BREEDING PLAN FOR SILVER BARB *(PUNTIUS GONIONOTUS)* IN VIETNAM: INDIVIDUAL (MASS) SELECTION TO IMPROVE GROWTH RATE

Report No. 3

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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 Silver Barb *(Puntius gonionotus)* 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 Mr. Nguyen Van Hao of Research Institute for Aquaculture No.2 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

Silver barb (*Puntius gonionotus*) is widely distributed in Southeast Asia. In addition to Vietnam, the species is found in Thailand, Cambodia, Lao, Malaysia, Indonesia, Bangladesh etc. In Vietnam, the species is abundantly represented in the Mekong Delta and has a wide distribution in Southern Vietnam (Dong Nai, Tien Giang, Vinh Long, Can Tho, Soc Trang, An Giang etc.)

Silver barb is one of the 40 economically important species in the Mekong Delta. The species has several characteristics that makes it suitable for culture. It grows well on low protein diets, whether feeding on certain aquatic plants or given supplementary feeding. If properly acclimatised, it can tolerate a wide range of environmental conditions: Water temperatures from 15 to 41.5 °C (suitable temperature from 25 to 30 °C), salinity levels from 0 to 7 ppm, pH levels from 5.5 to 9 (suitable pH from 7 to 8), and dissolved oxygen levels down to 0.656 mg/l (suitable oxygen levels above 2 mg/l). The species also seems to be resistant against diseases and tolerates handling and captivity. In pond culture, silver barb grows well during the first year and may reach an individual body weight of 150-200 grams in 12 months. In paddy fields with high water level during the rainy season, the body weight may reach 500 grams after 6-8 months. Some experiments suggest that the growth rate is lower during the second year.

In the Mekong Delta, the spawning season of silver barb is from March until September. Sexual maturity occurs at about 12 months of age in both males and females. Broodstock will normally be used for 2 to 3 breeding seasons, and spawning may be repeated 4 to 5 times in one season. The spawning interval may vary from 30 to 45 days.

Spawning is induced by environmental manipulation (high speed of running water) or by injecting both sexes with pituitary gland extract or manufactured hormones (HCG, LR-A from China). Females are injected twice and males are injected once at the same time as the second injection of the females. Males and females are then stocked together in a Chinese design spawning tank at a ratio of 1:1. Natural spawning occurs after 6-8 hours depending on the water temperature. All males and about 75-80 percent of the females will normally spawn after injection. The females will produce about 200 000 to 300 000 eggs per kg of body weight. The eggs will hatch after 17-18 hours at a water temperature of 27-29 °C. The hatchlings will be kept in the circular incubation tank for 3-4 days until the yolk sac has been absorbed.

Further rearing and grow-out will follow the techniques usually applied to farmed freshwater fish in Southern Vietnam.

Silver barb is a popular species among fish farmers in the Mekong Delta. The species is widely appreciated by the consumers because of it's long history from inland fisheries. In recent time, silver barb has become the main species in rice-fish culture in the Mekong Delta because of the favourable effects on the total income of the farmers. The impacts of a genetically improved farm stock of silver barb may consequently be substantial. The first priority breeding goal should be increased growth rate. In the long term, other traits like disease resistance, meat quality etc. should also be considered.

BASE POPULATION

At present, silver barb originating from various locations in the Mekong Delta is reproduced in captivity for aquaculture purposes. Broodstock will be collected from two such populations at Cai Be fish farm (Tien Giang province) and Can Tho fish farm (Can Tho province). However, to secure the genetic variability of the base population, and to eliminate possible inbreeding, wild silver barb broodstock will also be collected from 3 different locations in the Mekong River: Hau River in the Chau Doc district, Tien River in the Cao Lanh district, and plain of reed in the Hong Ngu district. Broodstock will also be collected from the Tri An reservoir in the Dong Nai River. The collected broodstock will be kept at the Cai Be fish farm of RIA 2 for mating. All together 100 males and 100 females from the 6 different locations (16-17 males and 16-17 females from each location) will be cross mated as shown in Table 1 to form a heterogeneous, outbred base population for the breeding program.

