

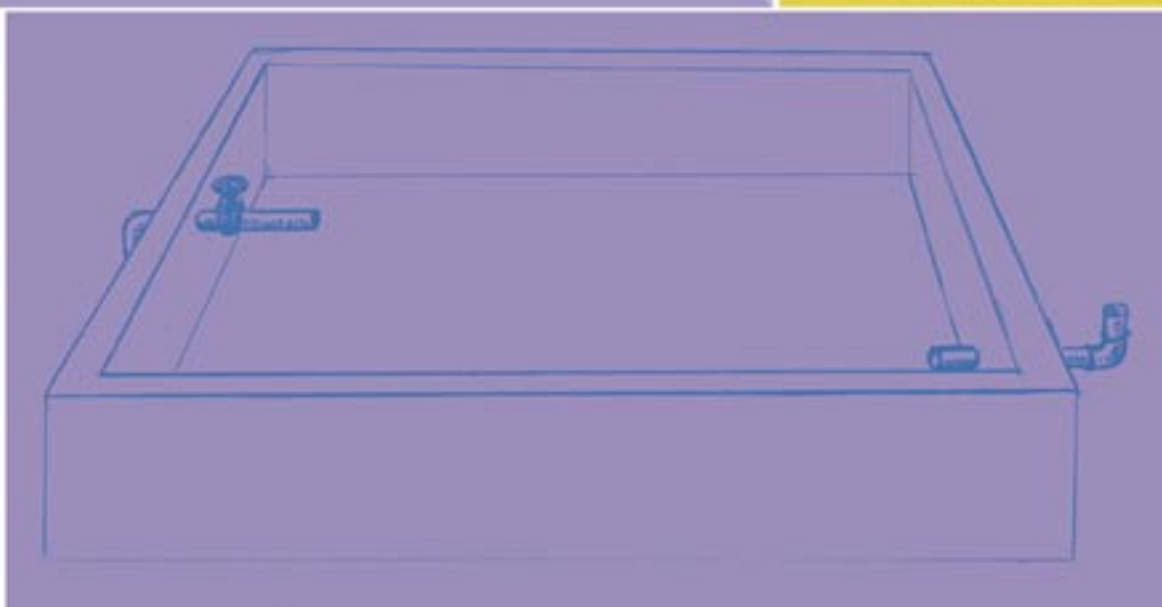
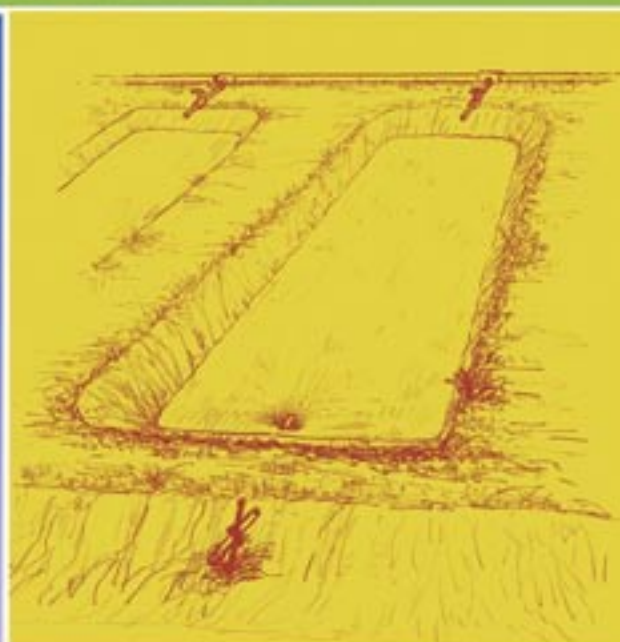
# Tilapia fish farming in Pacific Island countries

Volume: 1

## Tilapia Hatchery Operation

By  
Satya Nandlal

and  
Timothy Pickering





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Secretariat of the Pacific Community**

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## Preface

This book was developed from training workshops run in Fiji Islands from 2002 to 2004 jointly by the Marine Studies Program and the Institute of Marine Resources of the University of the South Pacific (USP) and the Aquaculture Programme of the Secretariat of the Pacific Community (SPC), with funding provided by the Government of Canada under the Canada – South Pacific Oceans Development Programme Phase II (C-SPODP-II). The book's production costs were met by C-SPODP funds granted to USP's Marine Studies Program and by AusAID funds granted to SPC's Aquaculture Programme.

The book is intended for use by fisheries extension officers, staff of rural community development projects, school teachers, or other people with some basic knowledge of biology, to help them impart fish-culture practices to people engaged in tilapia fingerling production. It can also be used by more advanced fish farmers who want to further improve their skills and their self-reliance by producing their own tilapia fry and fingerlings.

SPC and USP have also developed training materials on grow-out of tilapia in ponds. These assume less biological knowledge and have more emphasis on practical techniques, so are more suitable for people who are new to tilapia farming or only want to be involved in pond management.

Although there are many booklets and training materials for tilapia farming already available in the world, they are written mainly for Asian readers. This book is written for Pacific Island countries, and is based upon practical experience of what works in the varied environmental and cultural circumstances of the Pacific Island countries.

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## Introduction

Tilapia fish originated from the Nile Valley and spread to central and western Africa. Artificial introductions of this species in many Asian and some Pacific Island countries started in the 1950s. Tilapia farming is expanding world-wide in both developed and developing countries because this group of fishes can be cultured under very basic conditions and so is ideal for rural subsistence farming, yet is amenable to more sophisticated, market-oriented culture programmes. Tilapia culture requires minimal management and energy inputs. These fish have high reproductive and growth rates, are relatively disease free and hardy in nature.

Some past introductions of tilapia into lakes and rivers of Pacific Island countries have been inappropriate, causing problems in some places through predation on native fishes and competition with other farmed fish like milkfish. Another problem is that the early introductions were of Mozambique tilapia, which, because of its small size at first breeding, is nowadays considered less suitable for farming than the Nile tilapia.

Because of the problems that tilapia might cause, through predation or competition, we urge caution and restraint regarding any further introductions of tilapia into new countries, provinces or water catchments where it has not yet been introduced. However, where Mozambique tilapia is already well established and impossible to eradicate, Nile tilapia could be taken there to turn the presence of tilapia from a “minus” to a “plus” through well managed tilapia farming.

The Pacific Island region is the world’s smallest producer of farmed fish, producing just over 500 metric tonnes. By 2003 there were only about 100 ha of freshwater fish ponds, as well as some fish cages in lakes and reservoirs, throughout the region. However, although still in its infancy, farming of tilapia in the Pacific is on the increase. Commercial tilapia farming is most developed in Fiji Islands and to some extent in Papua New Guinea. Other countries in the region are also interested in this activity.

The production of tilapia fry and fingerlings is done largely with the use of either plastic lined tanks, or earthen ponds, or net enclosures called “hapa” in ponds. All three methods are in use in Pacific Island countries, depending upon local circumstances.

There are about 10 small-scale tilapia hatcheries in the region, mainly in Fiji Islands and Papua New Guinea, with an estimated annual production of 3 million fingerlings. This production is not sufficient to meet the increasing demand for fingerlings. To support our growing tilapia fish farming industries, this region needs more tilapia hatcheries and more people trained to operate the hatcheries.

This book provides practical information on breeding and fry-rearing up to fingerling stage (pond-stocking size), and other information needed for operation of a tilapia hatchery in Pacific Island countries.

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## Tilapia fish farming

Tilapia have good characteristics for farming, and are now so domesticated that they have earned the title “the aquatic chicken”. They are fast-growing, able to survive in poor water conditions, eat a wide range of food types, and breed easily with no need for special hatchery technology.

