GIFT TECHNOLOGY MANUAL

An aid to Tilapia selective breeding

formerly known as "ICLARM - The World Fish Center"

Our Commitment:
to contribute to food security and poverty eradication in developing countries.

A Way to Achieve This:
through research, partnership, capacity building and policy support, we promote sustainable development and use of living aquatic resources based on environmentally sound management.

We believe this work will be most successful when undertaken in partnership with governments and nongovernment institutions and with the participation of the users of the research results.

GIFT Technology Manual: An aid to Tilapia selective breeding

2004

Published by WorldFish Center
PO Box 500 GPO
10670 Penang, Malaysia

WorldFish Center. 2004. GIFT Technology Manual: An aid to Tilapia selective breeding. WorldFish Center, Penang, Malaysia, 56 p.

Perpustakaan Negara Malaysia Cataloguing-in-Publication Data

GIFT technology manual: an aid to Tilapia selective breeding
1. Tilapia—Breeding. 2. Freshwater fishes—Breeding.
3. Fish culture.
639.31

Dr Jorn Thodesen prepared a first draft of the manual. It was extensively edited, some sections were re-written and a few new ones introduced by Dr Raul W. Ponzoni. Ms Shirley Tan Saadiah prepared the data collection forms that appear in the Appendix.

Personnel of GIFT Foundation International kindly provided the photographs appearing in Figures 1 to 5, whereas Dr Jorn Thodesen took those appearing in Figures 6 to 24.

Editor: N. Puttaraksar
Layout and cover design: Garrick Tan
Cover photos: WorldFish photo collection

ISBN 983-2346-24-X

WorldFish Center Contribution No. 1715

Printed by Sun Printers, Penang, Malaysia.

CGIAR
FUTURE HARVEST

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.

Foreword

This manual is intended as an aid in the transfer of the selective breeding technology developed by the WorldFish Center and its partners. It was based upon experience and material accumulated during the 'Genetic Improvement of Farmed Tilapia' (GIFT) project (1988 to 1997). In its development particular attention was paid to the description of key husbandry operations in a sequential manner, using illustrations and photographs. There will be, however, instances in which some adjustment of the GIFT technology to local conditions is required.

The manual constitutes an integral part of the UNDP/Technical Cooperation among Developing Countries (TCDC) financed project called 'Transfer of selective breeding (GIFT) Technology for aquaculture improvement from the Philippines to Sub-Saharan Africa and Egypt'.

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1. Introduction

Aquaculture must grow rapidly as an alternative to the declining capture fisheries in the world. Genetically improved stock, such as the GIFT strain, has an important role to play in increasing aquaculture production in developing countries. However, in some cases, the introduction of already improved strains may not be possible or desirable due to health or bio-diversity considerations. In such cases it is important that, as an alternative, the technology for genetic enhancement of fish be transferred and applied to native species.

Several genetic techniques (e.g. selective breeding, cross-breeding, chromosome manipulation, YY-male technology) are available to enhance the productivity of aquaculture species, including Tilapias. However, with the exception of selective breeding, these technologies only result in a once-off gain, and not in continued improvement. A continuous improvement of relevant traits requires a well designed selective breeding program where the pedigree of brood fish is monitored to increase the accuracy of selection and to restrict inbreeding.

A ten-year collaborative research project (1988 to 1997) widely known as 'Genetic Improvement of Farmed Tilapias (GIFT)' was conducted by the WorldFish Center in cooperation with AKVAFORSK (The Institute of Aquaculture Research in Norway), and a number of national research institutions in the Philippines: the National Freshwater Fisheries Technology Research Center of the Bureau of Fisheries and Aquatic Resources, the Freshwater Aquaculture Center of the Central Luzon State University, and the Marine Science Institute of the University of the Philippines. The GIFT project demonstrated the potential of using selective breeding to genetically enhance the production performance of Nile Tilapia. After five generations of selection, the growth performance of the GIFT strain was improved by more than 80 per cent compared with the base population. The purpose of the present manual is to illustrate all major operations, as developed and applied in the GIFT project, to initiate and run selective breeding programs with *Oreochromis* (i.e. mouth breeding) species of Tilapia.

2. Selective breeding

2.1. Traits

A trait is any observable or measurable characteristic of an individual. In breeding programs one is often concerned with a range of characteristics. It may be conceptually convenient to group such characteristics in some logical way:

(i) Traits showing a relatively small number of classes, and often controlled by a small number of genes. Examples of this type of traits are some fish colors, and scale patterns in certain species.
(ii) Traits showing continuous variation, and assumed to be controlled by several genes, each one having a relatively small effect. There are traits in this category that are easily assessed or measured, such as size or weight, growth rate, body length, depth, width or shape. By contrast, there are some other traits that are difficult to measure, such as efficiency of feed utilization, yield of certain cuts (e.g. fillet), or flesh quality. The recording of this latter group of traits requires a special effort, and often it is carried out in relatives of the selection candidates especially designated for that purpose.
(iii) Traits that are discrete in their expression but assumed to be controlled by many genes. These traits usually take the form of 'Yes' or 'No', 'Present' or 'Absent', 'Dead' or 'Alive', and so on. 'Survival' and 'Achievement of sexual maturity at a particular age' may be used as examples of this type of trait. Generally, for the recording of these traits to be meaningful from a genetic evaluation point of view, it has to be carried out in groups of relatives, such as half-sibs, full-sibs, or progeny.

The above made classification is, of course, arbitrary to some extent. However, it is not only important from a conceptual standpoint, but also from a practical one, as the genetic improvement of traits in different groups may be the subject of different selection strategies.

2.2. Phenotypes, genotypes and breeding values

The observed category or measured level of performance for a specific trait in an individual is referred to as its phenotype. We may say that an individual has as many phenotypes as there are traits to be observed or measured on that individual. The purpose of selective breeding is to allow individuals with the best sets of genes to reproduce so that the next generation has, on average, more desirable genes than the current generation. By contrast, the genetic makeup of an individual is referred to as its genotype. Both the genotype and the environment an individual experiences determine its phenotype. Note, however, that an individual does not transmit to its progeny all of its genotype. Each progeny receives half of the genes from the female parent, and the other half from the male parent. The breeding value of an individual is the value of the genes it has to its progeny, that is, the value of the individual as a parent. On average, an individual will transmit the sum of the average effects of the alleles it carries, and this is its breeding value. A major challenge in selective breeding

is to estimate an individual's breeding value based on its own phenotype and (or) the phenotypes of its relatives.

2.3. Breeding goal

Selective breeding is a long-term strategy to improve the production performance and quality of Tilapias. Therefore, it is important to define a breeding goal, that is, a direction for the genetic improvement program. The breeding goal should be defined by a set of traits that are of economic and (or) social importance, that are to some extent inherited, and, preferably, that may be recorded in an applied selection program. Note, however, that there may be traits that are very difficult or sometimes impossible to record, but that still have to be included in the breeding objective. Disease resistance is in some circumstances one such trait.

2.4. Selection strategies

Several selection strategies are available for genetically improving the performance of Tilapias. The strategies differ in terms of which kind of relative provides information to estimate the breeding value of the selection candidates. The choice of selection strategy depends on the nature of traits (e.g. see earlier classification in section 2.1) and the heritability of traits (i.e. the relationship between an individual's phenotype and its breeding value).

2.4.1. Individual selection

Individual selection, or 'mass selection', is a strategy where the breeding candidates are selected based on their own phenotype, that is, on their own performance. It has been the most frequently applied selection strategy in fish due to its simplicity. However, it can only be used to improve traits that are recorded on the breeding candidates while they are still alive (e.g. growth, shape, color), and it is not efficient to improve discrete and lowly heritable traits such as survival rate.

If practiced without constraints, the accumulation of inbreeding can be a major problem of individual selection in fish. The selected broodstock can become more and more related to each other. If large families are naturally produced or stocked in the test environment, the wide genetic variation and common non-genetic effects (e.g. maternal and age effects) may easily result in a disproportionately high contribution of individuals from just a few parents to the next generation, and a consequent accumulation of inbreeding. Inbreeding reduces the genetic variation and the potential for further genetic improvement in the breeding population. Generally, it also lowers the performance of fish, due to the phenomenon known as inbreeding depression. To prevent inbreeding, only a restricted number of individuals from each full and half-sib family should be tested as breeding candidates when applying individual selection. However, this requires that each family be produced and kept separately from each other before testing.

2.4.2. Family selection

Family selection is a strategy whereby the breeding candidates are ranked based on records taken on their full and half-sibs, that is, individuals that have genes in common with them. This selection strategy can be applied to improve traits that cannot be recorded on the breeding candidates while they are alive (e.g. carcass quality traits), traits that can only be recorded for groups of fish (e.g. feed utilization), and discrete traits at high or low incidences (e.g. survival rate, age at sexual maturity). Furthermore, family selection is more effective than individual selection when the heritability of a trait is low. However, family selection requires that each family be produced and kept separately and that all fish are tagged before testing in common test environments.

2.4.3. Combined selection

When traits can be recorded on the breeding candidates, family selection can be combined with individual selection to increase the accuracy of the estimates of breeding values, and thereby achieve a greater selection response. Combined selection increases the rate of selection response relative to the earlier mentioned methods. It optimally combines all available sources of information used in the estimation of an individual's breeding value. With fish, most often this means information from full and half-sibs. More generally, it may mean information from parents, offspring, or any other group of relatives.

Combined selection may take the form of an index, in which individual, full-sib and half-sib information are included and weighted. It may also be implemented using Best Linear Unbiased Prediction (BLUP) procedures, which allow the simultaneous estimation of breeding values and adjustment for fixed effects. For relatively balanced designs, one may expect good agreement between the selection index and BLUP approaches.

2.5. The GIFT technology

What is broadly known as the GIFT technology includes a number of different and complementary tools to produce and test Tilapia of known ancestry, to statistically analyze collected grow-out data, and finally, to estimate breeding values for each individual, based on the performance of the breeding candidates themselves and their relatives in the population.

3. Forming a base population

3.1. Importance of a broad genetic basis

The genetic gain achieved by selective breeding (selection response) depends on the presence of additive genetic variation (variation in breeding values) in the breeding population. Assume that the two distributions shown in Figure 1 represent variation in breeding values. For a given proportion of individuals selected, we can expect greater genetic gain when the additive genetic variation in the breeding population is large (i.e. the distribution shown in the lower part of Figure 1). In fact, the success of a selective breeding program will, in no small part, depend on the genetic variation in the base population. Of course, it will also depend on other factors, such as how the population is maintained from one generation to the next.