BREEDING GOAL AND SELECTION METHOD

The most important breeding goal for an aquaculture stock of silver barb is to improve the growth rate. Since spawning of the breeders in the breeding nucleus may be synchronised (induced spawning), all the test fish in the following generation will be of the same age. Growth rate may then be recorded for each individual test fish as body weight on a fixed recording day. No tagging will be necessary to correct for age differences. The additive genetic variation of growth rate in silver barb has not been studied. If the variation is similar to that of several other fish species, growth rate may easily be improved by individual (mass) selection, i.e. without tagging. The breeding program should then be designed to avoid loss of genetic variation and to 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 (100 pairs), and a restricted number of progeny per pair (on average 20-30 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 nursing and rearing of the progeny groups through the early life phases, when the mortality is high. A fixed number of progeny per pair may then be counted and communally stocked for grow-out testing. The design is expected to result in a rate of inbreeding of less than 1% per generation.

Future broadening of the breeding goal to include less heritable traits or traits that may not be recorded in the breeding candidates will require development of tagging or branding techniques for pedigree recording. This may be done as a continuation of the proposed breeding program, since loss of genetic variation and accumulation of inbreeding will be kept under control.

START OF THE PROGRAM

The relative performance of the 6 collected strains in various farm environments will not be known at the start of the program. However, several strain comparison tests with other fish species have given little evidence of strong genotype by environment interactions. Rather than spending a lot of time and efforts (e.g. on developing tagging or branding methods) to carry out a strain comparison test in a range of farm environments, it is recommended to start forming a synthetic (mixed) base population for selection from the early beginning of the program.

If the collected strains are available at RIA 2 (Cai Be fish farm) before the end of 1996, mating may start in April/May 1997. All matings should be strain crosses, using all possible strain combinations in a 6 by 6 complete crossing design without pure-breds (Table 1). About 100 single pairs will be mated by stripping of eggs and milt and artificial fertilisation in trays. About 1 000 fertilised eggs from each pair will be transferred to separate incubation jars, where they will be hatched and kept until absorption of the yolk sac (Figure 1).

For each of the 30 reciprocal crosses, 3-4 pairs will be mated separately, producing about 100 full sib families (Table 1). This means that at least 23 females and 17 males from each location must be injected to induce spawning. Each male should be mated to one female only, and each female to one male only. All matings will take place within one day. The hatclings will be kept in the jars until the yolk sac is absorbed. The number of larvae per sib group may then be standardised.

Even if the number of larvae from each sib group is standardised after yolk sac absorption, the high mortality of larvae from yolk sac absorption until fry size (about 60 % in an ordinary nursery pond or hapa) may cause the number of fry per sib group to be quite variable at the end of the nursing period, and some sib groups may be entirely lost. Consequently, methods of separate nursing of larvae from each incubation jar until fry size should be investigated. A possible method may be to transfer the larvae from each jar to a separate, small nursery hapa (Figure 1). All 100 nursery hapas should then be installed in the same pond. The number of larvae to be transferred from each jar to the separate nursery hapas will depend on the mortality in the hapas until fry size. If the mortality is similar to that under communal nursing, 150 larvae should be counted from each jar and stocked in each nursery hapa. At fry size, 50 fry should then be counted from each hapa and communally stocked in a rearing pond. At fingerling size, all surviving fingerlings should be communally stocked in a grow-out pond until sexual maturation at 11-12 months of age (Figure 1). If nursing of the sib groups in separate hapas is found to be impossible, 125 larvae may be counted from each of the 100 jars (from each sib group), and communally stocked in a nursery pond or hapa until the fry may be transferred to a communal rearing pond and later on to a grow-out pond (Figure 1). At a stocking density of 100 larvae per square meter, this will require a nursery pond (or hapa) of 125 square meters.

The number of fry to be communally stocked for rearing in a rearing pond will be about 5 000, both if the sib groups are nursed separately (50 fry from each of 100 nursery hapas) or communally (125 larvae from each of 100 jars communally stocked in one nursery pond, 60 % mortality before transfer to the rearing pond). At a stocking density of 10 fry per square meter, this will require a rearing pond of 500 square meters. Assuming a survival rate of 60 % until stocking of the fingerlings in a grow-out pond, the total number of fingerlings will be about 3 000. At a stocking density of 2 fingerlings per square meter, one grow-out pond of 1 500 square meters will be required for testing and production of broodstock for selection. It is essential to secure the best possible environment (density, feeding, water quality etc.) during grow-out, to make sure that the test fish will express their growth potential and reach sexual maturity at about 11 months of age.