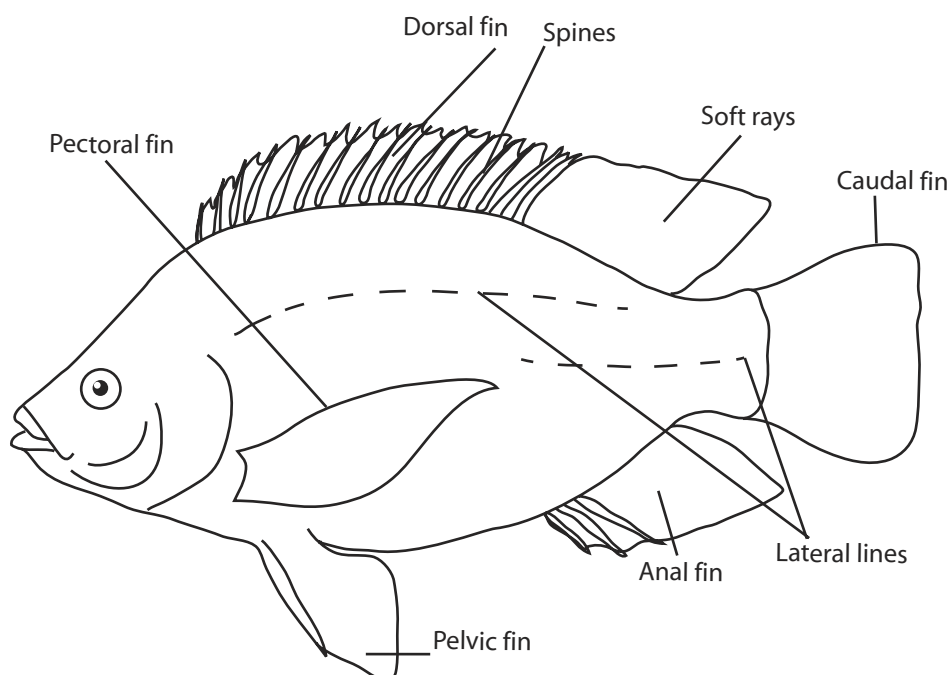
Tilapia are one of the best researched species for aquaculture, and there is a wealth of experience in their husbandry. Tilapia are tough and tolerate a wide range of environmental conditions: little environmental modification is needed, so aquaculture systems can be low-tech. Earthen ponds of appropriate design in non-flood-prone areas will be sufficient for tilapia farming. Concrete tanks or raceways can be used, but are more expensive to build and usually cannot be justified in rural areas. Cages in lakes, dams or rivers can also be used. All these systems are in use in Pacific Island countries, depending upon local circumstances.

Tilapia have some bad characteristics. Uncontrolled breeding in ponds can lead to overcrowding and stunted growth. Tilapia can be a pest that eats other fish species. Once tilapia are present in a pond, they are difficult to get rid of except by poisoning or by draining the place and leaving it to dry until the bottom has baked hard in the sun. They need to live in warm water and do not grow well if the water temperature is lower than 22°C.

The trick to efficient, successful and profitable tilapia farming is to stock with large batches of fingerlings of similar size and age. Only then can all fish in a pond be harvested at the same time, after which the pond must be completely dried out to kill any leftover, unwanted fish. A sign of inefficient farming is ponds filled with fish all of different sizes and ages. It is easy to farm tilapia badly, but to farm them well and produce large batches of similar-sized fish, management skills and a good supply of quality fingerlings are needed.

## Types of tilapia

Tilapia belong to a group of fish called cichlids \* and are native to Africa. Tilapia are shaped like snapper but can be identified by an interrupted lateral line, which is a characteristic of the cichlid family of fishes. They are laterally compressed (flat-sided) and deep-bodied with long dorsal fins. The front portion of the dorsal fin is spiny and the rear is soft rayed. Spines are also found in the pelvic and anal fins. The external anatomy of tilapia is given in Fig. 1.



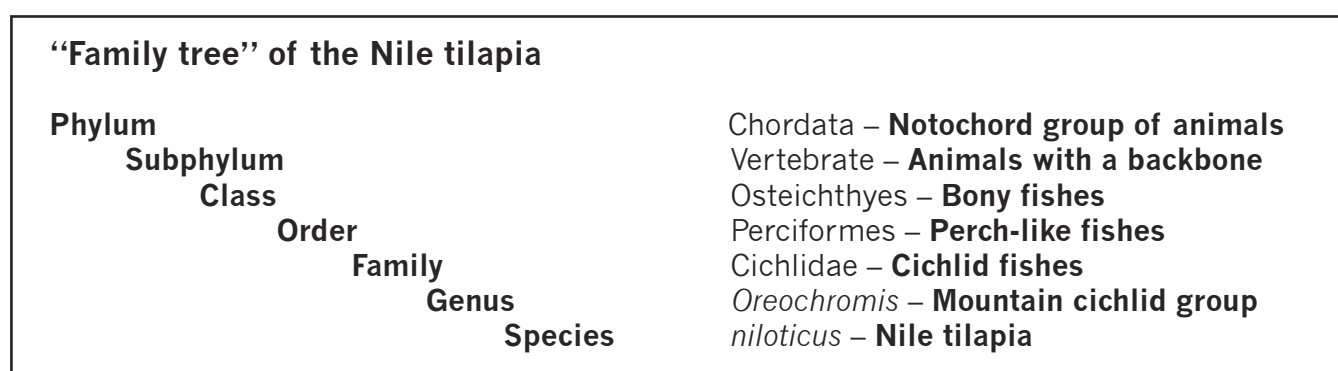
\* Cichlid is pronounced “sik-lid”.

**Fig. 1 External anatomy of tilapia**

The tilapia group consists of three important genera, *Oreochromis*, *Sarotherodon* and *Tilapia*. Several characteristics distinguish these three genera, but the most important one relates to reproductive behaviour.

Tilapia build nests and the fertilized eggs are guarded in the nest by a brood parent. Species of both *Oreochromis* and *Sarotherodon* are mouth brooders: eggs are fertilized in the nest but parents immediately pick up the eggs in their mouths and hold them during egg incubation. They continue to hold the fry in their mouths for several days after hatching. In *Oreochromis* species, only the females practise mouth brooding, while in *Sarotherodon* species either the male or both male and female are mouth brooders.

All commercially important tilapia outside of Asia and Africa belong to the genus *Oreochromis*, of which there are three main species used for farming. These are Nile tilapia\* (*Oreochromis niloticus*), Mozambique tilapia (*Oreochromis mossambicus*) and blue tilapia (*Oreochromis aureus*). The Nile tilapia is the most popular farmed species. Its classification is given in the Box.



These species can be differentiated from each other by examining their body colouration and fins (see Table 1). Nile tilapia have strong vertical bands on the caudal fins whereas Mozambique tilapia have weak or no bands on the caudal fins. Mature male Nile tilapia have grey or pink pigmentation in the throat region, while Mozambique tilapia have a more yellow colouration. However, colouration is often an unreliable method of distinguishing *Oreochromis* tilapia species because environment, state of sexual maturity, and food source greatly influence colour intensity.

**Table 1. Distinguishing features of the three main farmed species**

Species	Body	Fins
<i>O. mossambicus</i>	Dark colour	No bars on caudal fins
<i>O. aureus</i>	Bluish colour and anal fins	Red margins on dorsal, caudal
<i>O. niloticus</i>	Reddish to white	Prominent bars on caudal fins; white colour strips on dorsal and anal fins

\* The scientific names of Nile tilapia have been revised several times in the last 30 years, which can create confusion for fish farmers. The scientific name has been previously given as *Tilapia nilotica*, *Sarotherodon niloticus* and it is currently *Oreochromis niloticus*.