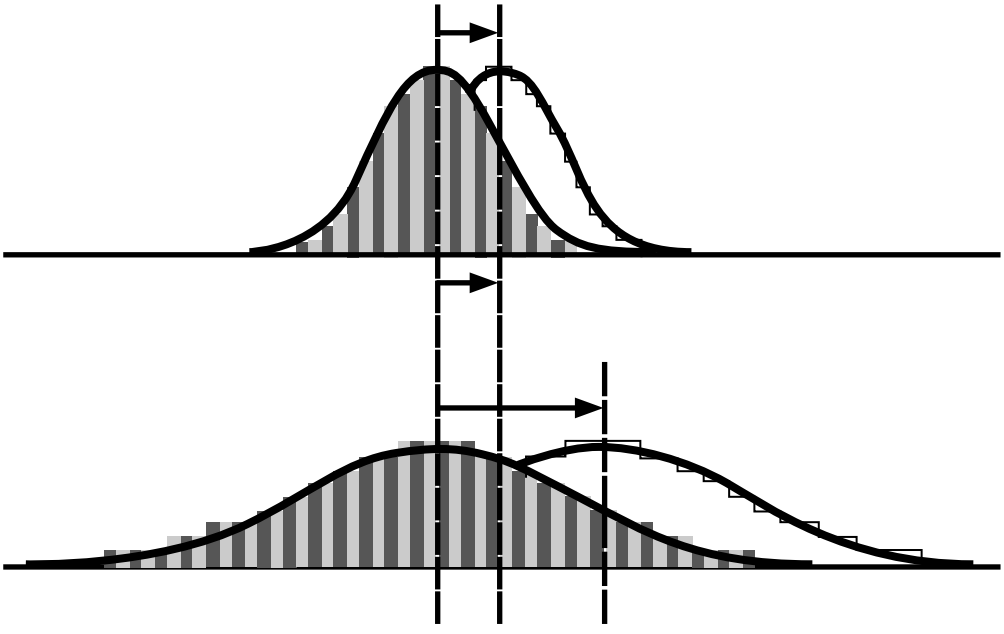


Figure 1. The selection response (represented by the arrows) depends (among other factors) on the additive genetic variation present in the breeding population.

3.2. A 'synthetic' or 'composite' base population

One way of ensuring the presence of abundant genetic variation in the base population is by assembling a 'synthetic' or 'composite' population, using breeders from different sources. Even if domesticated strains are available, wild populations may positively contribute to a synthetic base population, particularly in cases where there are only moderate genetic differences between wild and domesticated stocks of Tilapia. A useful way of combining fish from different sources is in a diallel cross design (Table 1). The different cross combinations can be tested in all prevailing production environments, and fish from the best combinations are then used to establish the base population.

Assume that strains A and B are wild strains, whereas strain C is a domestic one. In a full diallel cross, such as that described in Table 1, all strains are used as female parents, as well as male parents. The cells shaded green represent purebred progeny, whereas those shaded yellow are crossbreds. Making these crosses constitutes the first step in establishing the composite population. Following the evaluation of all the resulting progeny, the parents of the next generation are chosen from among this progeny, on the basis of their performance, irrespective of the mating that originated them. However, particularly in cases in which differences between strains are not large, it may be useful to have a minimum level of representation of each wild population and of each domesticated strain to ensure a broad genetic base in the population at the beginning of the selection program. Each male breeder can be mated to one or several females at the same time.

Diallel cross design		**Female parent strain**		
		A	**B**	**C**
Male parent strain	**A**	A	AB	AC
	B	BA	B	BC
	C	CA	CB	C

Table 1. Fish from different wild populations and domesticated strains can be used in a full diallel cross design to 'mix' their genes as a first step in the establishment of a base population.

4. Breeding and rearing

4.1. Synchronization of spawning

Spawning should be synchronized to produce progeny groups as uniform in size and age as possible. This will reduce the confounding effects of initial size and age during comparative studies of different genotypes (e.g. strains, cross combinations, family groups), and in the estimation of various genetic parameters (e.g. heritabilities and genetic correlations) and breeding values. Spawning of Tilapias is influenced by both environmental (e.g. photoperiod, temperature, food availability) and social factors (social stimuli exchanged between neighboring females). Therefore, the strategy for synchronizing spawning involves maintaining broodstock separated by sex in a suitable holding facility (e.g. hapas, see Figure 6), conditioning by proper feeding, and evaluation of the sexual maturity condition of females.

4.1.1. Holding in hapas

A 'hapa' is a fixed net enclosure, similar to an inverted mosquito net. It is made out of polyethylene netting with joints in nylon thread, double stitched to prevent splitting. The standard size is 1 m (width) by 1 m (length) by 1 m (depth). The mesh sizes are usually 1.0 to 2.0 mm (blue material in Figure 6), and 5.0 to 6.0 mm (black material in Figure 6, also called B-net). The thinner mesh (blue in Figure 6) is used to hold fry and small fish, whereas the broader mesh (black in Figure 6) may be used for larger fish.

Potential male and female breeders should be kept separately in hapas (Figure 6) installed in fertilized ponds at a stocking density of three fish per hapa if their live weight is up to 200 g, and two per hapa if the fish are heavier. Because of the generally greater live weight and more aggressive behavior of males, these may have to be stocked at a lower density per hapa. Individual breeders will then be exposed to the same reproduction-triggering factors at the same time. Furthermore, holding potential female breeders together might help synchronize spawning by exchanging social stimuli (e.g. pheromones). Stocking of breeders in hapas makes the handling and retrieval of fish during selection of breeders relatively easy.

Conditioning may also take place in larger hapas [e.g. 1.5 m (width) by 4 m (length) by 1 m (depth)]. In such cases the stocking density may be up to 20 fish per hapa.

4.1.2. Conditioning of breeders

Breeders should be conditioned at least two weeks prior to stocking in breeding hapas. During conditioning, Tilapia breeders are fed a balanced feed (about 30 per cent crude protein) at a feeding rate of 2 to 5 per cent of their body weight.

4.1.3. Evaluation of sexual maturity condition

After conditioning, the female breeders should be checked for their readiness to spawn by visually examining their morphological characteristics. Female breeders are then categorized in one of the following maturity conditions: 'ready to spawn' (RS), 'swollen' (S), 'not ready to spawn' (NRS), and 'has spawned' (HS). Descriptions of these four categories are given in Table 2 and Figures 2 to 5. Female breeders categorized as 'ready to spawn' are first selected for pairing with a male in a breeding hapa.

Category	Code	Morphological characteristics	Days until spawning
Ready to spawn	RS	Pink to red and protruding genital papilla, fully opened genital pore, and distended abdomen	3 to 7
Swollen	S	Pink to yellow genital papilla, slightly opened genital pore, and slightly distended abdomen	5 to 10
Not ready to spawn	NRS	White to clear and flat genital papilla, and normal abdomen	21 to 30
Has spawned	HS	Red genital papilla, and shrunken to compressed abdomen	15 to 30

Table 2. Different categories of sexual maturity of female Nile Tilapia and the expected number of days until spawning.

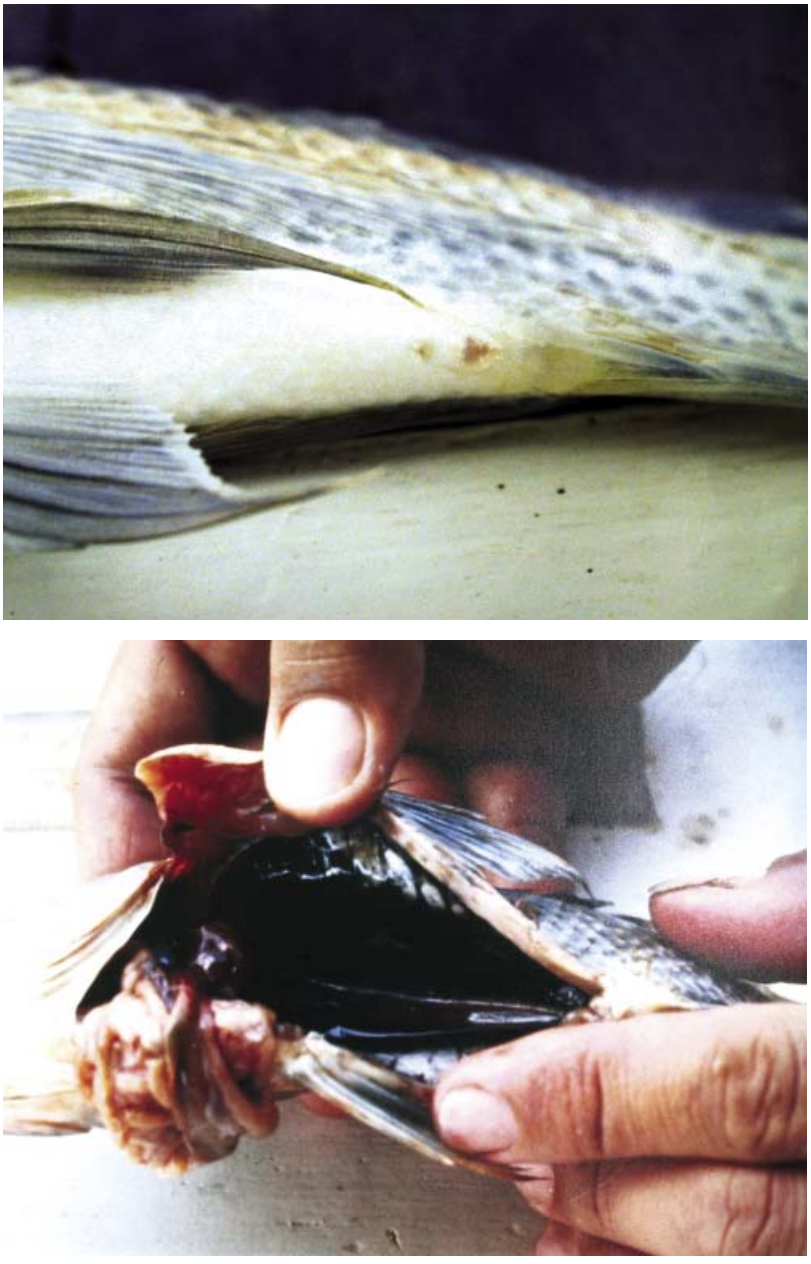


Figure 2. 'Not ready to spawn' (NRS) female of Nile Tilapia. Note the white, clear and flat genital papilla, and normal abdomen (top), and absence of egg development inside the visceral cavity (bottom).

