and lower body weight. Deformities has been observed in about 5-7 percent, but has not increased.

Before the start of a breeding program, the base population should be upgraded to broaden the genetic base and eliminate inbreeding. The possibility of acquiring wild mrigal directly from India should be investigated. RIA 1 has institutional contacts with Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, and Central Institute of Fisheries Education (CIFE), Bombay. If possible, a request for direct transfer of fry from three different river systems should be forwarded. A number of 2 000 fry per river strain will be sufficient. The imported strains should be grown separately at RIA 1 until maturation.

BREEDING GOAL AND SELECTION METHOD

The two most important features of an aquaculture stock of mrigal carp are a high growth rate and a high proportion of females. Males grow to 60-70 percent of the female body weight. An additive genetic improvement program will have to focus on growth rate in both sexes. Sex ratio will have to be manipulated by other techniques. The additive genetic variation of growth rate in mrigal has not

been studied. If the variation is similar to that of rohu and common carp, growth rate of mrigal may easily be improved by individual (mass) selection.

The breeding program should then be designed to avoid loss of genetic variation and avoid rapid accumulation of inbreeding. This may be done by securing a large effective population size. In the proposed breeding plan, a large effective population size is achieved by using a large number of breeders in each generation (2x75=150 pairs), and a restricted number of progeny per pair (on average 35-40 surviving progeny per pair at the end of the grow-out test). Furthermore, the variation between pairs in the number of progeny is kept as low as possible by separate incubation of the progeny groups through the early life phase, when the mortality is high. A fixed number of progeny per pair may then be counted and communally stocked for nursing, rearing and grow-out testing. The design is expected to result in a rate of inbreeding of less than 1% per generation.

Furthermore, maintaining two separate batches of breeding candidates originating from different sets of parents throughout every generation, as proposed in the present breeding plan, will permit a mating design that will exclude mating between sibs. This will prevent that mating between breeders from the breeding program could occasionally result in high inbreeding coefficients in some groups of progeny.

START OF THE PROGRAM

The relative performance of the RIA 1 population and the introduced strains in Vietnamese fish farms will not be known at the start of the program. However, in some strain comparison tests with other tropical species, little evidence has been found of strong genotype by environment interaction. Rather than spending a lot of time and efforts (e.g. on developing tagging or branding methods) to carry out a strain comparison test, it is recommended to start forming a synthetic (mixed) base population for selection from the early beginning of the program.

If the introduced material arrives at RIA 1 before the end of 1996, mating may start in April/May 1998. All matings should then be strain crosses, using all possible combinations in a 4 by 4 complete crossing design without pure-breds (Table 1). Single pairs will be mated by stripping of eggs and milt and artificial fertilisation in trays or by natural mating in hapas.

For each of the 12 reciprocal crosses, a first round of 6-7 pairs will be mated separately, producing about 75 full sib families. This means that at least 25 females and 20 males from each strain should be injected. Each male should be mated to one female only, and each female to one male only. Matings will take place within 2 days. About 1 000 fertilised eggs from each pair should be transferred to a separate incubation jar (Figure 1). The fertilised eggs will be hatched and the larvae will be kept in the jars until absorption of the yolk sac. After the absorption of the yolk sac, 100 larvae from each incubation jar should be communally stocked in a nursery pond. This will be the first batch of progeny. The procedure will then be repeated by mating another set of 75 pairs the following week. A second nursery pond will be used for the progeny from this set of breeding pairs. This will be the second batch of progeny. The two batches should be kept separately until the fish are sexually mature (Figure 1).

At stocking in nursery ponds, each batch will consist of 75 full sib families of 100 larvae, making up a total of 7500 larvae. At a stocking density of 100 larvae per square meter, this will require two nursery ponds (or hapas) of 75 square meters each. Assuming a larvae survival of 60 percent until transfer to rearing ponds, a total of 4500 fry will have to be stocked in each of the two rearing ponds. At a stocking density of 10 fry per square meter, this will require two rearing ponds of 450 square meters each. Assuming a survival rate of 80 percent until stocking in grow-out ponds (i.e. a total number of 3600 fingerlings in each of the two grow-out ponds) and a stocking density of 2.0-2.5 fingerlings per square meter, two grow-out ponds of 1300-1400 square meters each will be required for production of broodstock for selection.