## Male and female tilapia

Male tilapia are usually larger than females of the same age. To tell the difference between male and female fish, compare the illustrations in Fig. 2, which show the differences between the external appearances of the sex organs of a mature fish.

The **male** has two body openings situated just forward of the anal fins, of which one is the anus. The other is the opening of the urethra, at the end of the genital papilla (an oval-shaped lobe just rearward of the anus), from which milt (sperm) and urine are discharged.

The **female** has three body openings, of which one is the anus. The genital papilla of the female has two openings. They are the urethra, which is hardly visible to the naked eye, and the opening of the oviduct (a crescent-shaped slit), from which eggs are released.

These features are more visible and identifiable when the fish have grown to 10–20cm in length and 100–150g in weight. Mature Nile tilapia can also be distinguished by their colouration under the jaw — reddish in males and greyish in females.

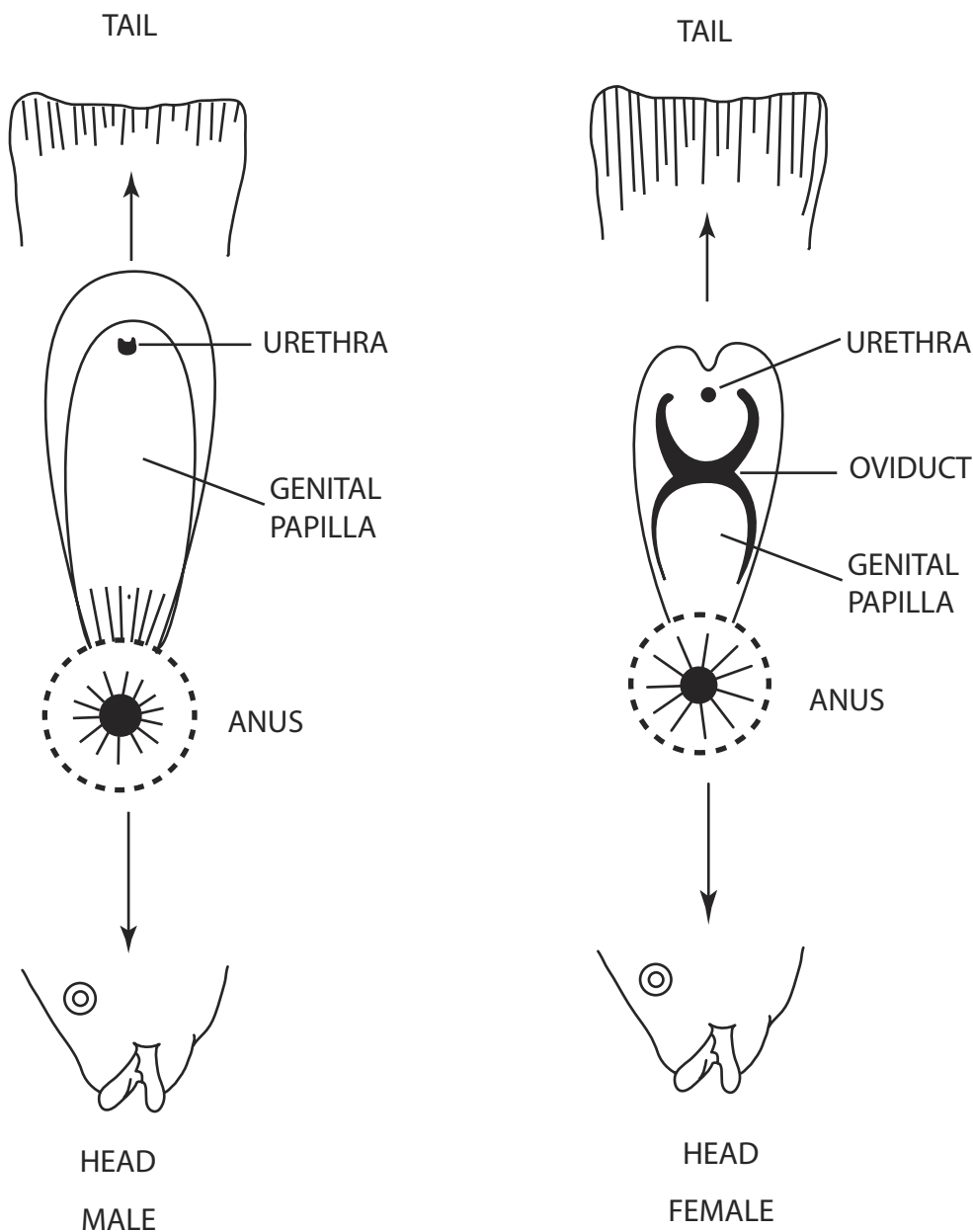


Fig. 2 Male (left) and female (right) sex organs of tilapia



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## Breeding characteristics

*Oreochromis* species usually seek out shallow areas and group together for breeding. Males develop bright colouration and set up territories in which they build their nest: they hollow out a small area on the pond bottom approximately 10–15cm in diameter. They display courtship behaviour and lure females to the nesting site. The nest is used as temporary site for courtship. If the female is receptive to the courting male, she will be induced to spawn.

In the nest, eggs are laid and fertilized by the male, who discharges sperm over the eggs. The female collects the fertilized eggs in her mouth, and mouth-broods the eggs for around 6–10 days. After hatching, the newly hatched fry continue to shelter in her mouth for another 4–7 days. See illustration in Fig. 3. The fry begin to swim freely in schools, but may return to the mouth of the mother when threatened. Females do not feed while they are incubating the eggs or mouth-brooding the newly hatched fry.

Male tilapia can mate with several females (polygyny) and females may mate with several males (polyandry). In ponds, Nile tilapia become sexually mature at three to five months of age (150–200g weight), while Mozambique tilapia mature earlier and at smaller size. The fact that Nile tilapia mature late is why they are a better fish for farming. They have a longer time to feed and grow to a good size before they breed and so come into competition with their own offspring for food, space and oxygen.

As soon as sexual maturity is attained, most female tilapia are able to undergo successive spawnings to produce a new brood every 4 to 6 weeks. Temperature plays an important role here. If water temperature remains at 22°C and above, tilapia will spawn throughout the year. Temperature in the range of 25–30°C is considered ideal.

The number of eggs per clutch increases as the female fish grows. On average each clutch of eggs will produce 100–500 fry. As female fish get older (more than one year old) they produce less fry compared with when they were younger. The best size of female for breeding is 150–300g. On average a 200g breeder would produce 200–500 fry per month. Fry production will also depend on the condition and health of the breeders. In ponds or any confined conditions, egg size and clutch size may vary.

In general fry production decreases as salinity of the water increases. Adult Nile tilapia can tolerate salinity of up to 30 parts per thousand (ppt), while fry and small juveniles are much less tolerant of saline water (they prefer less than 10 ppt). This suggests a potential for brackish water culture from the point of view of reproductive control, to lessen the problem of adults competing for food with their offspring.