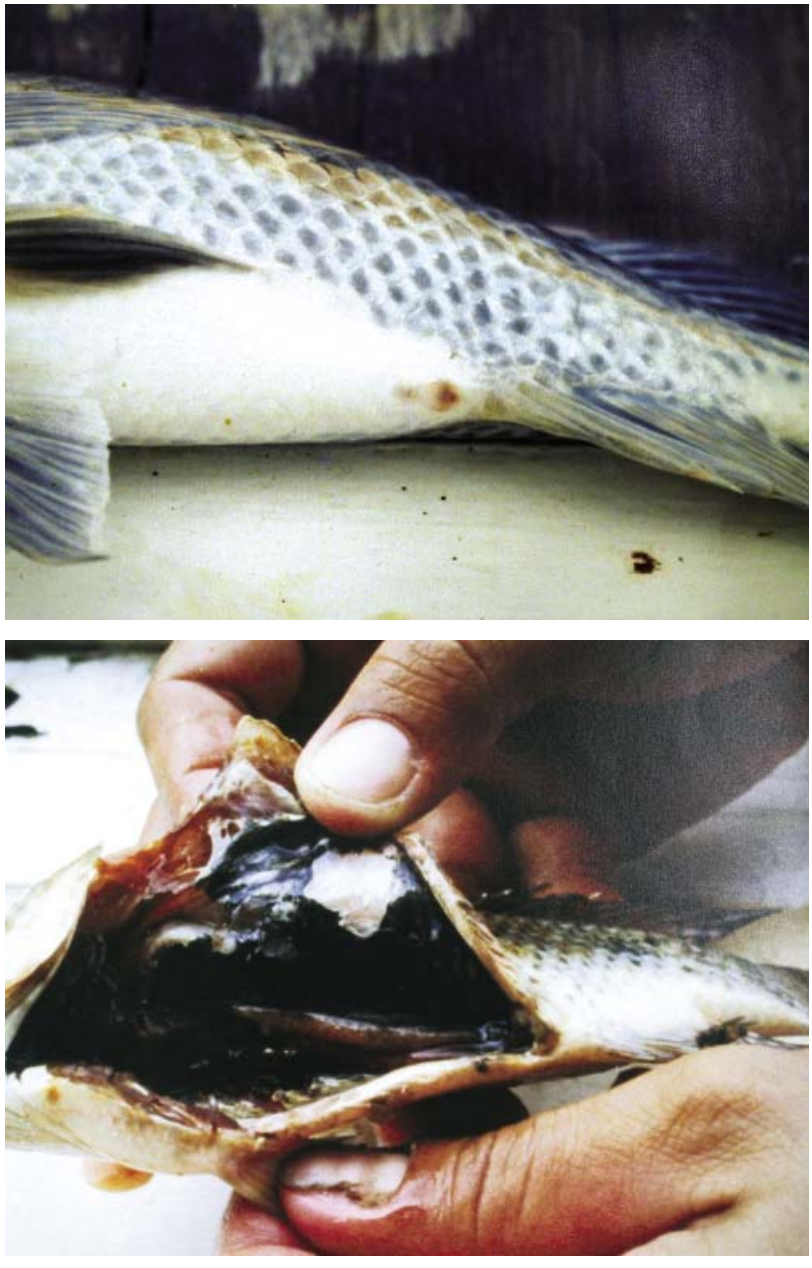


Figure 3. 'Swollen' (S) female of Nile Tilapia. Note the pink to yellow genital papilla, slightly opened genital pore and slightly distended abdomen (top), and eggs beginning to develop inside the visceral cavity (bottom).

Figure 4. 'Ready to spawn' (RS) female of Nile Tilapia. Note the pink to red and protruding genital papilla, fully opened genital pore and distended abdomen (top), and eggs developed inside the visceral cavity (bottom).

Figure 5. 'Has spawned' (HS) female of Nile Tilapia. Note the red genital papilla and shrunken to compressed abdomen (top), and that eggs have been released (bottom).

4.2. Breeding in hapas

Tilapias are known to spawn in tanks, ponds or in small cages (breeding hapas) within ponds. The GIFT technology uses breeding hapas (1 x 1 x 1 m^3) installed in a pond to enable a controlled production of a large number of full and half-sib families.

4.2.1. Preparation of facilities

The breeding pond should first be totally drained, and then allowed to dry for at least two weeks before liming and refilling with water at a level of 80 cm. Liming is usually carried out at a rate of 100 to 300 g per square meter. The water inlet and outlet should both be covered with a fine-meshed wire screen to prevent the entry of predators into the pond. One or two weeks prior to stocking the breeders, the pond should be fertilized [2 000 kg chicken manure and 100 kg inorganic fertilizer (N:P:K in the ratio 16:20:0) per ha] to stimulate the production of natural food. Breeding hapas should then be installed in the pond in rows with enough space to enable water circulation (Figure 6). Breeding hapas for two females to be mated with the same male are installed opposite each other to facilitate an easy transfer of the male.

4.2.2. Mating design and procedure

A mating plan should be prepared combining all selected breeders. Generally, each male breeder is mated at random to two female breeders in a nested mating design to produce paternal half-sib families. This will allow the calculation of phenotypic and genetic parameters (i.e. heritability, phenotypic and genetic correlations), which are necessary for calculating breeding values. It is important to avoid mating of closely related individuals (i.e. full-sibs, half-sibs or cousins) to prevent inbreeding depression.

4.2.3. Stocking of breeders

The female breeders should be stocked into the breeding hapas before the males. The males are then transferred to the females that are most ready to spawn. After fry are produced and collected, the males are separated from the female and immediately transferred to the hapa where the second female is stocked. Spent females that produce less than 200 fry should be conditioned (by proper feeding) in the breeding hapa and mated again with the same male until they produce a satisfactory number of fry. The Tilapias being mated should not be fed when the female breeders are expected to spawn since this might cause the females to swallow the eggs.

Figure 6. Breeding and nursery hapas (blue) and B-net rearing hapas (black) installed in rows with enough space to enable water circulation.

4.2.4. Mouth clipping of males

To avoid, or at least reduce, mortalities the body weights of female and male breeders should be as close as possible to each other. If the male breeders are much larger (say, greater than 30 per cent) than their female counterparts, it is necessary to carry out mouth clipping of male breeders before transferring them to the breeding hapas (Figure 7). The male breeders should be anesthetized before removing their upper lips, and the wounds should be disinfected using an antiseptic preparation (e.g. Betadine, 10 per cent solution).

4.2.5. Fry collection

The first fry collection may be done 10 to 14 days after stocking the breeders (Figure 8). The hapa is first divided into two compartments to separate the female and male breeders to reduce stress when checking the females. The fry should be collected early in the morning to avoid stress and mortalities. Females with incubating eggs or yolk sac fry in their mouth should remain in the breeding hapa until yolk absorption is complete or until the fry are in the free-swimming stage. Eggs or yolk sac fry that have been accidentally released from the mouth of the female can be collected and transferred to artificial incubators. Both fry and eggs should be rinsed and counted before transferring them to the nursery hapas or artificial incubators.

4.2.6. Artificial incubation

Eggs or yolk sac fry that are accidentally released from the female breeders can be transferred to artificial incubators (Figure 9). It is important to ensure a constant flow-through of water to the incubators to optimize the environment for the eggs or yolk sac fry. The eggs usually hatch after 2 or 3 days. The fry are incubated until yolk absorption is complete and are then transferred to nursery hapas.

Figure 7. Mouth clipping of male Nile Tilapia that are much larger than the female breeders to reduce their aggressive behavior.

Figure 8. Fry collection from breeding hapas and rinsing of fry before transferring them to the nursery hapas.

Figure 9. Accidentally released eggs should be transferred to artificial incubators.

4.3. Rearing of families

Fry that are collected from the breeding hapas should be reared separately until they are large enough to be tagged. Individual tagging will ensure that the pedigree of all fish can be recorded in the breeding program.

4.3.1 Rearing in nursery hapas

Fry are transferred to nursery hapas (1 x 1 x 1 m) at a density of 150 to 200 fry/m^3. Families with more than 200 fry are stocked in several nursery hapas. All nursery hapas should be installed in the same pond and a similar density of fry should be maintained in all hapas to reduce environmental differences between families. The fry are reared for 21 days in nursery hapas and then transferred to B-net cages.

4.3.2. Rearing in B-net cages

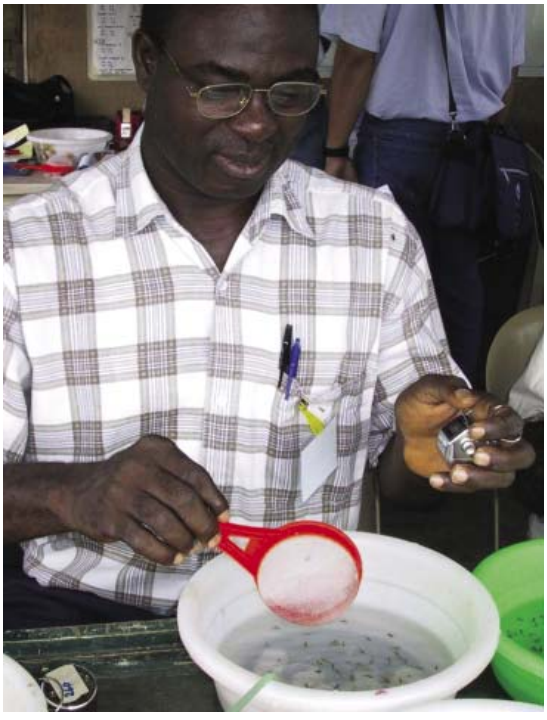
The fry are transferred to B-net hapas (1 x 1 x 1 m) to allow better water circulation and improved growth. The density of fry should be reduced to 100 to 150 per m^3, and the fry should be reared for another 21 days until they reach a live weight of at least 3 to 5 g, and are ready to be tagged.

4.4. Collection of breeding data

A successful breeding program will partly depend on a well-organized collection of data. Recording the live weight of all breeders to ensure matching males and females by size is highly desirable. With experience, the allocation of males to females may be done based on a visual assessment of their size. Furthermore, it is necessary to keep records of the number and mean body weight of fry collected from each breeding hapa (Figure 10), of fry stocked in each nursery hapa, and of fingerlings transferred to and collected from each B-net hapa. These records are important to ensure that enough fingerlings are produced and that the rearing conditions are standardized for all full-sib families. A number of forms used to record these and other data in the GIFT project are given in Appendix A. These forms may, of course, be modified to suit the specific needs of each individual project. They are presented as a guide to researchers initiating their data collection.

4.5. Anesthetization

The fish should be anaesthetized to reduce or eliminate the stress experienced during handling in connection with sampling and tagging. The Tilapia fingerlings or breeders should be conditioned in tanks with aeration for 1 to 2 days before the anesthetic is applied. The fish should not be fed during this conditioning period. In the GIFT project, tricaine methanesulphate, or MS222 (at a concentration of 0.33g l^{-1}) was found to be the most effective anesthetic for Tilapias. However, the fish should be removed immediately from the anesthetic solution when they turn on their side and allowed to recover in basins with aerated freshwater. Prolonged exposure to the anesthetic solution may irreversibly damage fish health. The fish are expected to regain full mobility after 2 to 3 minutes.