SELECTION OF BROODSTOCK

Assuming a survival rate of 80 % in the grow-out pond from stocking of the fingerlings until the fish has reached an age of 11-12 months, 2 400 breeders will be available for selection (no pre-selection). Body weight, sex, and sexual maturity should then be recorded for all fish. Among the sexually mature breeders that are ready to spawn, the 110 largest males and the 130 largest females should be

selected and injected to induce spawning (Figure 2). This will result in an expected selection intensity of about 8-10 %. With an assumed heriabilty for body weight of about 0.3 and an assumed coefficient of variation of 30 percent, as shown in many fish species, the expected genetic gain in the progeny should amount to about 15-17 % compared to the mean of the parent generation (15-17 % genetic gain per generation). Preferably, selection and mating should be carried out at 11-12 months of age to maintain a generation interval of about one year. This will make it possible to carry out the mating of the selected breeders at about the same time during the spawning season every year. If the generation interval has to be extended to more than a year, mating will have to take place later in the spawning season for every generation that passes, and eventually it will have to be postponed with 6-7 months until the next spawning season occurs.

At the same time as the largest breeders are selected, 20 sexually mature males with average male body weight and 25 sexually mature females with average female body weight should be selected and injected to induce spawning. These breeders will be used to produce a control group.

PRODUCTION OF THE NEXT GENERATION

The production of the next generation should be carried out by single pair mating of 100 of the largest selected males and 100 of the largest selected females and 15 average males with 15 average females (Figure 2). Again, separate incubation and hatching of 1 000 eggs counted from each pair into incubation jars and nursing of 150 larvae counted from each incubation jar into separate nursery hapas should be carried out following the design in Figure 1. At fry size, 50 progeny from each of the 100 selected pairs should be communally stocked in a rearing pond until fingerling size and then transferred to a grow-out pond for testing.

If separate nursing of the progeny from each jar until fry size is not possible, 125 larvae should be counted from each jar for each pair of selected breeders and communally stocked in one nursery pond or hapa, and all surviving fry after the nursing period should be transferred to a rearing pond and later to the grow-out pond for testing as shown in Figure 1.

For comparison with a genetic control, 30 additional larvae should be counted from each incubation jar containing progeny of selected breeders and 200 larvae should be counted from each jar containing progeny of average breeders. The further procedure for control of genetic gain is described below and in Figure 3.

DISSEMINATION OF IMPROVED SEED TO THE FISH FARMERS

The surplus eggs from the 100 selected females may be incubated in ordinary, large scale circular tank incubators for hatching. The larvae may then be sold directly from the breeding station to fish farmers (Figure 2). The selected males and females may also be used in repeated spawnings for commercial mass production of postlarvae throughout the spawning season. Assuming that a total of 100 females with an average body weight of 0.3 kg will spawn, that 200 000 eggs will be spawned per kg of body weight per spawning, that each female will spawn 3 times during the spawning season, and that the survival from fertilisation to postlarvae will be 60 %, this should result in a production of about 10 million postlarvae that will be available for the grow-out farmers. This will all be progeny of the best selected breeders. If the demand from the farmers exceeds this supply, the best non-selected broodstock may be reproduced commercially at the breeding station or sold to collaborating hatcheries for production of postlarvae (Figure 2). Progeny of the best selected breeders may also be reared and used as broodstock by collaborating hatcheries.