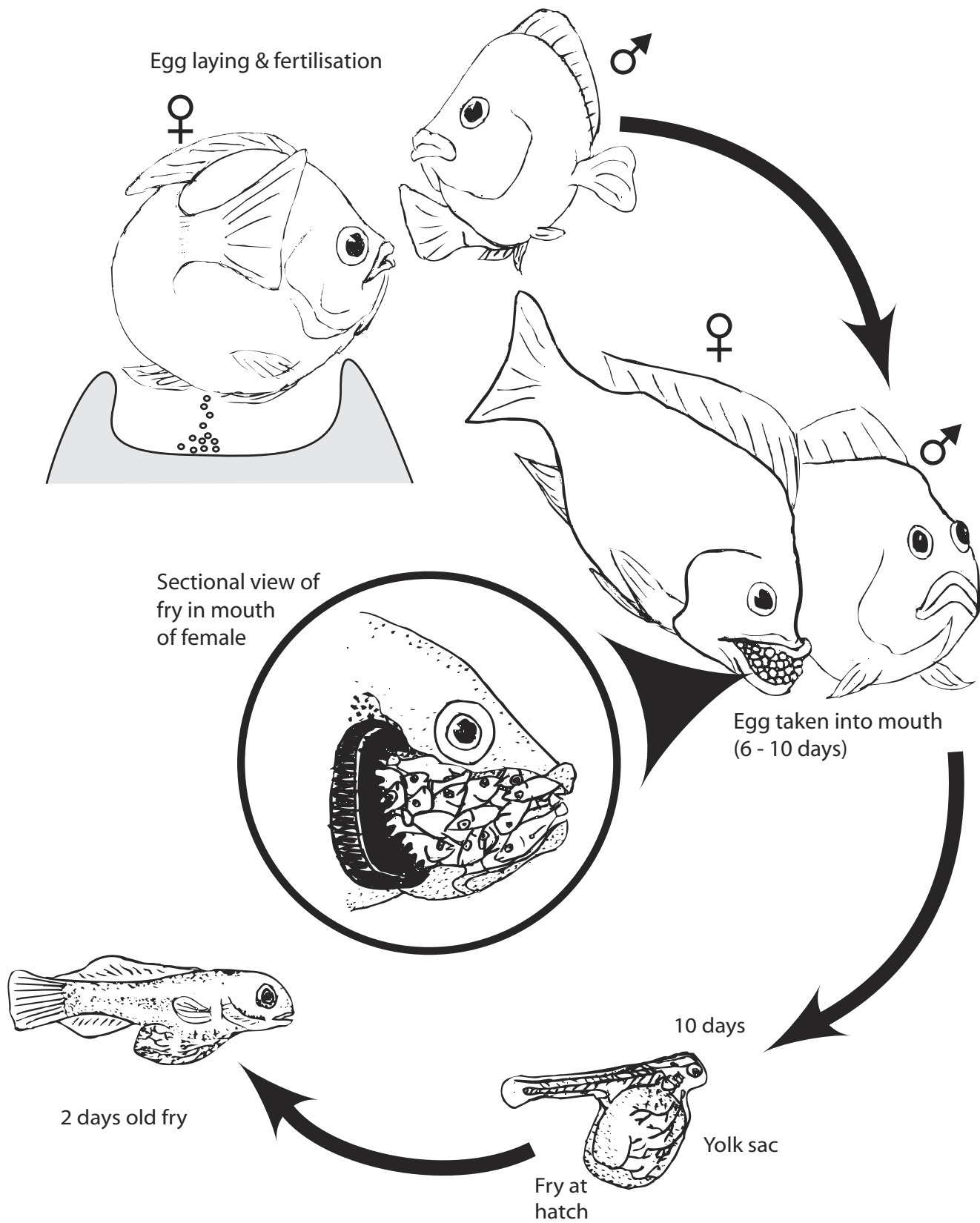


Fig. 3 Diagrammatic sequence showing the breeding of tilapia

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## Tilapia hatchery operation

A “hatchery” is a facility for breeding fish to produce fry and fingerlings for grow-out.

“Fry” are the very young tilapia soon after hatching.

“Fingerlings” are juveniles 1–3g in weight that have completed a nursery phase of culture and are ready to be stocked into grow-out ponds.

“Grow-out” is stocking tilapia fingerlings into ponds and feeding them until they are big enough to be eaten or sold.

The goal of the hatchery is to produce a consistent quantity and quality of fingerlings for various types of tilapia grow-out operations. The fingerlings can be produced in suitably designed ponds, or net enclosures (called “hapa”) in ponds, or tanks (made of concrete, plastic, fibreglass, metal, glass, wood). These different systems require different levels of inputs and management. The choice of system depends on the circumstances of the area and the number of fingerlings that will be required.

### The basic facilities required for tilapia fry and fingerling production are:

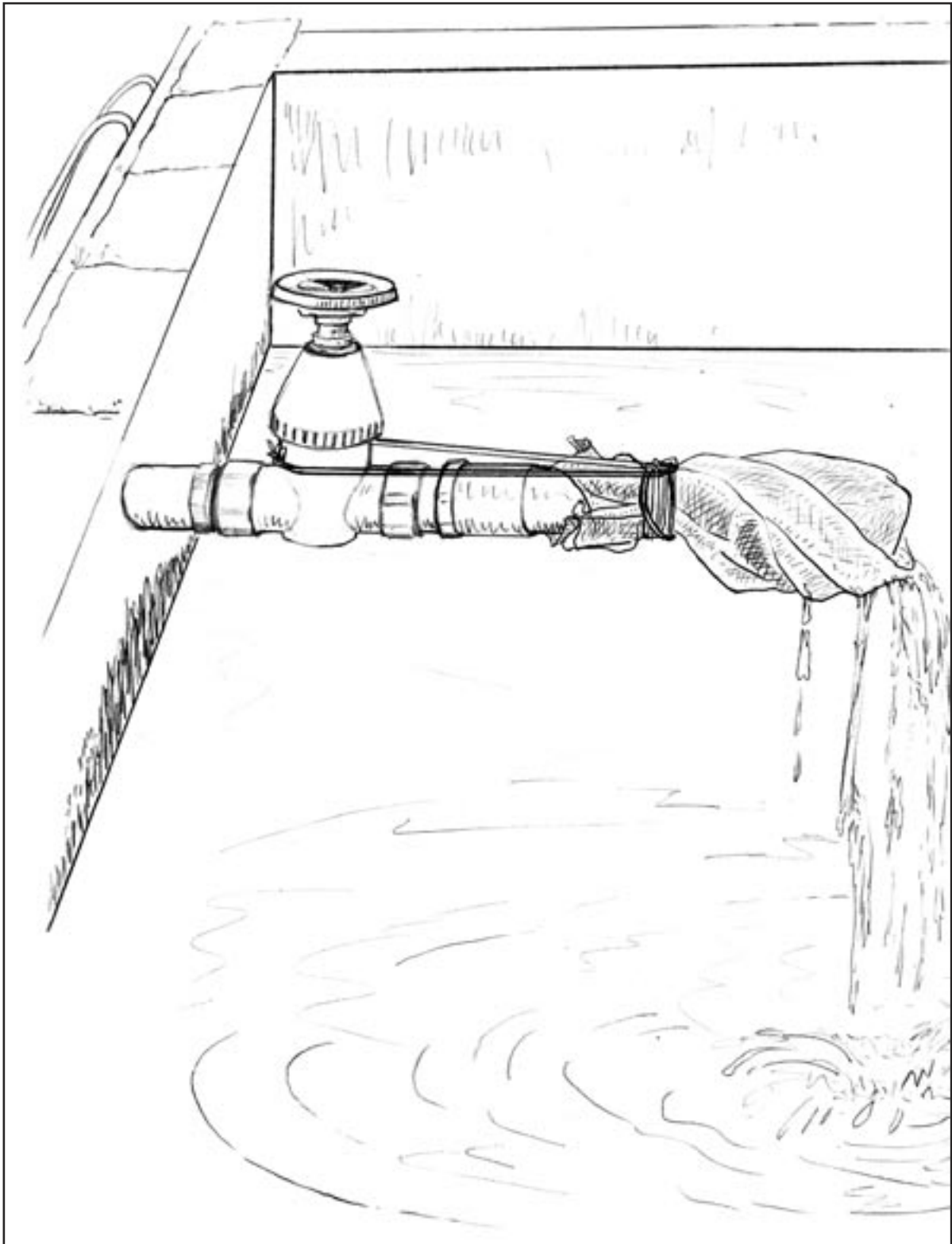
1. Ponds or tanks for holding and rearing an adequate number of broodstock fish
2. Spawning pond, tanks, or hapa
3. Nursery pond, tanks, or hapa
4. Holding tanks with hapa for recovery from handling stress prior to distribution of fingerlings
5. Water supply system
6. Air (aeration) system (where applicable)
7. Basins, buckets, containers
8. Seine nets, scoop nets, grading basket
9. A good scale for weighing fry and fingerlings
10. Accessories for packing of fry and fingerlings
11. Packing and loading area

### Water

Water quality, as well as quantity, is one of the most important factors affecting the success of a hatchery operation. If water quality is good, then good results can be achieved easily. In hatcheries, water quality should be managed to regulate environmental conditions so that they are within the desirable range for survival, growth and reproduction of fish.