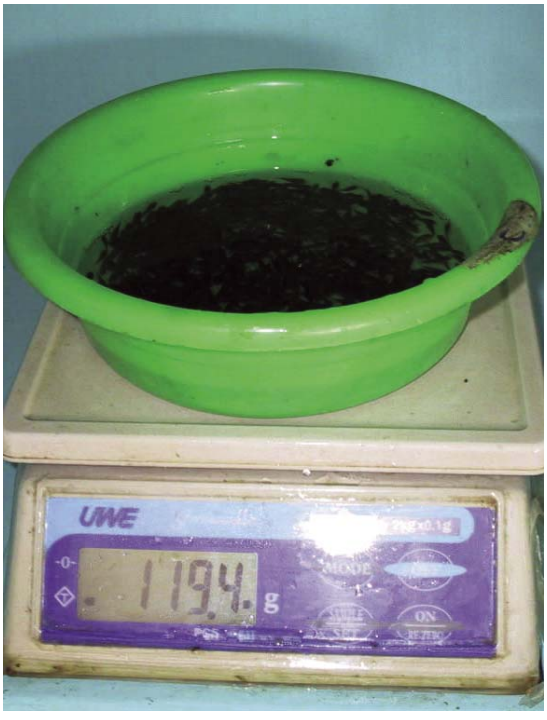


Figure 10. Counting and bulk-weighing full-sib families of fry collected from separate breeding hapas.

5. Testing

5.1. Individual marking

The accurate testing of Tilapias in common farm environments requires that groups (e.g. full-sibs, half-sibs, strains, and strain combinations), and in some instances individual fish, have a unique identification. A limited number of groups or individual fish can be marked by fin clipping, which involves the removing of fins or spines. However, the individual identification of a large number of fish requires tagging of some sort. This involves attachment, insertion, or injection of a foreign object to or into the body of the fish. The GIFT technology has involved external tagging, using Floy fingerling tags, to uniquely identify each one of the large number of fish tested.

5.1.1. Floy fingerling tags

The Floy fingerling tag, which is a small, flat, oval-shaped piece of plastic, is tied to a vinyl thread that holds it. The tag is especially designed for fingerlings. Individual identification is based on numbers and (or) letters printed on either side of the tag (Figure 11). For instance, one side may indicate the full-sib group (i.e. the family) to which the individual belongs, whereas on the other side we may find the individual's own number. If the family number is preceded by the generation (e.g. 1, 2, …, n) number to which the test fish belong, then we have a unique identification for all fish across generations. This is required for some analyses that use the full pedigree of all individuals in the population. It is recommended to use tags of different colors (e.g. 20 to 25 full-sib families per color) to facilitate a rough sorting by families when harvesting the fish. However, it is not recommended to use red or any other dark color since it might be difficult to read the numbers at harvest.

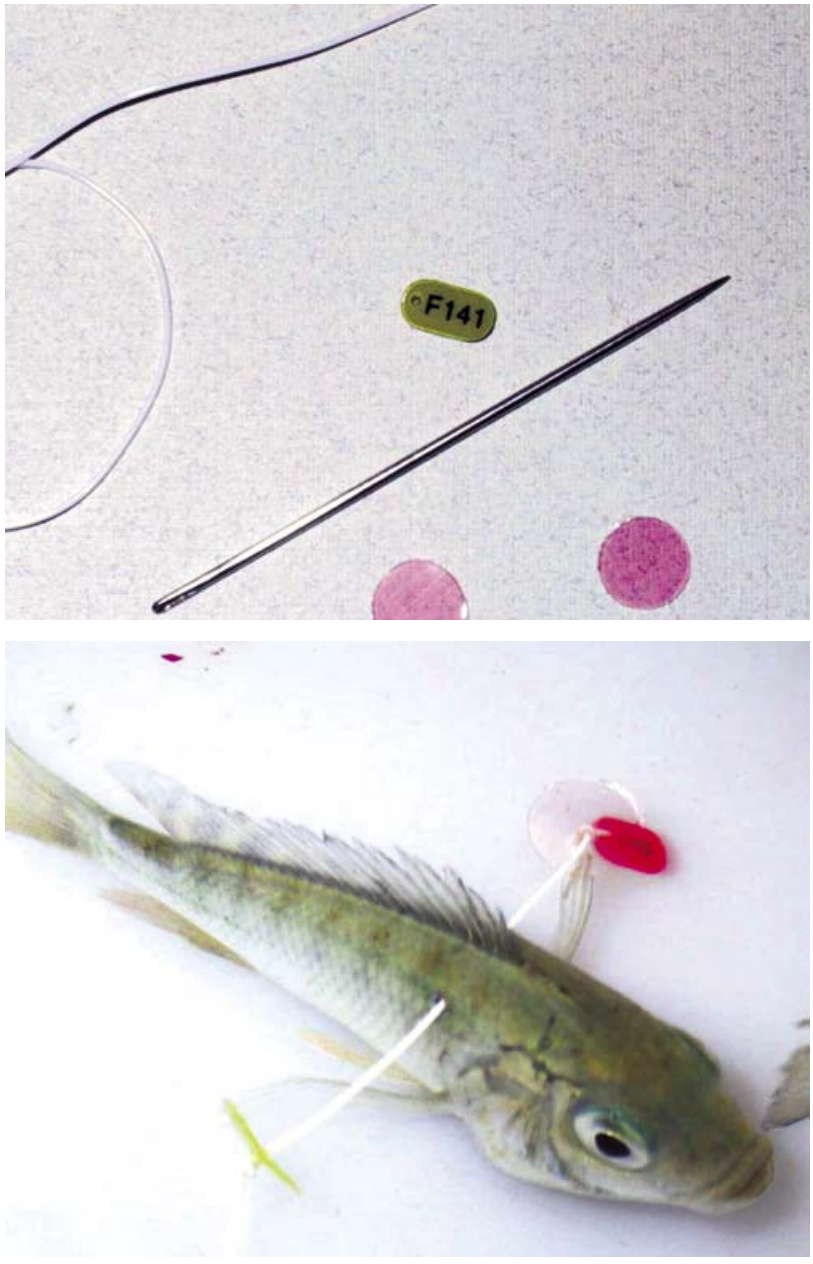


Figure 11. The test fish may be tagged using Floy fingerling tags with the family number on one side (distinguished with the letter 'F') and an individual number on the other side.

5.1.2. Tagging fingerlings with Floy tags

The Floy fingerling tags have been found convenient for the tagging of Tilapias. However, there may be other suppliers of suitable tags. In the section that follows we describe the routine procedure of affixing the tags to fingerlings.

1) Cut several pieces of 15 cm vinyl thread.
2) Make a single knot on one end of the thread to serve as anchorage for the tag.
3) Attach the tag and make another knot to lock the tag in place.
4) Insert the thread in the needle and attach a round plastic disc (made of ordinary plastic material) by pushing the needle through its center, to serve as anchorage on the other end of the thread.
5) Arrange the fixed tags in sequence on a prepared cardboard (Figure 12).
6) Prepare a master list of tag numbers for each test environment and family replicate to facilitate data editing.

5.1.3. Insertion of Floy fingerling tags

Prior to insertion of Floy fingerling tags, the following materials should be prepared: anesthetic solution, scoop net, fixed Floy fingerling tags, extra plastic discs, scissors, towel, basin with clear water, and an aerator (Figure 12). The insertion of the tags should then be conducted in the way described below:

1) Hold the anesthetized fingerling in a swimming position facing the left hand (Figure 13).
2) The insertion site is located on the left side of the fish body between the sixth and seventh spines of the dorsal fin, above the lateral line.
3) The needle is inserted underneath one scale and carefully passed through the tissue without hitting any bones or veins (Figure 13).
4) A round plastic disc is attached to the vinyl thread (Figure 14).
5) A three-loophole knot is made to secure the tag (Figure 14).
6) Cut the thread. The thread should have a length of 3 to 5 cm to allow for the fish's growth (Figure 15). The length of the thread can be decided based on the anticipated maximum growth (e.g. as determined by the age or weight at harvesting).
7) Adjust the tag (Figure 15) and immediately place the tagged fingerling in a recovery basin with aerated freshwater.

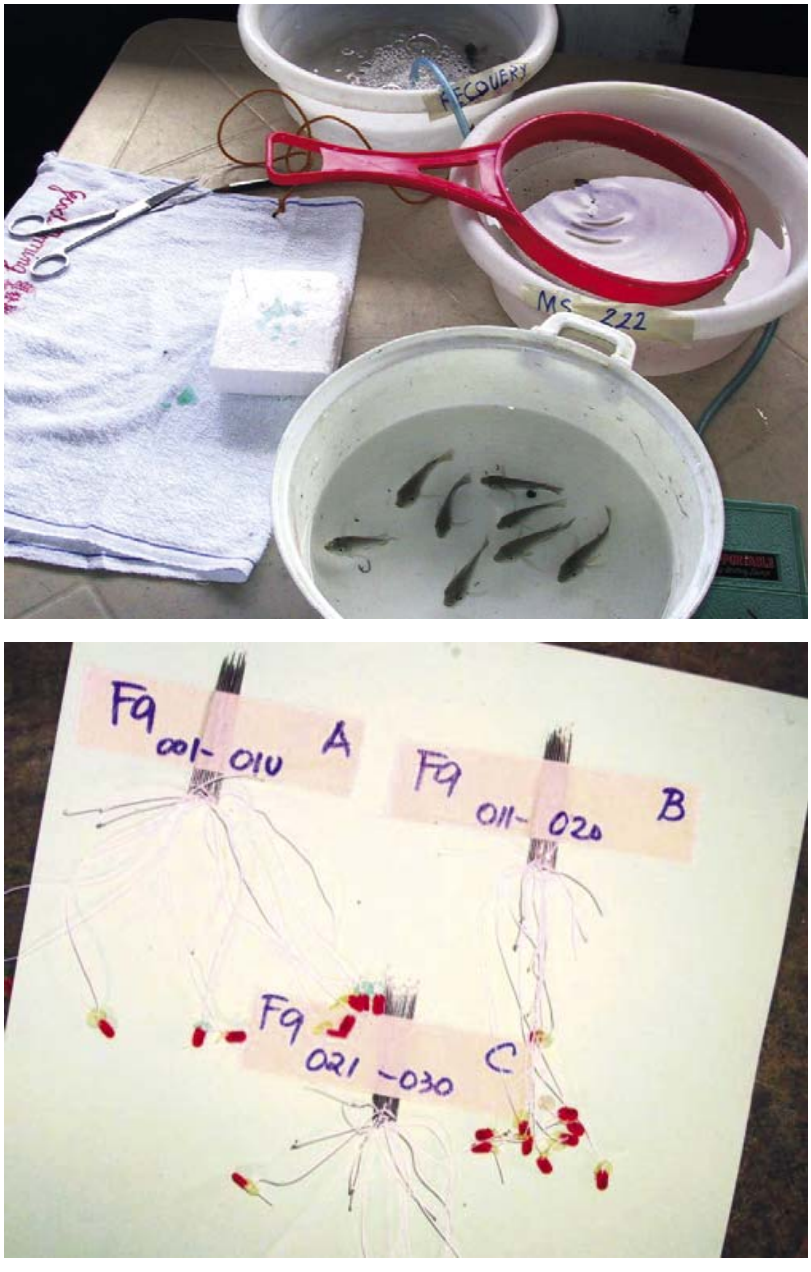


Figure 12. All necessary materials should be well organized before initiating the tagging of test fish.