CONTROL TO ESTIMATE 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. After the progeny of single pair matings of selected and average broodstock has been hatched in separate jars, 30 larvae from each jar containing progeny 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 3. 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 3. The same procedure should be repeated with the progeny of the average breeders. The 6 nursery hapas should then 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. The difference in mean body weight between the two groups will then represent the response to the previous round of selection. 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	5	6
1	-	X	X	X	X	X
2	X	-	X	X	X	X
3	X	X	-	X	X	X
4	X	X	X	-	X	X
5	X	X	X	X	-	X
6	X	X	X	X	X	-

X: Mating of 3-4 pairs for each reciprocal cross

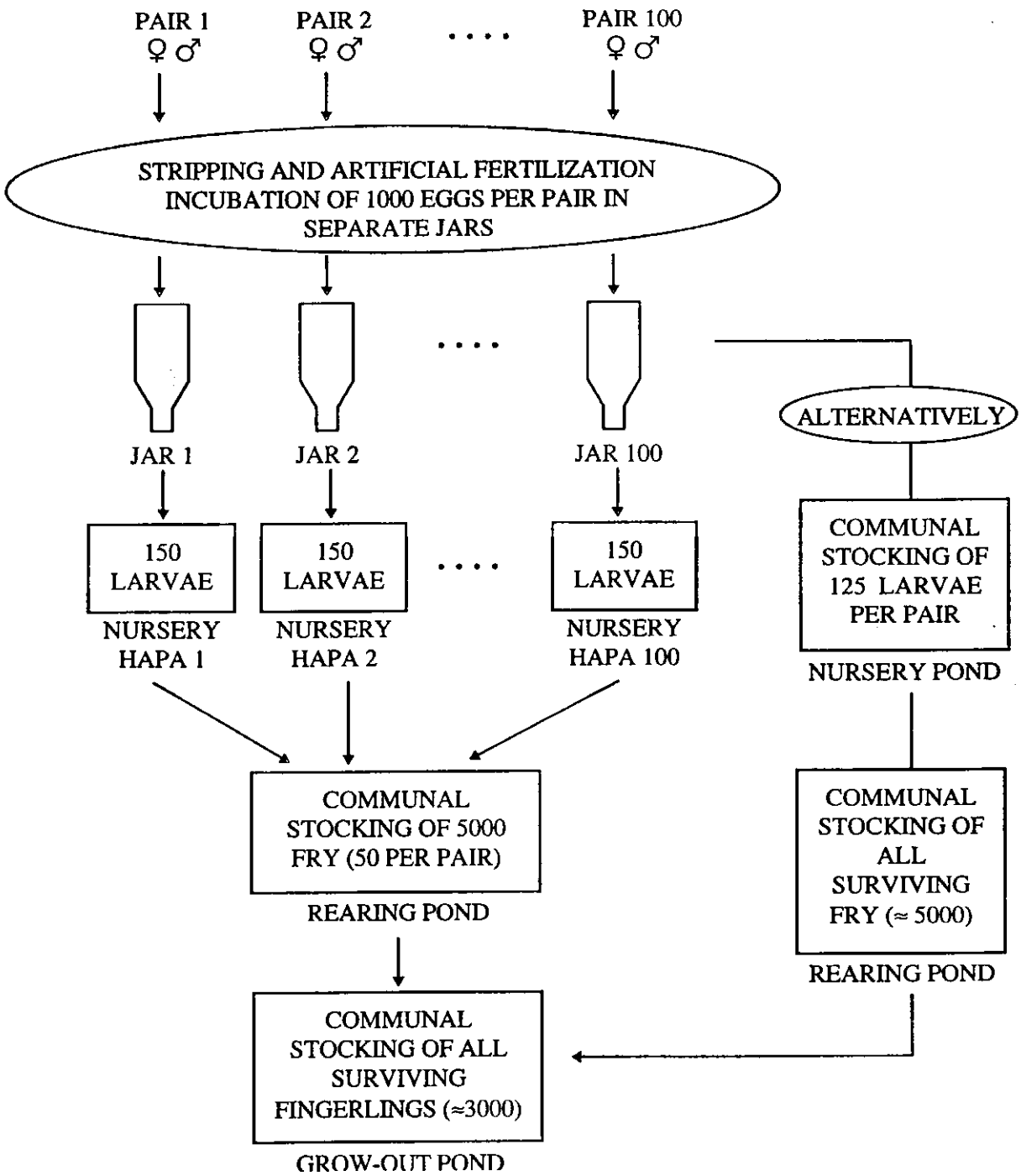

FIGURE 1. MATING AND REPRODUCTION OF THE PARENT BROODSTOCK AND REARING AND TESTING OF THE PROGENY

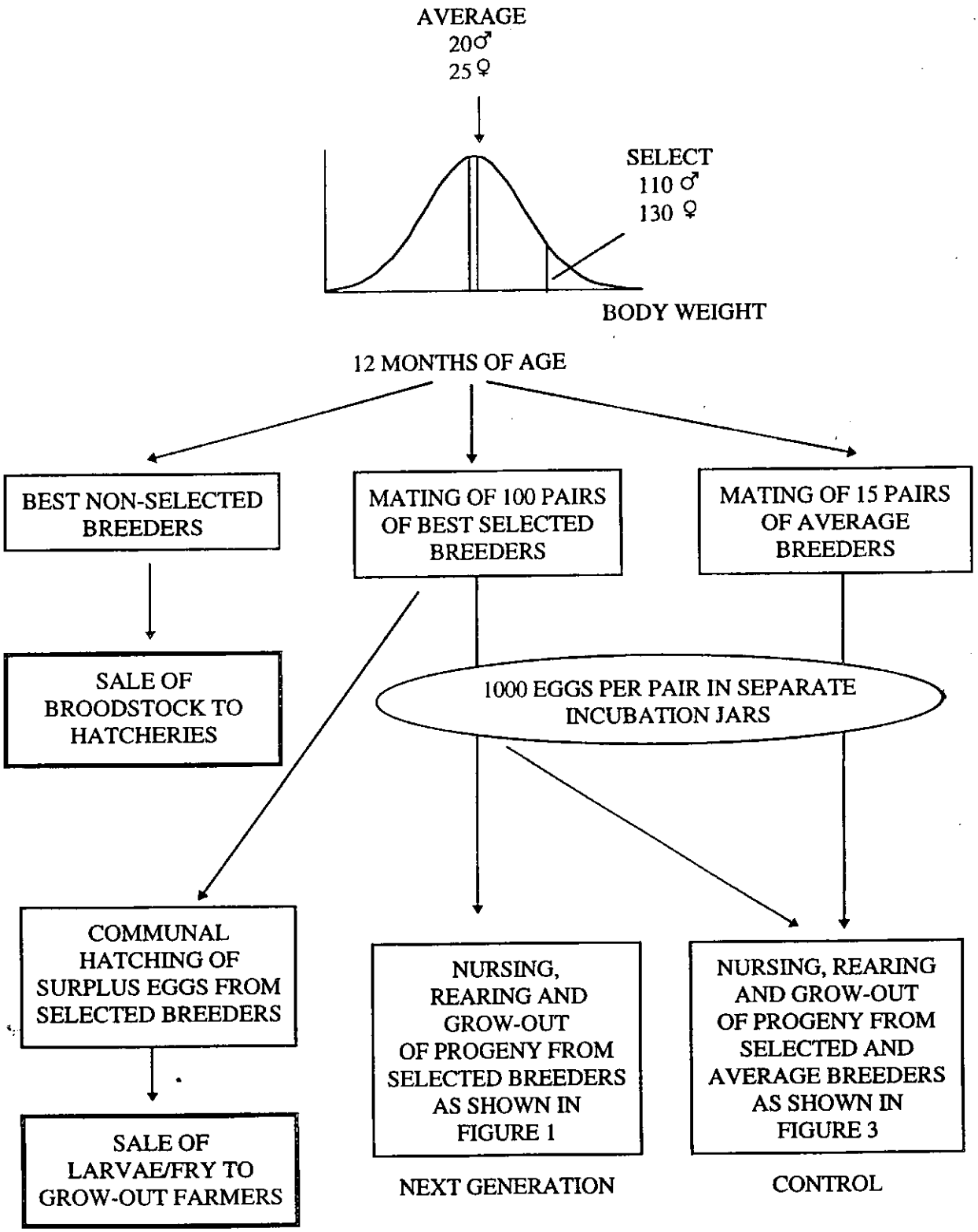

FIGURE 2. SELECTION OF BROODSTOCK FOR PRODUCTION OF THE NEXT GENERATION. DISSEMINATION OF IMPROVED BROODSTOCK TO FARMERS

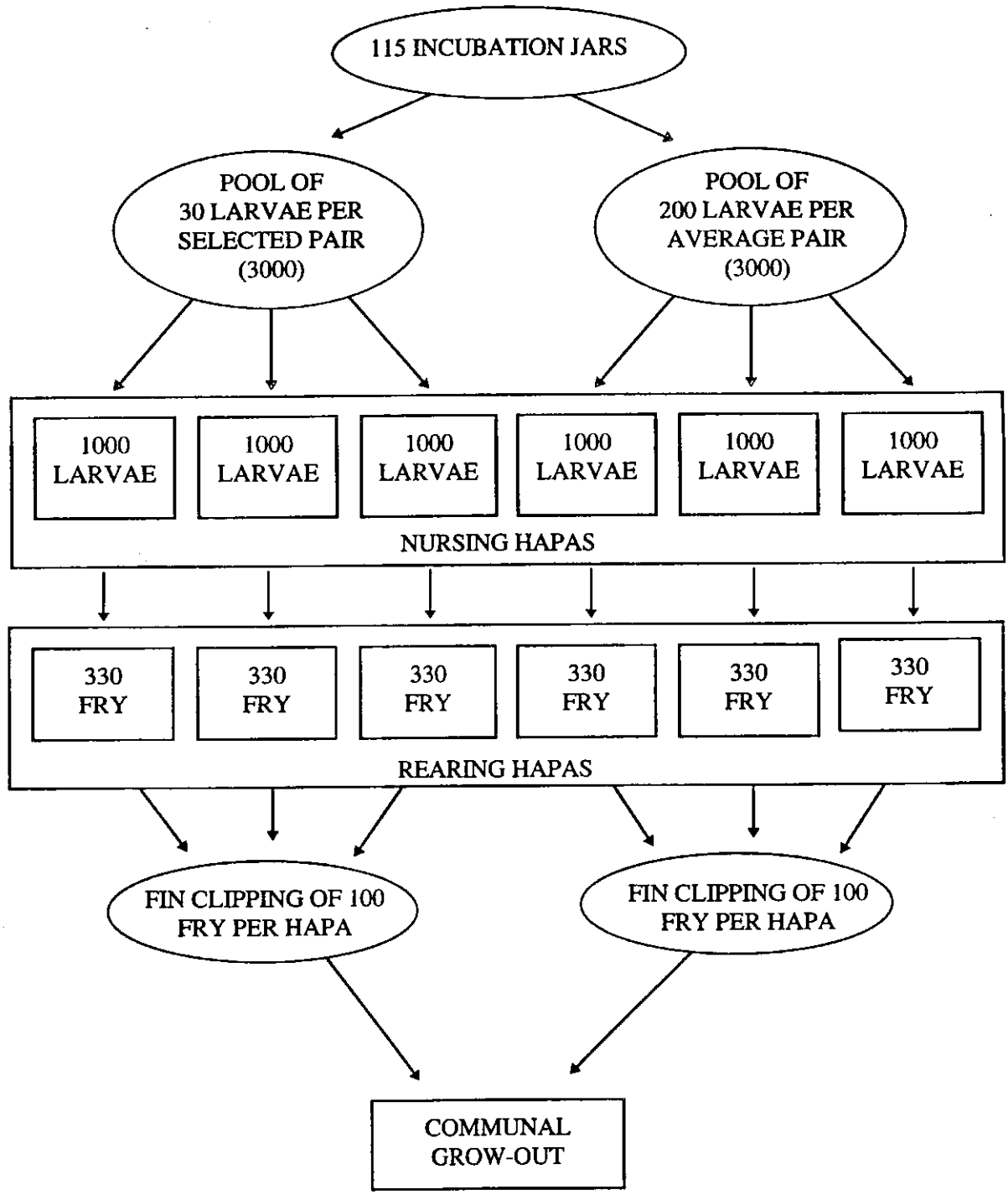

FIGURE 3. CONTROL COMPARISON OF PROGENY OF SELECTED BREEDERS WITH PROGENY OF AVERAGE BREEDERS