Water from river, stream, lake or groundwater can be used in hatcheries. A fine-mesh “sock” screen (Fig. 4) is fitted over the water inlet to prevent introduction of unwanted fish from outside the system. Similarly, a “sock” screen is fitted in the entrance of the outlet pipe to prevent escape of fish from the pond or tank.

It is strongly recommended to filter the water and where possible store it in an overhead reservoir or large tank that allows gravity flow of water to the fish ponds or tanks. It is desirable that water temperature be in the range of 25–32°C and pH be 6.5–9.0.



**Fig. 4** *Mesh sock on inlet pipes will prevent entry of unwanted fish*



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## Rearing of fry and fingerlings

The fry, which have left their mother's mouth a few days after hatching and begun to eat food, are reared for 15–25 days until they become fingerlings (about 1–3g), the usual size supplied for grow-out ponds in Pacific Island countries.

Fry are very delicate and small. Their movement and ability to feed are weak. Their diet is restricted and they have a high metabolic rate. Rearing should be carefully managed to maximize survival rate and produce healthy fingerlings.

Growth rate is very rapid during fry and fingerling stages. During these stages they have different biological characteristics from adults, especially in terms of feeding habits, growth and habitat preferences. The growth rate declines as the fish get older. At the fingerling stage, the growth rate is 5 to 6 times less than at the fry stage.

### Feeding

In the wild, tilapia are generally omnivores, feeding on plankton (phytoplankton, zooplankton), benthic organisms, detritus, small fish, and aquatic plants. In captivity, tilapia readily accept artificial diets such as powder mash, crumbled pellets and pelleted feeds, if sized appropriately to fit into their mouth. This means that the fry and fingerling stages need plankton, mash, or crumble feed, rather than a pellet.

Chicken manure is often an important component of food production for fry and fingerlings. Manure stimulates phytoplankton production, and also bacteria production on the bottom of the pond (detritus). The phytoplankton is food for zooplankton. These processes are interlinked, since phytoplankton is a major source of detritus for bacteria production. Also phytoplankton, through photosynthesis, is the chief producer of dissolved oxygen in the pond, which is used by all organisms including fish.

A combination of rice pollard (50%) and fish meal (50%) is commonly used as fry and fingerling feed. Other examples of feeds include copra meal, wheat bran, wheat pollard, rice bran and meat meal. These feeds can be ground, sieved, and mixed in various combinations giving a crude protein level of 40–50%, depending on availability in the area.

Food requirement is expressed as a percentage of the fish body weight per day: for example, feeding at a rate of 40% of body weight per day for fry and reducing to 20% per day for fingerlings.

#### Worked example of calculating feed required by fry and fingerlings

- (1) Take a sample of about 20 fry (or fingerlings). Weigh them, and calculate their average body weight (ABW) by dividing this weight by 20 (the number of fish in the sample).
- (2) Assume that each mother fish contributes 200 fry. Estimate the total number of fry by multiplying the number of mother fish by 200.
- (3) Calculate the total amount of food needed per day from these figures and the percentage food requirement:

Daily food ration (DFR) = ABW x No. of fry x % food requirement

Example: 5 females contribute 5 x 200 = 1000 fry, of average body weight 1g.

$$\begin{aligned} \text{DFR} &= 1\text{g} \times 1000 \times 40/100 \\ &= 400\text{g per day} \end{aligned}$$

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The retention time of food in the gut of fry and fingerlings is related to water temperature. When water temperature reaches 30–32°C, food is digested in a very short time. This means that the daily food ration should not be given all at once, but divided up into at least four feedings spaced out during the day (for the example above, four feedings of 100g each). Tilapia are not very active during the night, so it is no use feeding them after dark.

## Environmental tolerances of fry

The main factors that influence growth of tilapia under farming conditions are the quality of the fish, the number stocked per unit area, feed quality and quantity, and water quality factors like dissolved oxygen, temperature, salinity, turbidity and pH (acidity).

Tilapias are hardy fish for culture and can be grown in very harsh environments. However, for successful management it is necessary to know their tolerance limits. At-least occasional checks should be made of water temperature, salinity, dissolved oxygen and acidity (pH), to ensure that values remain in the range known to be good for tilapia growth. If you do not have the necessary equipment to carry out these checks, you will need to make a request to your Fisheries Department for assistance, or hire the services of a professional water analytical laboratory.

**Temperature.** Tilapia do not thrive in low water temperatures but are very tolerant to high temperatures. Note, though, that too much handling at high temperature could result in high mortality. Activity and feeding of tilapias are reduced below 20°C.

**Salinity.** Tilapia are much more tolerant of salt water than most other freshwater fishes. Adult Nile tilapia can tolerate salinity of up to 30 ppt if the salinity increases gradually so they have time to get used to it. Fry and small juveniles are happiest at less than 10 ppt and will all die if salinity goes above 14 ppt.

**Dissolved oxygen.** Tilapia can survive extremely adverse conditions. The dissolved oxygen (DO) limit for Nile tilapia has been found to be 0.01 parts per million (ppm). A well known fact when harvesting tilapia from ponds is that any fish left behind can survive for several days in small mud puddles. If the pond is refilled and restocked without killing these fish (for example by drying the pond hard in the sun, or by application of poisons) then they will survive and will be very aggressive during feeding time. Although tilapia can survive very low oxygen conditions, it does not mean they will grow well. The DO level for good growth of fish should ideally be above 2mg/L. Ponds should be managed to maintain a reasonable DO level for good growth of fish.

**Acidity.** Tilapia can survive in wide range of water acidity — pH between 3.5 and 12. However, the recommended pH for good growth of tilapia is 6.5 to 9. Acidic water (with a low pH) will not support the growth of phytoplankton, zooplankton and detritus-digesting bacteria. All these organisms are important as they collectively provide an environment conducive for fish growth.



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## Broodstock management

Obtaining quality broodstock requires sound knowledge on managing and selecting breeders that are unrelated (not close relatives). It is imperative to keep records of the breeders to manage the stocks. These records need to be collected for each generation, and include, for example, numbers, sex, holding facility, growth rate, survival, fecundity and deformities.

The key to good broodstock management is obtaining and maintaining good quality broodstock. The hatchery should obtain parent stocks of known origin, usually from research stations of Fisheries Departments. They should have good performance — that is, good growth rate, lowest feed conversion ratio, disease resistant etc.

Broodstock are a valuable asset, and they need to be protected through management practices that prevent loss of quality. A government hatchery should maintain standard reference strains at all times. The effective population size ( $N_e$ ) of the breeders should be between 400 and 1000.