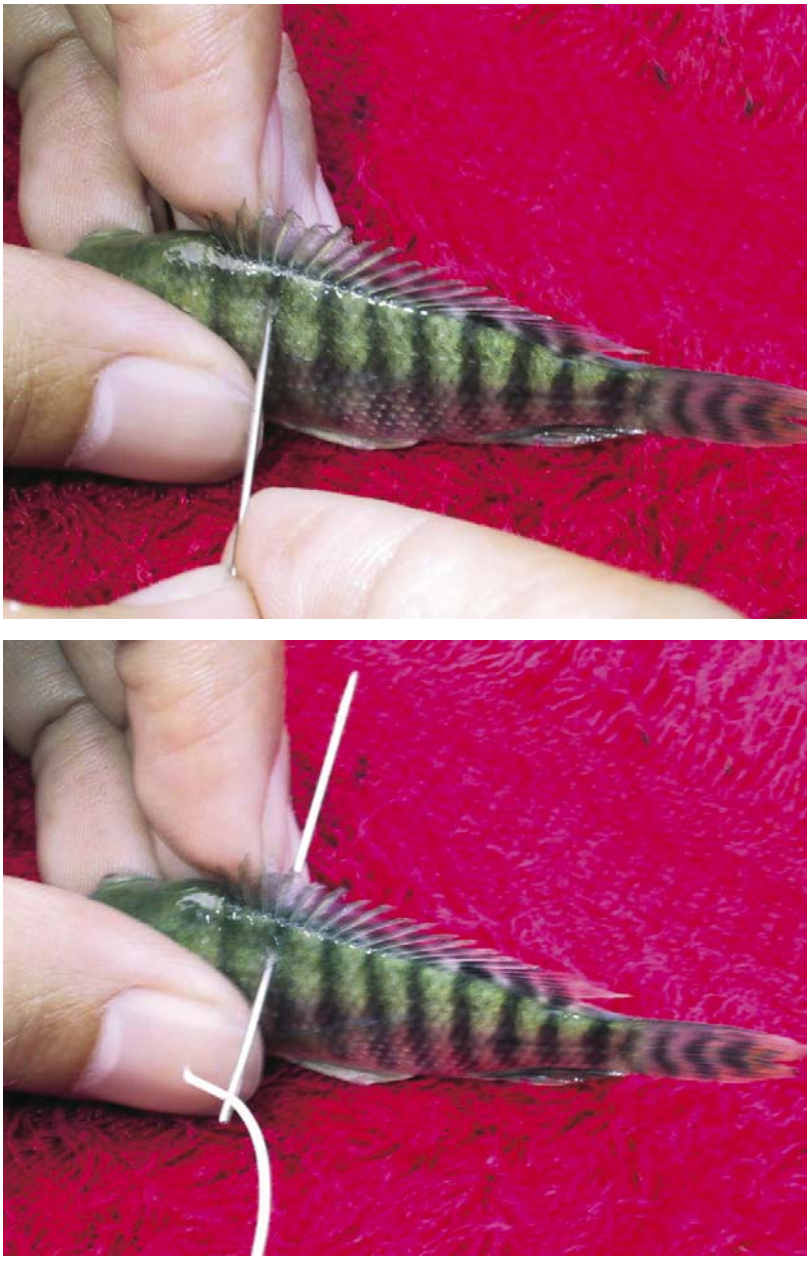


Figure 13. Floy tagging: The fingerling should be held in a swimming position facing the left hand, and the needle should be carefully inserted underneath the scale between the sixth and seventh spines of the dorsal fin, above the lateral line.

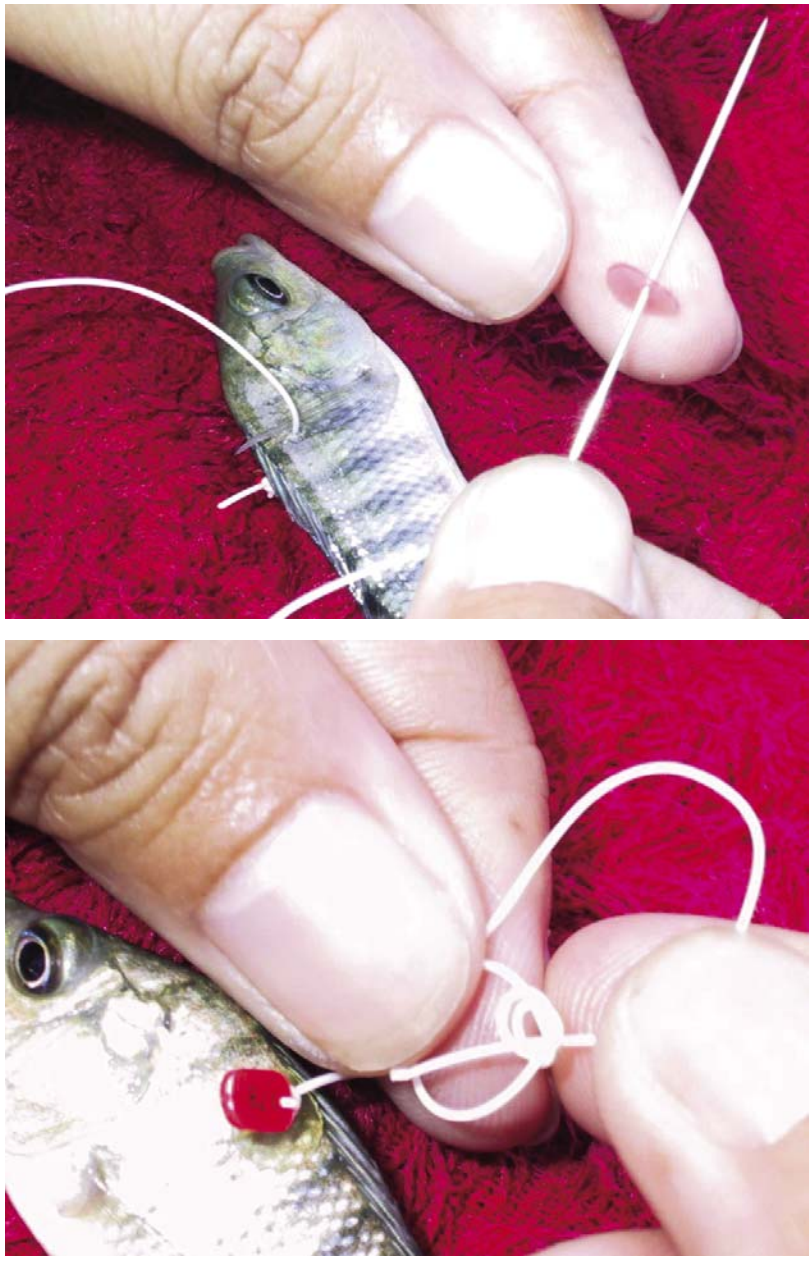


Figure 14. Floy tagging: A round plastic disc, through which the thread passes, is attached and a three-loophole knot is made to secure the tag.

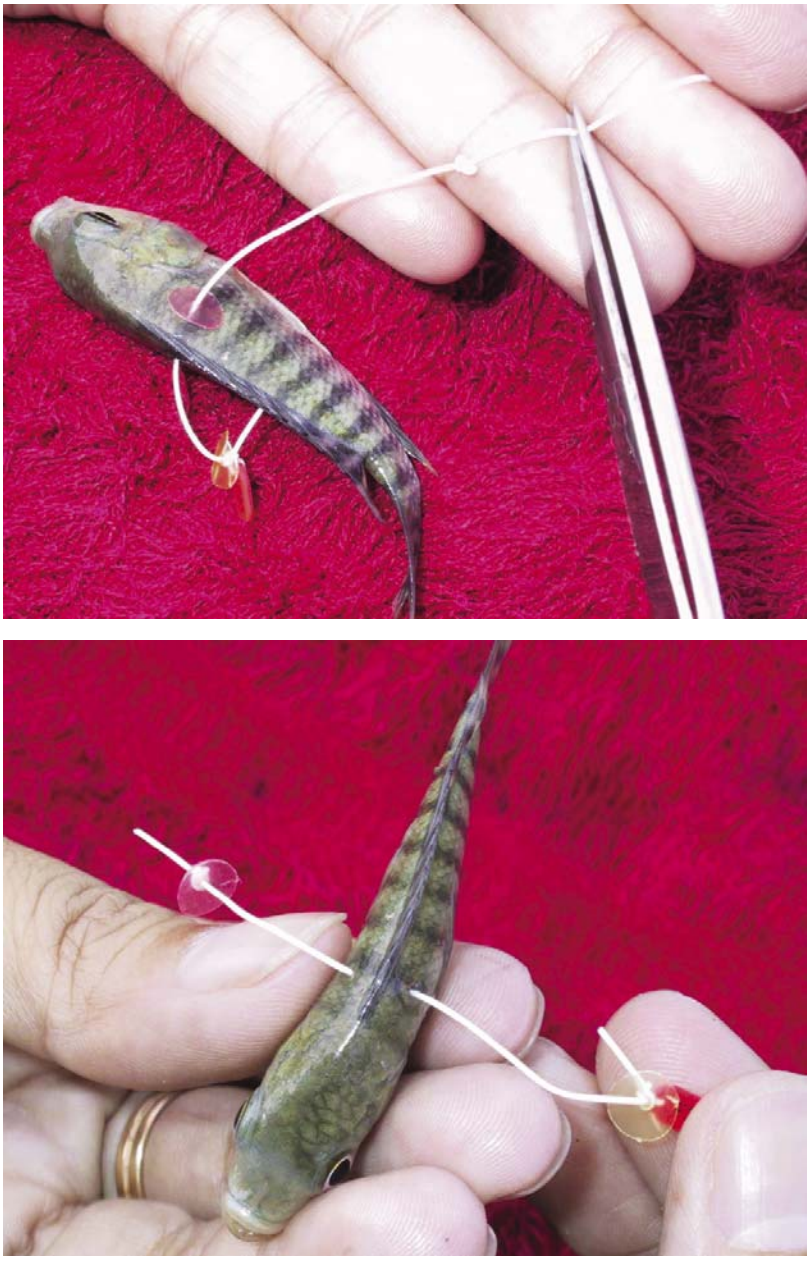


Figure 15. Floy tagging: The thread is cut at a length of 3 to 5 cm and the tag adjusted to allow for the fish's increase in size.