SELECTION OF BROODSTOCK

In commercial mrigal farming, production target is to harvest the fish in January, at an age of 8 months or a size of 300-500 grams. Consequently, the body weight of all fish in the two grow-out ponds should ideally be recorded some time in January. However, since it is difficult to determine the sex of the fish at this age, and since sexual dimorphism in body size has already started to occur, strong selection at this stage may result in a low number of males among the selected broodstock. Assuming a survival rate of 80 percent from stocking of fingerlings in the grow-out ponds until 8 months of age, each of the two grow-out ponds will contain about 3300 fish. By applying a pre-selection of 50 percent (discarding the 50 percent of the fish with the lowest body weight), the stocking density in the grow-out ponds will be reduced to about 1 per square meter. At the same time as the pre-selection, 40 fish with an average body weight (average of all fish before pre-selection) should be fin-clipped and restocked with the pre-selected broodstock in each pond (Figure 2).

Final selection should then be carried out in March/April at 22 months of age. Body weight and sex should be recorded for all fish, and the 80 largest males and the 110 largest females from each of the two grow-out ponds should be stocked separately by pond and sex in hapas (4 hapas) until mating. All the selected fish should be sexually mature and ready to spawn. The final selection intensity is then expected to be about 6-8 % (Figure 2). With an assumed heritability for body weight of 0.3 and a coefficient of variation of 30 percent, as shown in many other fish species, the expected mean genetic gain in the progeny should then amount to about 16-18 % compared to the mean of the parent generation (16-18 % genetic gain per generation).

At the same time as the final selection, all fin-clipped males from the first pond (batch 1) that are sexually mature and ready to spawn should be kept and stocked together with the selected males from the same pond until mating. All fin-clipped females from the other pond (batch 2) that are sexually mature and ready to spawn should be kept and stocked together with the selected females from this pond until mating. These breeders will be used to produce a control group (see below).

PRODUCTION OF THE NEXT GENERATION

The production of the next generation should be carried out by single pair mating of 75 selected females from the first grow-out pond with 75 selected males from the second grow-out pond to produce a new batch 1 (Figure 3). This means that 110 females and 80 males should be injected to induce spawning. Again, each full sib group will be hatched in a separate incubation jar and 1000 larvae will be kept in the jar until the yolk sac is absorbed. From each jar, 100 larvae will be counted and pooled together in one nursery pond. In the following week, the routine will be repeated, this time with 75 selected males from the first grow-out pond and 75 selected females from the second grow-out pond, to produce a new batch 2 (Figure 3). The two batches should again be kept separate all the way until sexual maturity, selection and mating (Figure 1).

DISSEMINATION OF IMPROVED SEED TO THE FISH FARMERS

The surplus eggs from the all together 150 selected females may be incubated in ordinary, large scale circular tank incubators for hatching. The larvae may then be sold to fish farmers through collaborating hatcheries. Assuming that a total of 150 females with an average body weight of 1.2 kg will spawn, that 250 000 eggs will be spawned per kg body weight per spawning, that each female

will spawn two times during the spawning season and that the survival from fertilisation to post larvae will be 60 percent, this should amount to 54 mill post larvae available for the industry. This will all be progeny of the best selected breeders, and may be sold directly to grow-out farmers (Figure 3). The best non-selected broodstock at the final selection may be sold to collaborating hatchery operators. To avoid inbreeding in the hatcheries, males from one pond should be supplied together with females from the other pond to each individual hatchery operator.

CONTROL TO ESTIMATE REALISED GENETIC GAIN

Establishing a procedure for control of genetic gain is not required to obtain response to selection in a breeding program. However, a lot of assumptions have been made about unknown parameters in the present program. Including a routine for genetic control will make it possible to check if these assumptions are valid, or if the program needs adjustments for other reasons.

The progeny of the average breeders (see above) may serve as a control to estimate genetic gain from each generation of selection. At the same time as the new batch 1 is produced (see above), about 15 pairs of fin-clipped breeders (males from pond 1 and females from pond 2) should be mated, and 1 000 fertilised eggs from each pair should be hatched in separate jars. After absorption of the yolk sac, 40 larvae from each jar containing progeny of the first batch of selected breeders and 200 larvae from each jar containing progeny of a pair of average breeders should be counted from the jars as shown in Figure 4. The 3 000 progeny of the selected breeders should then be pooled and randomly divided in 3 equally sized groups of 1 000 larvae for stocking in 3 nursery hapas (replicates) as shown in Figure 4. The same procedure should be repeated with the progeny of the average breeders. The 6 nursery hapas should be placed together in the same pond and given the same treatment.