### To maintain the quality of broodstock, the operator should:

- prevent any deliberate or accidental introduction of inferior tilapia types (especially Mozambique tilapia) into the breeding system
- eliminate from the breeding system any fish that have questionable characteristics, for example slow growth, deformities such as curved backbones, signs of disease or parasites, blindness, or unusually large and hardened belly
- ensure parentage is known; for example, introduce breeders from known sources.
- ensure no fish remain in the pond from the previous breeding cycle before starting the next cycle
- keep stock lines in secure and separate holding facilities
- reduce inbreeding (breeding between closely related fish) by maintaining a large population of breeders and ensuring that a large proportion of them get a chance to breed
- replace broodstock fish every 18–24 months, if possible
- when replacing aging broodstock, choose offspring originating from as many parents as possible for breeding
- maintain a systematic record of stocks

The following is a list of the requirements to successfully manage broodstock. If a broodstock provider lacks any of these requirements, it will be difficult to manage stocks properly.

- The hatchery operator and the assistant staff need to be well trained in overall hatchery operation including genetic aspects of broodstock management
- There need to be enough ponds, tanks, and hapas (B-net) to hold broodstock fish of different varieties, ages and sexes and keep them separate so there is no mixing and uncontrolled breeding between them
- The hatchery facilities need to be safe from flooding, theft, or any other disturbances that can result in broodstock fish getting lost or mixed up together or mixed with wild stocks
- If a pond is used, it must be drained so that leftover stock in the pond can be destroyed before introducing new stock, to prevent mixing of stocks
- The breeding pond should be away from main roads, free from any noise. Noise affects the brooding female and eggs may be expelled from her mouth

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## **“Inbreeding” is the mating of relatives.**

The subject of inbreeding often comes up when broodstock management is explained to fish farmers. Tave (1999) states that:

Inbreeding, along with selection and crossbreeding (hybridization), is one of the three major breeding programmes that have been traditionally used to improve livestock and plants. While inbreeding is an important breeding programme that can be used to improve a population when it is planned and directed, unplanned and uncontrolled inbreeding can ruin a population through something called “inbreeding depression,” which is a decrease in growth and viability coupled with an increase in abnormalities.

You will know whether inbreeding is leading to “inbreeding depression” among your farmed fish if, after a few generations, you start to see the following characteristics:

- wider variation in sizes of fish among a batch
- higher occurrence of deformed or abnormal fish
- production performance not up to expected levels

The main strategy for the hatchery operator to reduce inbreeding is to maintain a large population of broodstock fish, and ensure that a large proportion of them get a chance to breed and contribute to the next generation. If possible, more broodstock should be used than just the minimum necessary to meet fingerling production targets, so that as many broodstock as possible will get a chance to breed.

At least a few fry from all of these broodstock should be retained and grown out for use as the next generation of broodstock, before the previous generation gets too old and is discarded. This requires a lot of small ponds, tanks or hapa, and good record keeping to know identity of parent fish. This activity is usually carried out by a hatchery specialist, normally at the Department of Fisheries in your country.

## **Rearing and conditioning of broodstock**

*“Conditioning” is a short period of rest and recuperation.*

Fingerlings intended for use as broodstock should be grown out at low stock density (1–2 fish per m<sup>2</sup>) in ponds. They take 4–6 months to reach breeding size of 150–300g. Fish kept for broodstock should not be used for more than 1.5–2 years.

Ponds can be fertilized to encourage natural food production (plankton). The fertilizer may be, for example, chicken manure applied at 300kg per ha per month or an inorganic fertilizer like ammonium phosphate at 150kg per ha per month.

Supplementary feed (for example, a commercial pellet feed or a grower mash with around 30% crude protein content) should be given in the morning and afternoon at a daily ration equal to 3–5% of total fish body weight until they reach breeding size.

Female breeders should be placed separately from male fish. Both males and females are conditioned for spawning for at least 10–12 days, feeding a lesser amount of supplementary feed (2–3% of body weight daily). The female fish should be examined, and only those in “ready to spawn” breeding condition should be introduced to male fish for spawning. There are five categories of breeding condition: ready to spawn (RS), swollen (S), not ready to spawn (NRS), had spawned (HS) and immature. A detailed description of how to recognize each category of breeding condition is provided in Table 2.



Choose males of similar size to females. If males are significantly larger (30–40% of female size), clip the upper lip (see section on mouth-clipping).

The reason for (1) holding female fish separately from males, (2) conditioning them by proper feeding, and (3) choosing only those most ready to spawn for introduction to males is to achieve synchronicity of spawning. “Synchronicity of spawning” means large numbers of fish spawning at about the same time. These three steps are the secret to producing large batches of similar-sized fingerlings for grow-out.

**Table 2. Description of breeding-condition categories for female tilapia fish**

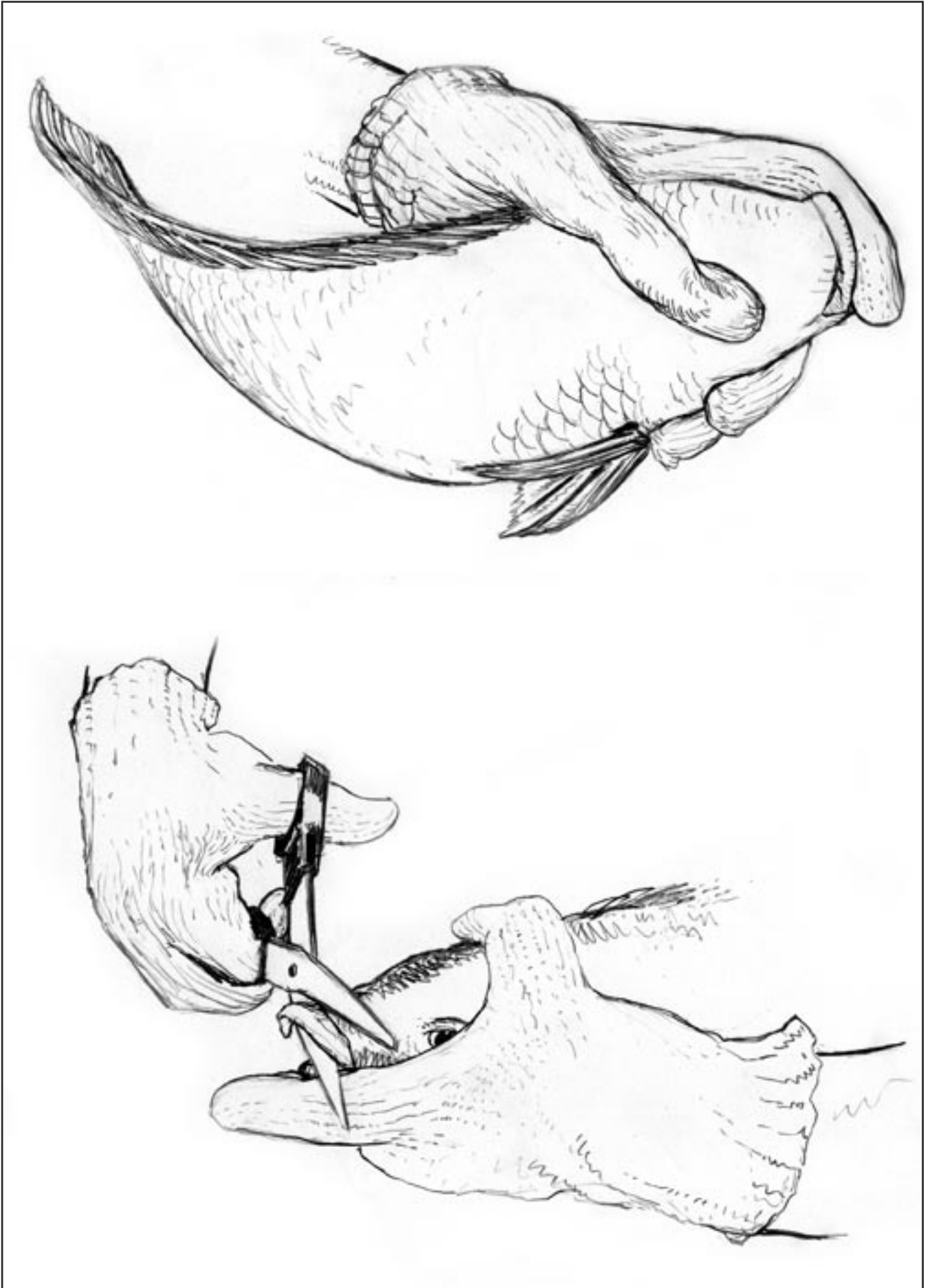
Category	Days to spawning	Description	Timing of fry collection (days)
Ready to spawn (RS)	3 to 7	Pink to red and protruding genital papilla Fully opened genital pore Distended abdomen	10 – 14
Swollen (S)	5 to 10	Pink to yellow genital papilla Slightly opened genital pore Slightly distended abdomen	12 – 16
Not ready to spawn (NRS)	21 to 30	White to clear and flat genital papilla normal to swollen abdomen	Further conditioning
Had spawned (HS)	15 to 30	Red genital papilla Shrunken or compressed abdomen	Further conditioning
Immature	Unpredictable	Papilla very small No sign of maturity	Further rearing