5.2. Communal testing

The tagged fingerlings should be pooled together and conditioned in a tank for 1 to 2 days without feeding before stocking. When testing in different environments, a minimum of 15 fish per full-sib group is recommended. However, researchers are encouraged to consult a quantitative geneticist-statistician to decide on the necessary number for the specific circumstances. In selection programs, the population of potential breeders should have a minimum of 20 to 30 fish per full-sib group. This should ensure that as many sires and dams as possible are represented at harvest time among the progeny, and will allow a high selection intensity. As suggested above, seeking expert advice is recommended before making final decisions.

5.2.1. Growth

Individual growth is recorded as body weight and (or) body length at harvest. Ideally, fish from all full-sib groups should be tested during the entire production period in all common farm environments (e.g. ponds, cages, rice-fish production systems). This would ensure that the genetically improved strain(s) are the best for any particular Tilapia farmer supported by the breeding program. Note, however, that apart from the fish used in any on-farm testing, it is wise to keep a good representation of potential male and female breeders at the research station or 'breeding station'. This constitutes an insurance against losses that can take place at the farm level. If the potential breeders are allowed to grow to more than 200 g there may be difficulties during mating (e.g. more difficult to handle, display greater aggressiveness). Therefore, for selective breeding purposes, harvesting at or about that weight is recommended, even if it were below the desired weight for some markets. One may assume that there is a positive, and most likely a high, genetic correlation among weights at different ages.

Before stocking in different test environments, the live weight, body length, and any other measurements or observations deemed necessary of all tagged fingerlings may be recorded, together with their individual tag numbers. Since Tilapias are farmed in a very wide range of production environments, it is also important to describe the respective test environments: production system (e.g. pond, cage), fertilization and (or) feeding regime (e.g. what kind of fertilizers or feeds, how much is used each time, how often during the test period), water temperature, and other factors perceived as having a potential impact on performance. This information is important when verifying whether the tagged Tilapias are tested in representative farm environments as targeted by the breeding program. In principle, Tilapias should be tested following commonly used production cycles and systems, until they reach the common harvest weight. In the GIFT project, the fish were tested for four months in ponds, cages within ponds, and rice-fish production systems. At the end of the grow-out period, all test fish should be harvested as carefully as possible, and the individual tag numbers, sex, sexual maturity condition, body weights and body length, and any other measurements or observations should be recorded.

5.3. Harvesting

Harvesting of Tilapias, especially the potential breeders, should be done as carefully as possible to reduce stress and mortality. Conducting the harvest from ponds in two steps is recommended: an initial harvest by seining in a maximum of three drags to reduce the number of fish in the pond (Figure 16), and then a final harvest the following day by draining the pond completely (Figure 17). The fish should be harvested early in the morning before sunrise or in the evening after sunset to minimize stress due to heat. All harvested fish should immediately be transferred to large hapas for 1 to 2 days of conditioning without feeding, before recording the individual identification (tag number), sex, sexual maturity condition, live weight, length, width, depth, and any other measurements or observations made at the time.

The forms used to record growth and other data in the GIFT project are shown in the Appendix.

Figure 16. Initial harvesting of test fish from a pond by seining.

Figure 17. Final harvesting the following morning by draining the pond completely and transfer of harvested Tilapias to large conditioning hapas.

5.4. Ensuring the identity of potential breeders

After recording, the potential breeders are sorted by tag color and sex before transferring them to large hapas. The sex of Tilapia can usually be determined when the live weight is above 15 grams. The males have two openings: a large opening (anus) and a smaller opening (urogenital pore). In females (see Figures 2 to 5), the genital papilla is usually smaller and has three openings (anus, oviduct and urethra). After 1 to 2 days, the potential breeders (i.e., those selected because of their superior breeding value) are transferred to B-net cages for conditioning until breeding. The identification of fish should be ensured before transferring them by up-rooting the dorsal spines or PIT (passive integrated transponder) tagging.

5.4.1. Up-rooting of dorsal spines

Dorsal spine uprooting is an easy method to mark individual fish within a small group. Each potential breeder will have a unique spine or combinations of spines removed as a second identification in case they loose their Floy fingerling tags. It is not recommended to up-root the first dorsal spine since it serves as a reference point for counting which spines have been removed. The dorsal spine uprooting should be conducted as follows:

1) Hold the anesthetized tilapia in a swimming position facing the left hand by covering the head (Figure 18).
2) Spread the web of dorsal spines and count from the first anterior dorsal spine to identify the spine(s) to be removed.
3) Rip the membrane on both sides of the spine(s) to be up-rooted (Figure 18).
4) Hold the spine to be up-rooted and bend it carefully until the bone breaks (Figure 19).
5) Loosen the attached broken spine by twisting and moving it sideways and, when loosened, carefully up-root it (Figure 19).
6) Disinfect the wound(s) to prevent infection (Figure 20).
7) Record the second identification and immediately place the up-rooted Tilapia in aerated freshwater for recovery before transferring it to the B-net cage.

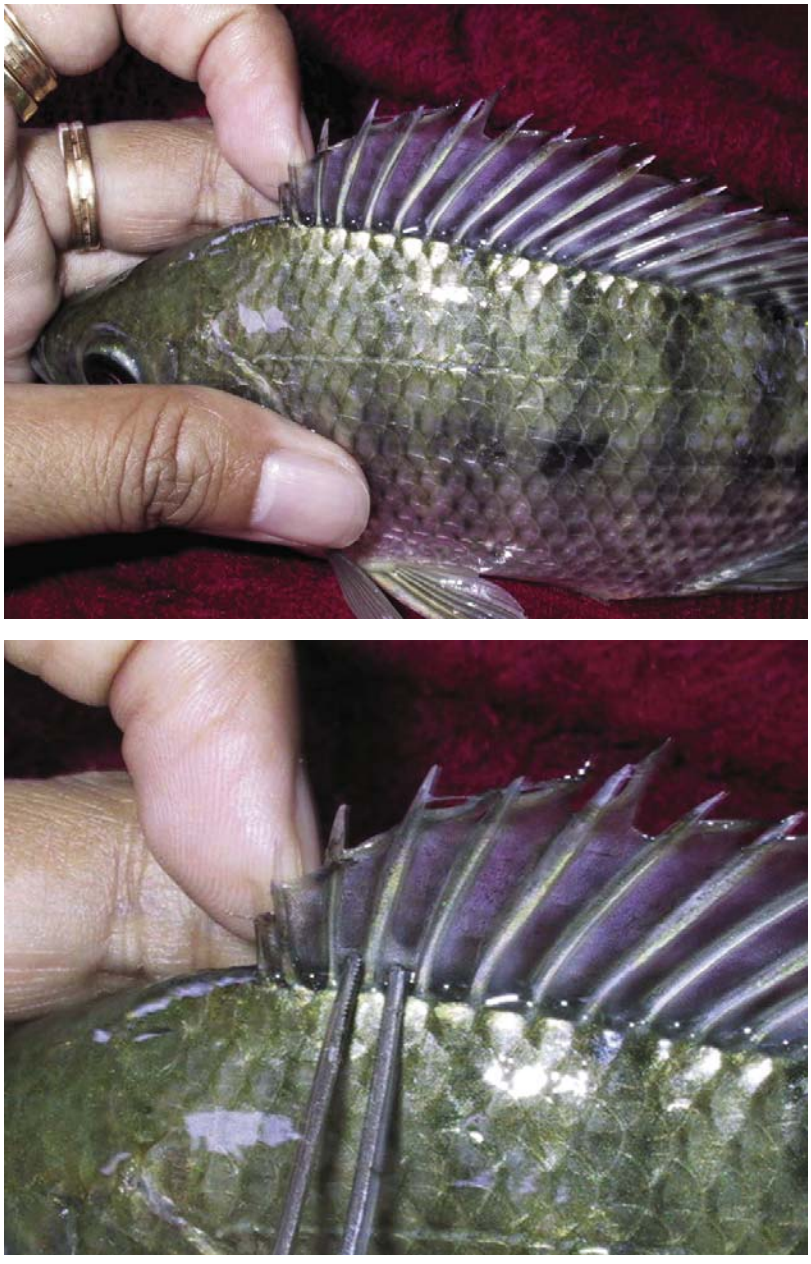


Figure 18. The anesthetized tilapia is held in a swimming position facing the left hand, while the membrane is cut on both sides of the spine(s) to be up-rooted.

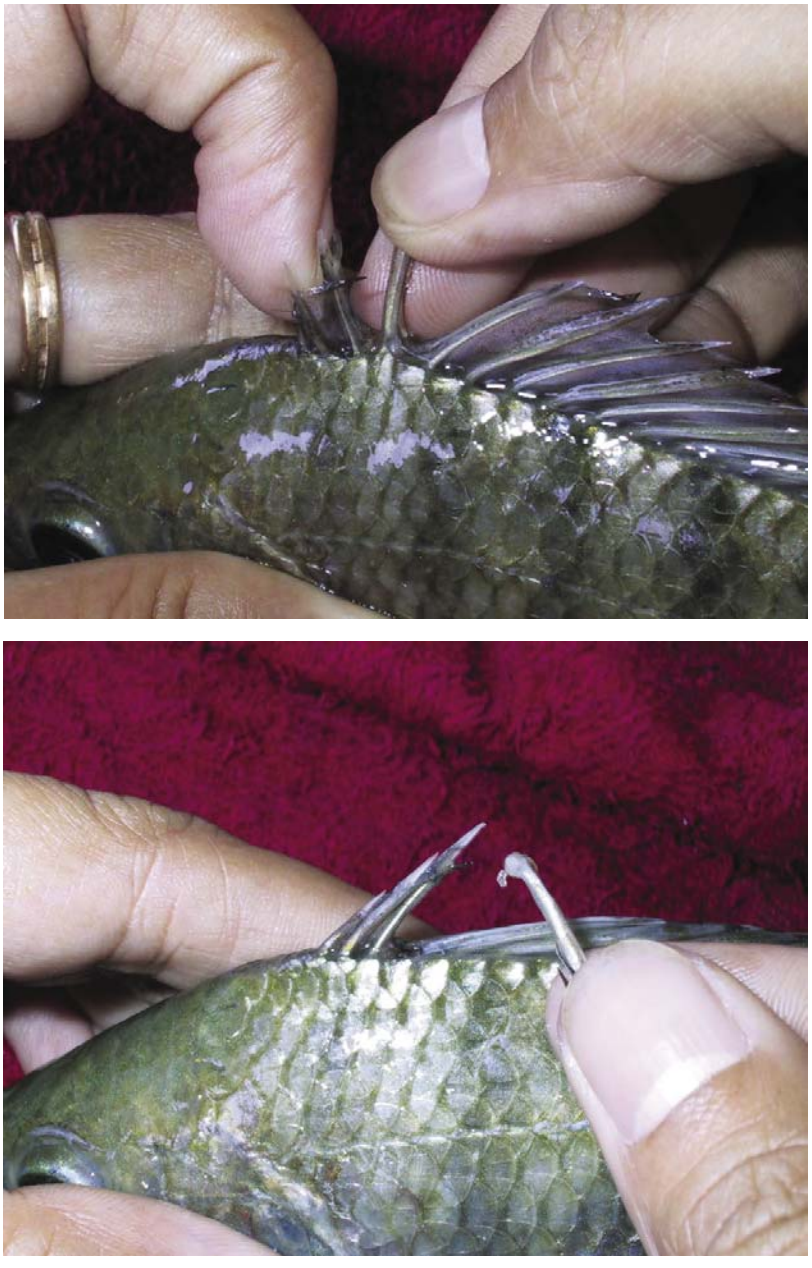


Figure 19. The spine to be up-rooted is bent carefully until the bone breaks, then it is twisted and moved sideways until it is loosened, and lastly, carefully up-rooted.