At fry size, about 330 fry from each nursery hapa should be transferred to separate rearing hapas and reared until fingerling size. The fry should be reared in the hapas until they have grown to a size when they may be fin clipped or branded to separate the two progeny groups from each other (e. g. by clipping the pectorial fin on one side in the progeny of selected breeders and the other side in progeny of average breeders). About 100 fin clipped fingerlings from each hapa (i. e. 300 progeny of selected breeders and 300 progeny of average breeders) should then be communally stocked in a grow-out pond until market size. The fish should be stocked at a low density and under proper feeding and management conditions to ensure good growth performance. At 8 months of age, the comparison test may be terminated by harvesting all fish and recording of individual body weights and fin clip marking to compare the two groups (Figure 4). This should provide an estimate of the realised response to selection in this generation. The procedure may be repeated in each generation.

TABLE 1. MATING DESIGN FOR PRODUCTION OF THE BASE POPULATION.

Females from location No.	Males from location No.			
	1	2	3	4
1	-	X	X	X
2	X	-	X	X
3	X	X	-	X
4	X	X	X	-

X: Mating of 6-7 pairs for each reciprocal cross

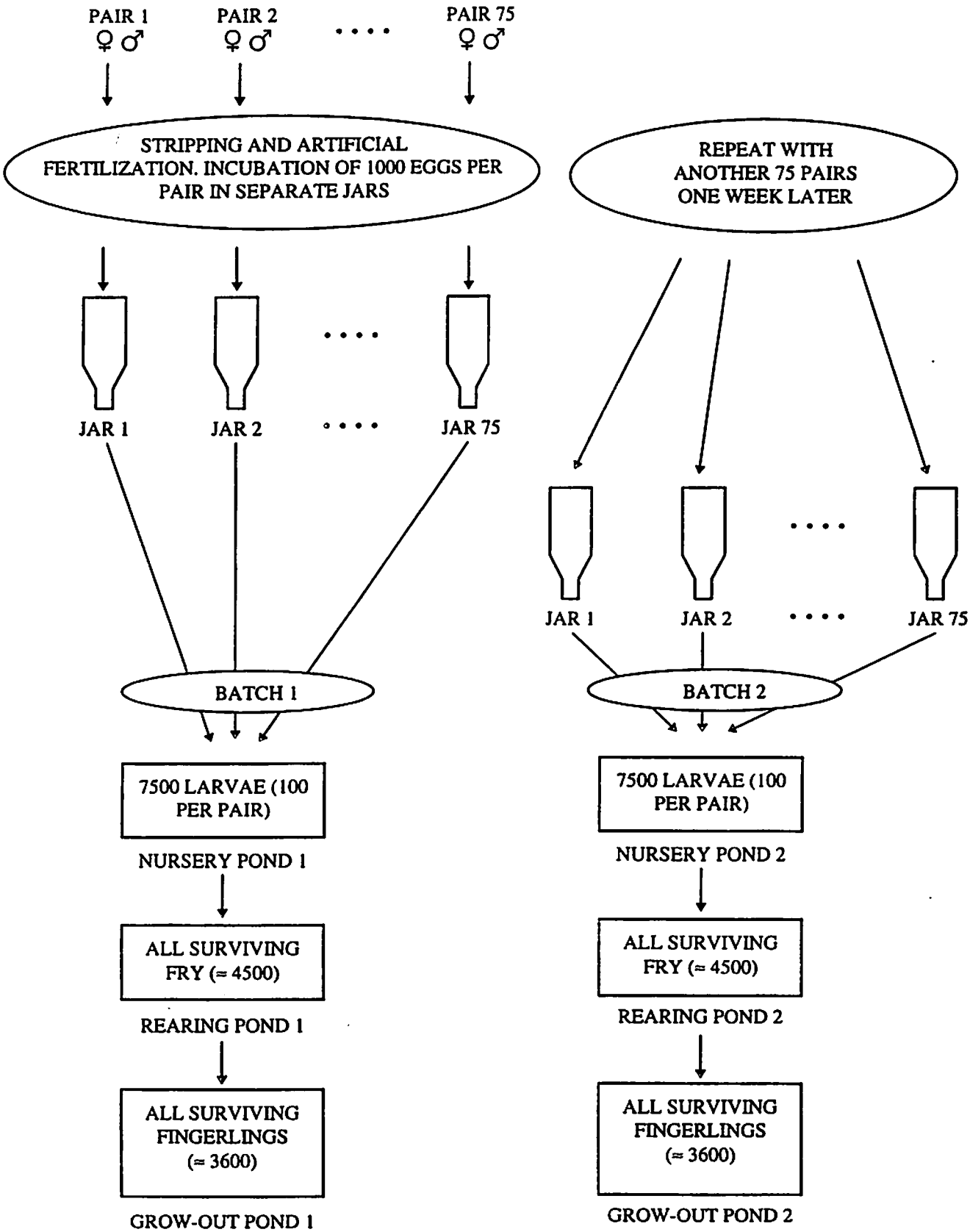