## Mouth-clipping of males

Male tilapia can be aggressive in their mating behaviour, and can pursue and nip at female fish. In a pond environment stocked at low density the female fish are able to escape this type of harassment, but in the confined space of hapa nets or tanks they become exhausted or suffer scale damage that forms sores, leading to their death.

For hapa or tank hatchery methods, mouth-clipping of male fish with sharp scissors to remove the pre-maxilla bone of the upper lip (Fig. 5) is one method to reduce their ability to inflict damage. The wound can be disinfected (for example, with a 10% solution of Betadine) to avoid infection.

Another strategy to reduce harassment of female fish is to mate female fish with similar-sized males, in particular avoiding use of males that are very much larger than the females.



**Fig. 5** *Mouth-clipping of male fish. Hold the mouth open with one finger to move the upper lip out from the fish's head*

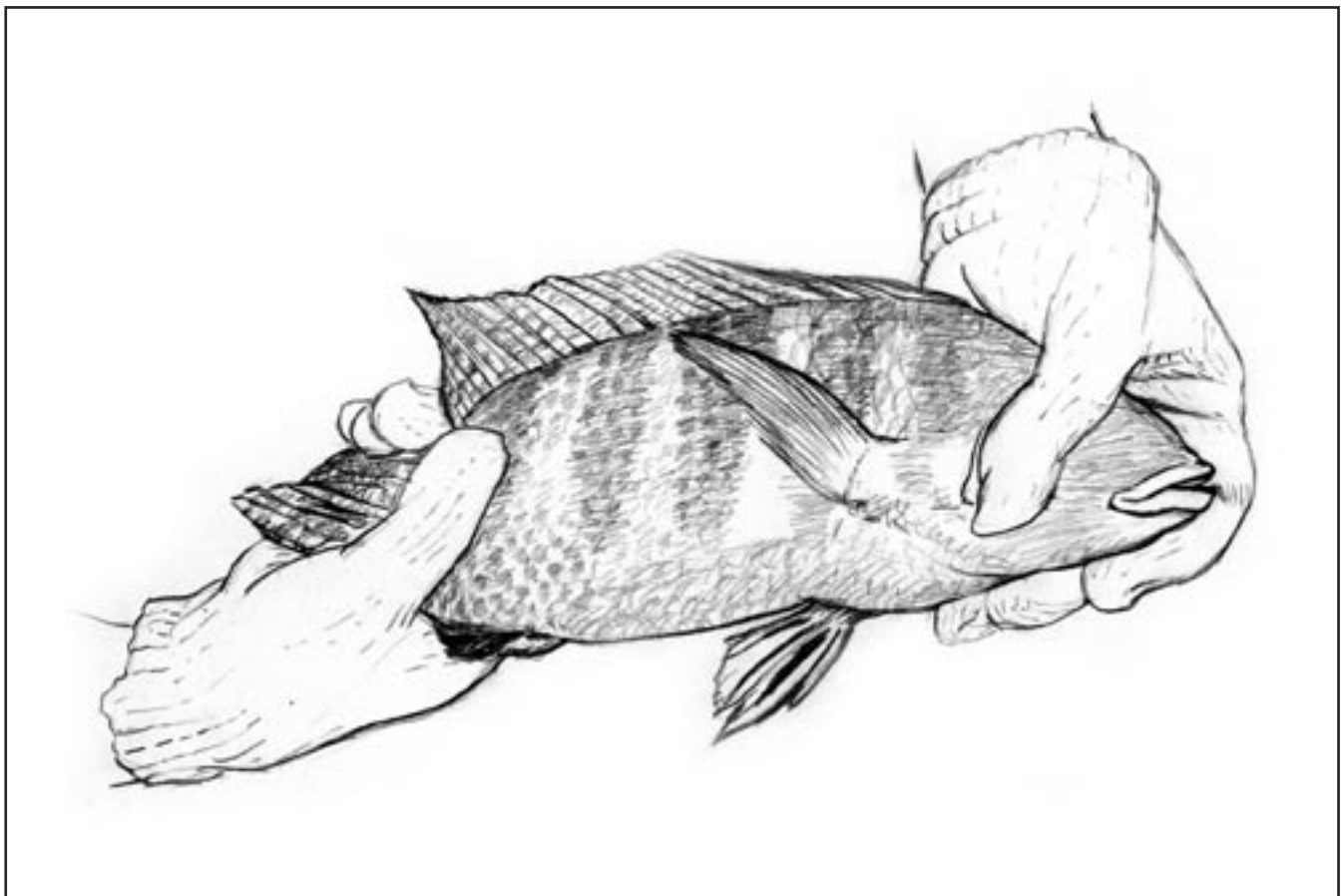
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## Fish handling

*“Fish handling” is taking your fish out of water and carrying them about, for example when transferring them from one tank or pond to another.*

It is important to be gentle with your fish and keep the amount of handling to a minimum, to avoid injury and stress that can lead to disease or death. The following guidelines will help keep your fish in good condition.

- Handle the fish in the cool of morning or afternoon or under shade.
- If holding them crowded in containers for a time, make sure water is clean, and has air bubbling through or running water flowing through the container.
- Fry are delicate and squash easily. Keep them in the water so they can swim. Avoid leaving them piled up together in a net or at the bottom of a container with no water to swim.
- Fish in small containers should not be too overcrowded (no more than 200 fry per litre of water).
- If you see fish in a small container all crowding at the surface to gulp air (piping), then either provide vigorous air bubbles through the water or transfer some of the fish to another container.
- Scoop nets should be of soft material that will not bruise or hurt the fry. Nets should be fine-mesh, as coarse-mesh nets will catch the fish by the gills and cause injury.
- Avoid dropping fish on the ground, or making them panic so they start flapping around.
- When holding or carrying a broodstock fish in your hands, wear gloves and cover the eyes with one hand so that the fish will remain calmer (see Fig. 6).