Figure 20. The wound should be carefully disinfected.

5.4.2. PIT-tagging

As an alternative to dorsal spine uprooting, the Tilapias can be identified using Passive Integrated Transponder (PIT) tags (Figure 21). The capsulated PIT tags are implanted within the visceral cavity of the fish, thus providing a secure and permanent identification of potential breeders. The unique number on each tag is read by using an electronic tag scanner. The implantation of PIT tags should be conducted as follows:

1) Insert the tag into the hole of the syringe needle (Figure 21).
2) Lay the anesthetized fish on a wet hand towel with the head facing the left hand.
3) Locate the insertion site on the left ventral side of the fish adjacent to the anus (Figure 22).
4) Insert the tip of the syringe underneath one scale until the visceral cavity is encountered.
5) Fully push the plunger of the syringe to securely lodge the tag in the visceral cavity (Figure 22).
6) Hold on the insertion site while the needle is gently withdrawn (Figure 23).
7) Check that the implantation was successful by reading the PIT-tag number using the electronic tag scanner, and immediately place the tagged fish in aerated freshwater (Figure 23).

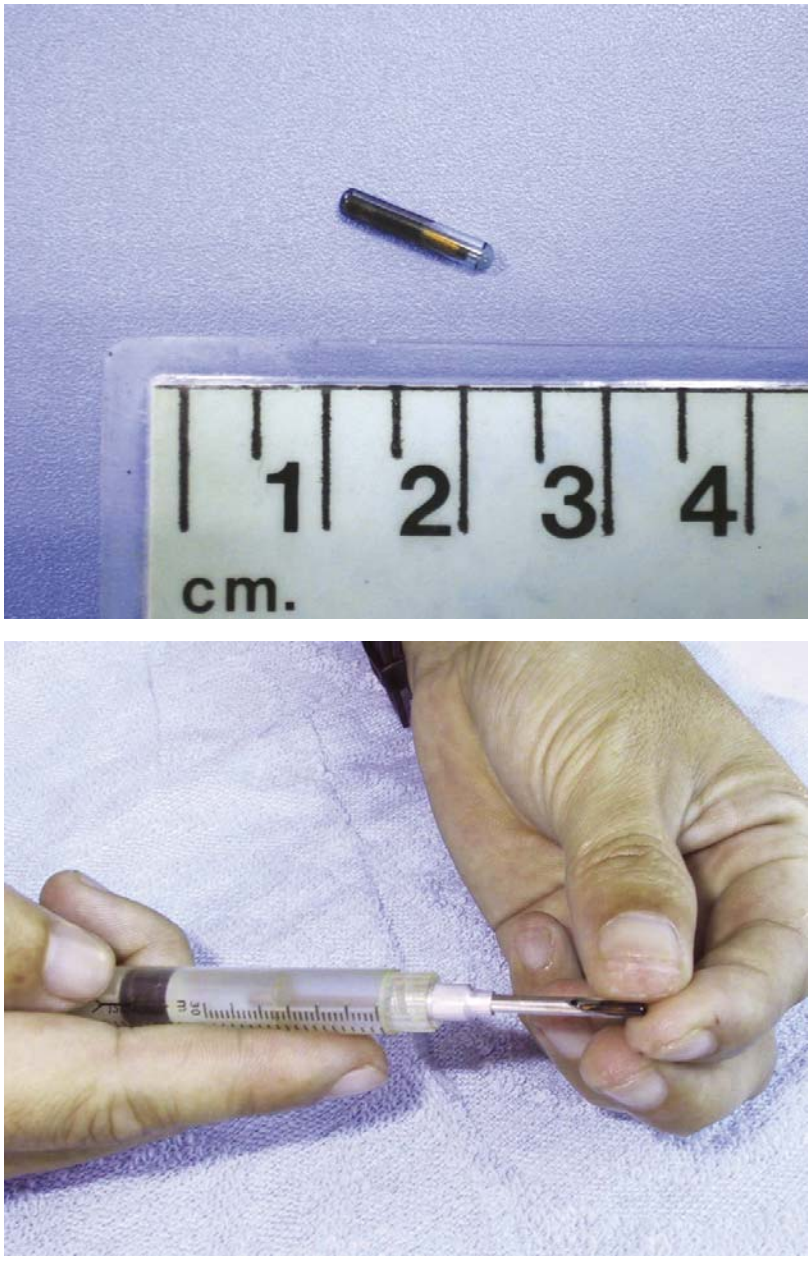


Figure 21. The PIT-tag is inserted into the hole of the syringe needle.

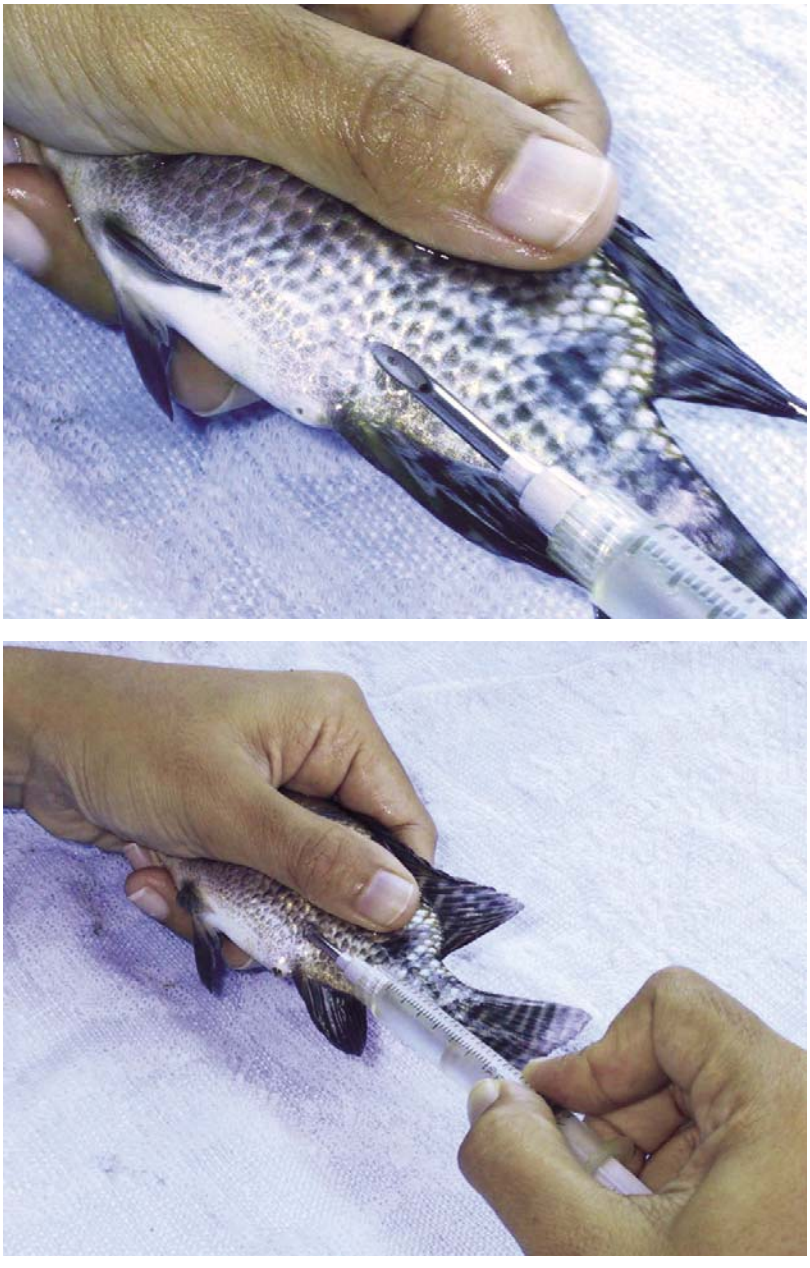


Figure 22. The insertion site is on the left ventral side of the fish, adjacent to the anus, to safely and permanently lodge the tag into the visceral cavity.