FIGURE 1. MATING AND REPRODUCTION OF THE BROODSTOCK AND REARING AND TESTING OF THE PROGENY

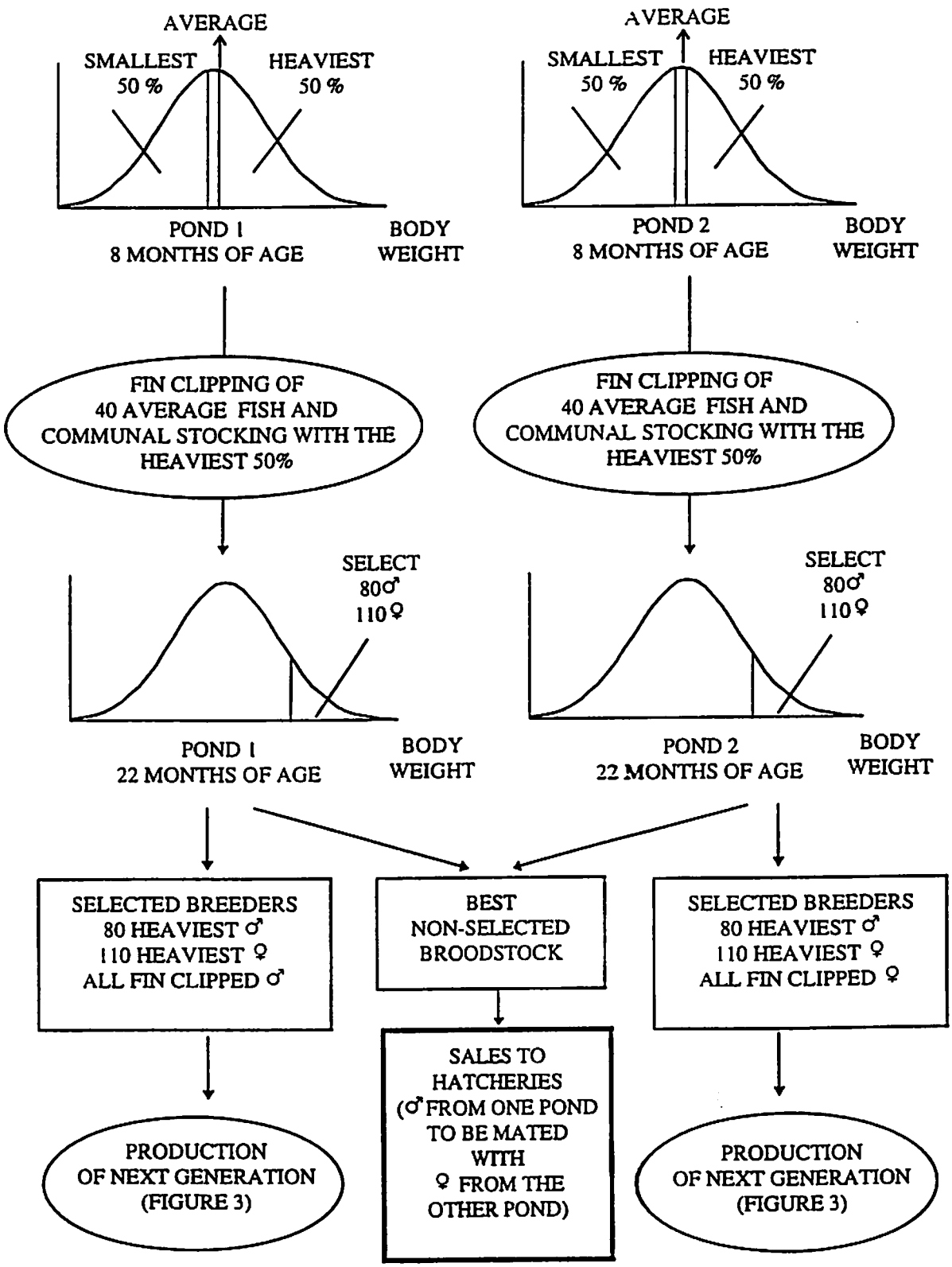

FIGURE 2. SELECTION OF BROODSTOCK FOR PRODUCTION OF THE NEXT GENERATION

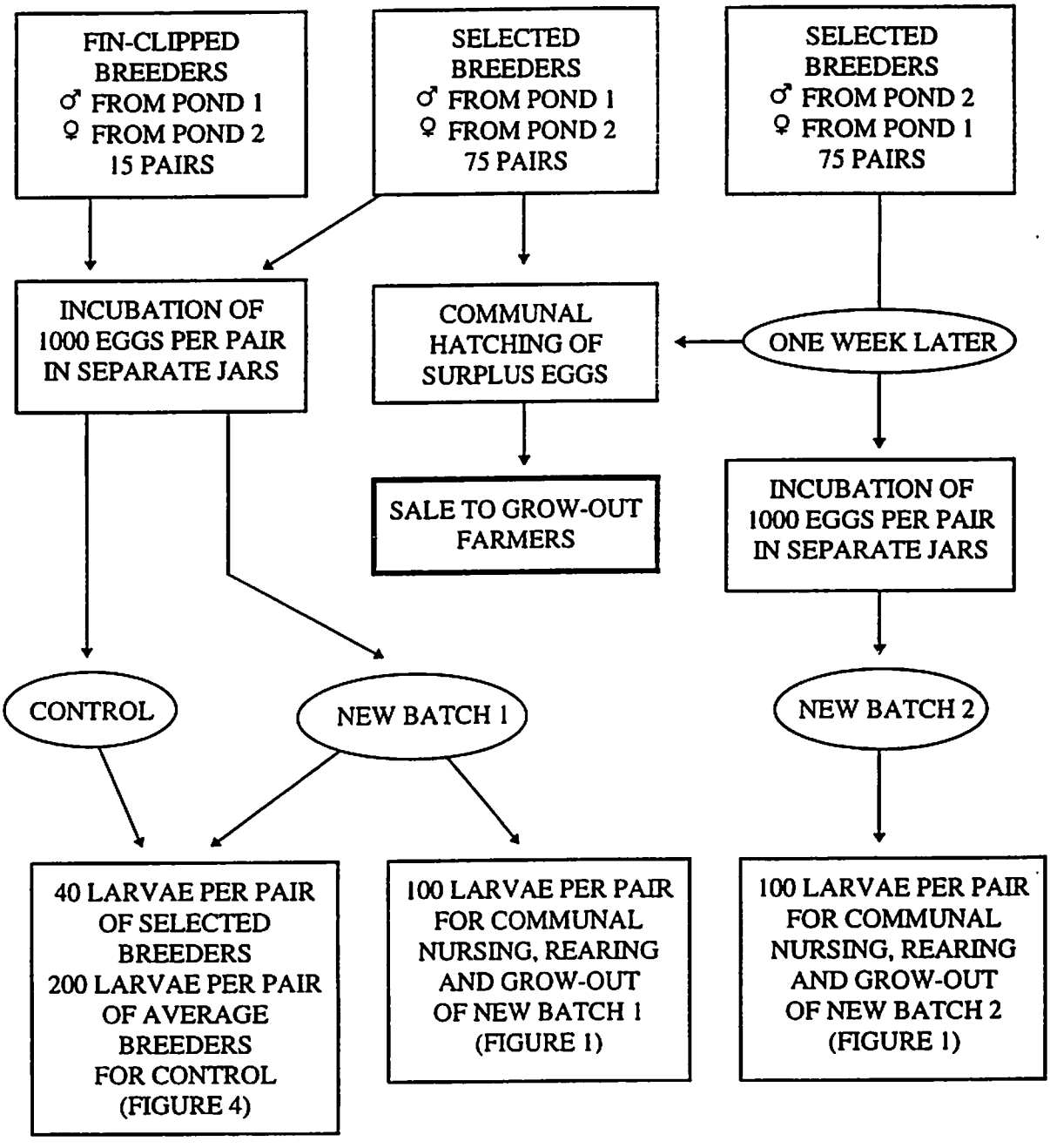

FIGURE 3. PRODUCTION OF THE NEXT GENERATION

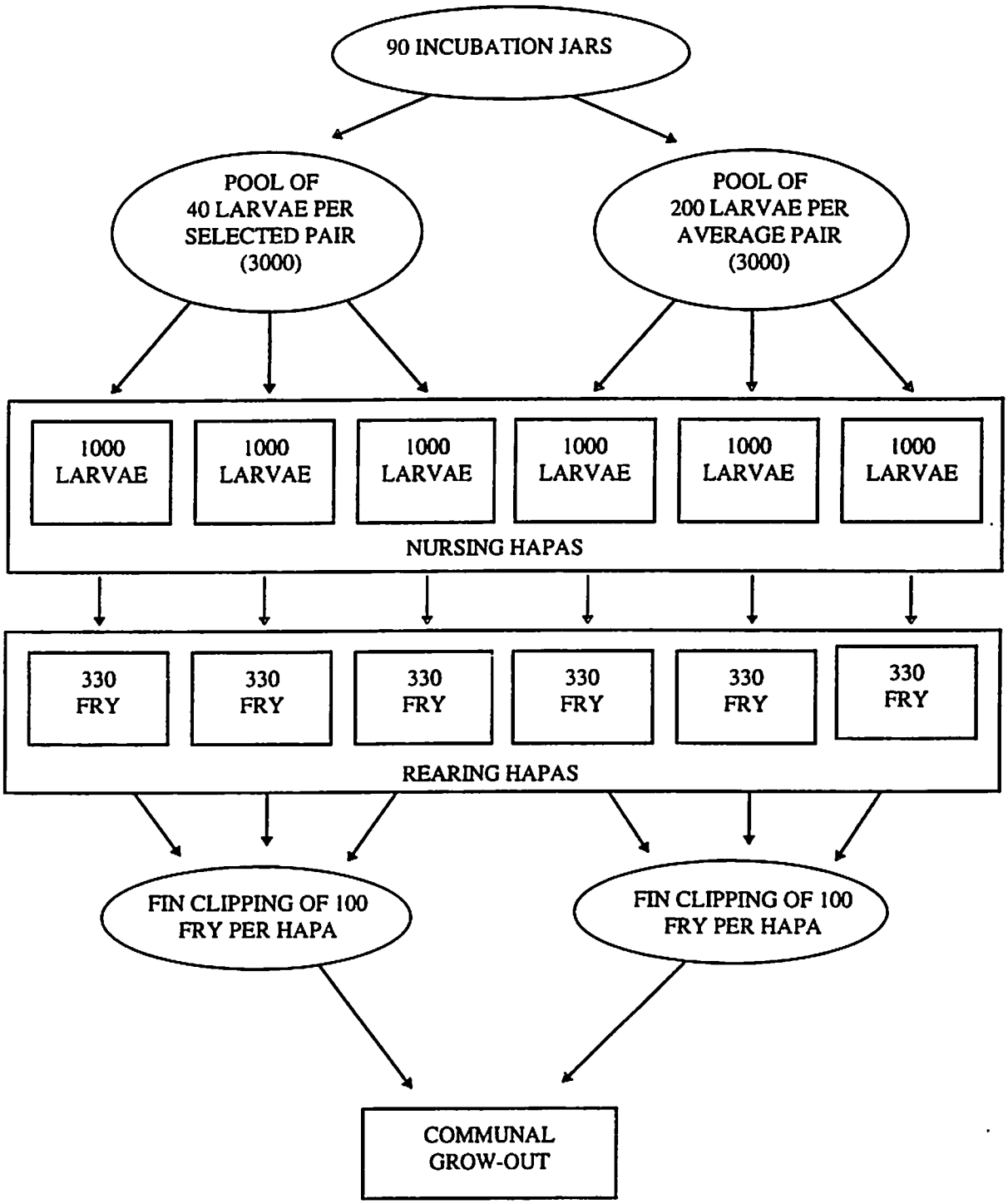

FIGURE 4. CONTROL COMPARISON OF PROGENY OF SELECTED BREEDERS WITH PROGENY OF AVERAGE BREEDERS