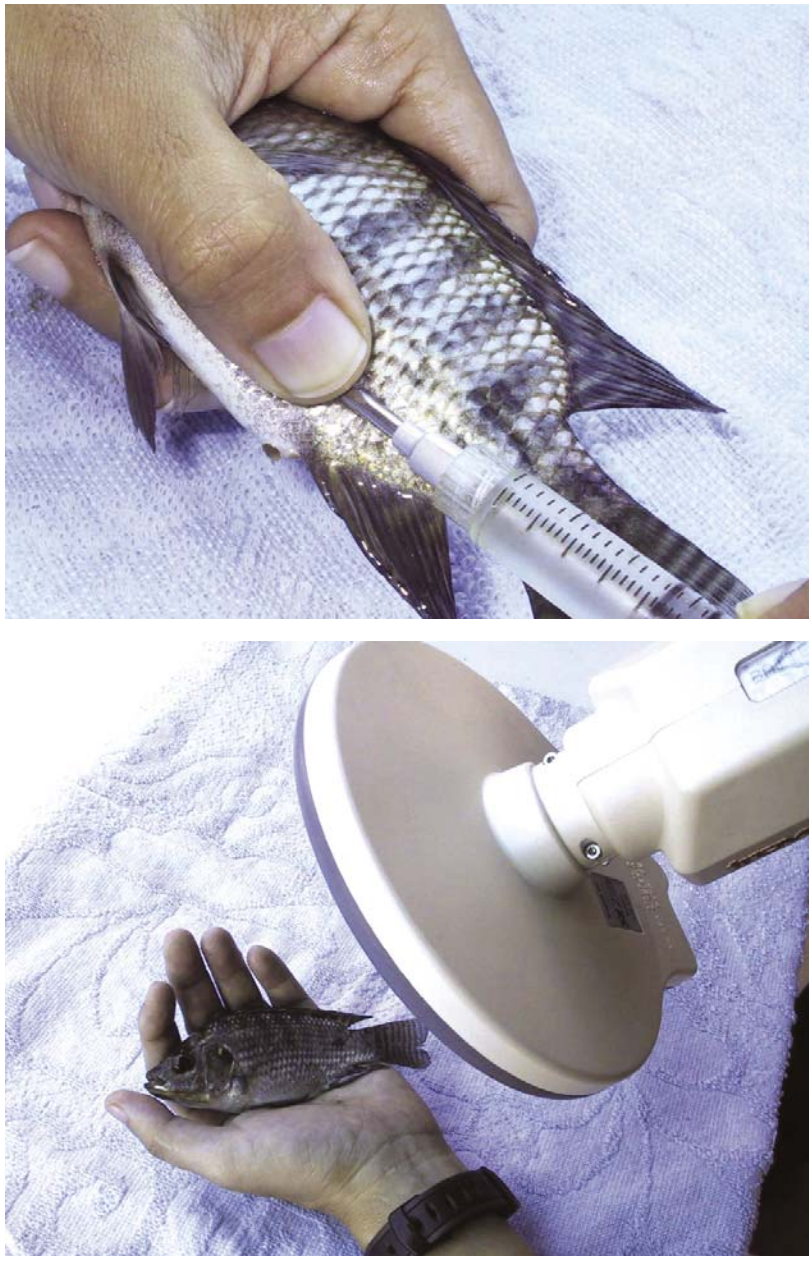


Figure 23. The thumb is placed on the insertion site while the needle is gently withdrawn, and the successful implantation is finally checked using an electronic tag scanner.

6. Selection

The potential breeders will be ranked according to their estimated breeding values based on information of their own performance and the performance of their full-sibs and half-sibs.

6.1. Data analysis

In order to estimate the individual breeding values, the data collected have to be rigorously analyzed. Usually, it will be necessary to 'correct' the grow-out records for major environmental effects, such as environment, batch, and replicate. Because males generally grow faster than females, sex of the fish should also be taken into consideration. The test fish will often be produced over a period of about 60 days, so that an adjustment for age at harvesting will also be desirable.

There are different ways in which the information can be processed, and a variety of models that may be fitted to the data, to a large extent, dictated by the specific structure of the records. For instance, we may decide to pre-adjust the data for environmental effects and use conventional selection index theory to combine the information from the individual and its full and half-sibs. Alternatively, we may use BLUP procedures, which enable the simultaneous adjustment for environmental effects and estimation of breeding values.

Details on the procedures used in the estimation of breeding values and adjustment for environmental effects can be found from several sources. Reddy et al. (1999) provide an excellent account of the use of conventional selection index methodology applied to a selection program for fish. Cameron (1997) and Kinghorn, van der Werf and Ryan (2000) provide introductions to BLUP methodology. Bourdon (1997) provides a comprehensive coverage of animal breeding theory, whereas Ponzoni (1992) gives details on the overall aspects of design of genetic improvement programs.

Researchers that have undergone the necessary training in animal breeding and genetics may feel confident about doing the analysis themselves. By contrast, those that do not feel confident enough or lack the necessary software, may decide to seek help from a genetics-statistics group or person. In any case, the WorldFish Center, through the Breeding and Genetic Enhancement section of the Biodiversity and Genetic Resources Research Program, can provide support to relevant partners in this area.

6.2. A comment on heritability estimates used in the calculation of breeding values

The heritability of a trait may be defined as the proportion of parental superiority that is transmitted to the next generation. It is estimated as the ratio between the additive genetic variance and the phenotypic variance of the trait in question. The heritability is used in the estimation of breeding values for selection purposes.

Typically, in genetic evaluation, the heritability estimates used in the estimation of breeding values do not come from the same data set for which the breeding values are being estimated. Rather, they come from other, generally earlier and comprehensive studies of phenotypic and genetic parameters for the trait(s) in question. In the case of tropical fish we often face the problem of lack of prior estimates of heritability, and some researchers have resorted to estimating heritability from the same data set for which breeding values are being estimated. Researchers following this approach should be cautious because the heritability estimates from single data sets may have large standard errors and be subject to bias due to selection of the parents.

Estimating the heritability from the data set for which we want to estimate breeding values is, nevertheless, useful but the estimates thus obtained should be compared with those reported by other researchers for strains and environments as close as possible to the one in question. Sometimes, a decision based on common sense has to be made when choosing the estimate to be used. The WorldFish Center can provide assistance to researchers facing uncertainties in this area.

When more than one trait is involved, correlations (phenotypic and genetic) are also required for multi-trait estimation of breeding values. The same comments as those made with regard to heritability apply to the choice of correlation values.

6.3. Selection of breeders

Ideally, we would produce the next generation from only the very best individuals in terms of breeding values. In practice, however, we need to balance selection intensity with the effective population size, which is directly related to the accumulation of inbreeding.

To obtain an acceptable genetic gain and to hold the accumulation of inbreeding at a low rate, the effective population size (Ne) must be relatively high. Ne is not necessarily equal to the population census size, and it is calculated as:

$$Ne = 4\ N_f\ N_m\ /\ (N_f + N_m)$$

where N_f and N_m represent the number of females and males used as parents in each generation. For example, if 50 males and 100 females were used, Ne would be equal to approx. 133. Note that when $N_f = N_m$, $Ne = N_f + N_m$. An Ne of 100 is usually considered as a minimum. This population size would contain inbreeding at a rate of less than 1.0 per cent per generation, which would be satisfactory.

With combined and BLUP selection, it is likely that the best animals turn out to be highly related to each other. The selection of individuals that are highly related reduces the effective population size. As an additional precaution to contain the accumulation of inbreeding, it is wise to ensure that no less than 30 to 40 sires and dams of the previous generation are represented in the selected individuals. There are computer programs that, given the estimated breeding values and the pedigree relation among the selection candidates, can help in achieving a balance between genetic gain and

increase in inbreeding. In the interest of the long-term sustainability of the genetic improvement program, this is an area in which researchers responsible for such programs are encouraged to seek advice.

Note that in addition to considerations related to breeding values and pedigree relations, it will usually be necessary to set up matings in such a way that male and female breeders are not too dissimilar in size from each other, to prevent the larger individual from injuring the smaller one.

6.4. Back-up of potential breeders

Individuals with breeding values ranked slightly below but next to those selected as breeders from each set of parents of the previous generation should be kept as back-ups in case of tag losses, mortalities or escapes during breeding. These fish can be kept in B-net hapas during the breeding period and conditioned in the same way as the selected breeders, so that they are ready in case they are needed. It is wise to keep no less than three back-ups of each selected individual.

7. Dissemination

Prior to dissemination, the genetically enhanced Tilapias should be tested on-farm with the commonly used strains of Tilapias to demonstrate the potential benefits of the selected line to farmers. Furthermore, it is important to develop strategies regarding the dissemination of genetically enhanced Tilapias to all fish farmers that the breeding program is planned to service. The dissemination could be organized with a centralized breeding station and, depending on the need for genetically enhanced fingerlings, one or several hatcheries functioning as multipliers.

7.1. Breeding station

At least initially, the genetic improvement program will usually be implemented by a government agency at a breeding station. Under normal circumstances, approximately between 400 and 1 000 progeny can be expected from the mating of each pair of selected parents. This should generate a surplus of fish after the necessary number (e.g. 100 per full-sib group) has been tagged. This surplus can be used in a multiplier role, either at the breeding station itself or at collaborating hatcheries. If the surplus were insufficient, further matings could be set up using the fish chosen as back-ups of the selected parents.

When distributing broodstock to hatcheries, sending males and females from a different sire group is a good idea. In this way inbreeding in the first mating at the hatchery will be minimized. For example, this can be easily achieved by sending males from sire groups 1 to 6, but females from sire groups 7 to 12. As soon as the fingerlings are large enough to be sexed (about 15 grams), they can be distributed to the collaborating hatcheries.

7.2. The multiplier level (Hatcheries)

The collaborating hatcheries should receive fingerlings produced by fish of the latest generation of selection in the breeding program, produced in the above described manner (i.e. surplus stock from the latest generation, or from matings of the back-ups of the selected parents). When demand from hatcheries is high, surplus stock may not be enough, and special purpose matings may have to be carried out to satisfy the requirements. During the mass production at the hatchery level, the breeders can be stocked in large breeding hapas (5 x 1.5 x 1 m^3) at a stocking density of 5 males and 15 females (Figure 24). In this manner, the mass production of high quality fingerlings to be distributed to the tilapia farmers gets underway.

Figure 24. Mass production of Tilapia fingerlings at one of the sites of the Bureau of Fisheries and Aquatics Resources (BFAR), Philippines.

8. Research facilities and equipment

The magnitude of the research facilities and equipment required to initiate and run a selective breeding program for tilapias will depend on the size of the program, namely, how many sires and how many dams are used, and how many progeny are produced and tested in each generation. As earlier stated, to restrict the accumulation of inbreeding in the breeding population, the use of about 50 sires and 100 dams can be recommended. Furthermore, it may be desirable, at least initially, to test fish from all full and half-sib groups in two or more common production environments. In this way we can ascertain whether genotype by environment interaction will or will not be an issue in the selection program.

Estimates of the infrastructure and consumable requirements to initiate and run a selective breeding program as described in this manual are given in Tables 3 and 4.

Infrastructure	**Quantity**
Ponds	
Holding and conditioning (2 000 m^2)	1
Breeding (1 000 m^2)	1
Rearing (2 000 m^2)	1
Grow-out (1 000 m^2)	1
Sewing machine (optional)	1
Water pump	1
Aerator (aquarium size)	5
Counters	2
Weighing balance	1
Computer and printer	1

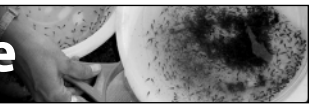
Table 3. Infrastructure requirements needed to initiate a selective breeding program for Tilapias as described in this manual.

Consumables	Quantity	Units[1]
Hapa polyethylene nets		
Breeding and nursery	1 800	m
B-net	1 800	m
Hapa hangers [2]	1 000	pcs
Fingerling tags		
Floy tags	7 000	pcs
Vinyl thread	2 250	m
Needles	5 000	pcs
Scoop nets	10	pcs
Seine nets	2	pcs
Basins	10	pcs
Anesthetics	200	g

[1] Units: m = meter (100 cm); pcs = pieces; g = gram
[2] Stakes made of wood or galvanized iron, to which the hapa nets are affixed

Table 4. The minimum requirement of consumables to initiate and run a selective breeding program for Tilapias as described in this manual for three generations. It is assumed that 100 families of 50 fish each are tested in each generation.

9. Recommended literature



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FORM 1

Appendix