**Fig. 6** *When handling broodstock, wear cotton gloves to obtain better grip and cover the eyes so fish remain calm*

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## Open pond method of breeding

In the open pond method, ponds (Fig. 7), usually 100–1000m<sup>2</sup> in size with a depth of 60–100cm of water, are used for both the spawning and the fry and fingerling rearing steps. In summary, fish are placed in the breeding pond when they are ready to spawn (RS) or swollen (S), allowed to breed for 25–45 days, and the fry or fingerlings are harvested. The breeders are then removed to another pond and rested while the breeding pond is completely drained, dried out to kill any remaining unwanted fish, and set up again.

This is the simplest and least expensive breeding method and requires the least amount of equipment. However, during harvest the fry get stressed, which can result in low survival. Some fry and fingerlings get stranded on the pond bottom as water is drained out, where they are difficult to collect without damage.



**Fig. 7** A breeding pond. Attract fingerlings by adding some feed just before you harvest, to make them easier to net



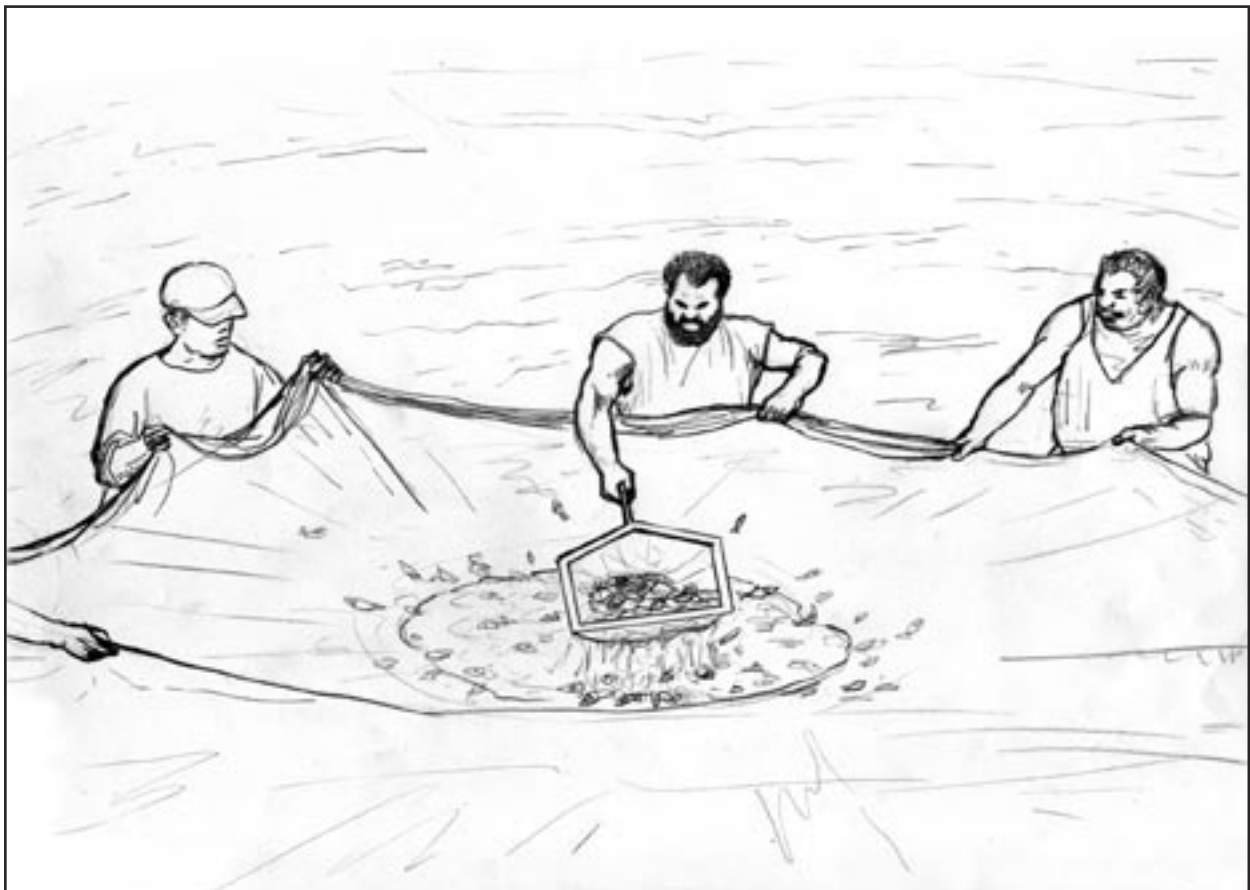
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## Steps involved in the open pond method of fingerling production

1. Completely drain the breeding pond and let it dry out for at least two weeks.
2. While the pond soil is wet, spread lime uniformly over the pond bottom at 100–300g per m<sup>2</sup>, to kill any remaining fish as well as to moderate the acidity of the soil.
3. Spread chicken manure over the pond bottom at approximately 2000kg per ha to stimulate a plankton bloom (make the water turn green).
4. Fill the pond with water to about 60–100cm depth, ensuring that the inlet and outlet are screened properly (see Fig. 4). Place chicken manure in “teabags” (hanging sacks, see Fig. 8) in the pond to continue fertilizing the pond. Use about 1000–2000kg per ha per month.
5. Meanwhile, condition male and female broodstock in separate ponds, tanks or hapa for at least 10–12 days.
6. Transfer ready to spawn (RS) or swollen (S) females (see Table 2) along with active-ripe male fish to the breeding pond, calculating the number for the correct stocking density as shown below.
7. Feed the broodstock with a supplementary ration in the morning and afternoon at 3–5% of body weight daily.
8. Collect fry using seine net, starting 10–14 days after stocking, and transfer them gently in water-filled containers to the nursery pond, hapas or tanks.
9. Collect fry at least every 2 days to reduce losses to predation by broodstock, and to ensure collected fry are of similar size.
10. At the 21st day, remove the broodstock to the conditioning pond or tank for conditioning again.
11. Drain the breeding pond again and dry it ready to begin the next cycle of breeding.
12. Feed the fry in the nursery pond with a powdered mash at 40% of body weight daily until they reach fingerling size. Divide the daily food ration into 3–4 feedings a day. Feed can consist of 50% rice pollard and 50% fish meal.
13. Alternatively to using a nursery pond, you can wait longer until fry reach fingerling size before starting to collect fingerlings. Fingerling collection using seine net can start from about the 25th day until the 45th day (Fig. 9), when broodstock are removed and the pond is drained.
14. The fingerlings will range in sizes and ages, so will need to be size-graded through plastic mesh grader baskets of different mesh sizes (see Fig. 10). Fingerlings can be transferred directly to grow-out ponds.



**Fig. 8** *Fertilizer tied in a sack will give a steady slow release of nutrients into the water to maintain a plankton bloom*



**Fig. 9** *Seine net being used to harvest fingerlings from a breeding pond*



**Fig. 10** *A size-grader mesh basket can be used to gently separate fingerlings of different sizes. To avoid injury, fish should be graded while swimming in water in a basin*

## Worked examples of calculating stocking density in breeding ponds

**Set method** — 1 set is 3 female and 1 male fish. Stock 1 set per 5–7m<sup>2</sup> of pond surface area.

*Example:* A 150m<sup>2</sup> pond will be stocked with 1 set per 7m<sup>2</sup>.

$$\begin{aligned} \frac{150\text{m}^2}{7\text{m}^2} &= \text{approx. 20 sets} \\ &= 80 \text{ fish} \\ &= 60 \text{ females and 20 males at 3:1 sex ratio} \end{aligned}$$

**Number per unit area method** — Stock 1 fish for every 2 – 4m<sup>2</sup> of pond surface area, at a female: male sex ratio of 3:1.

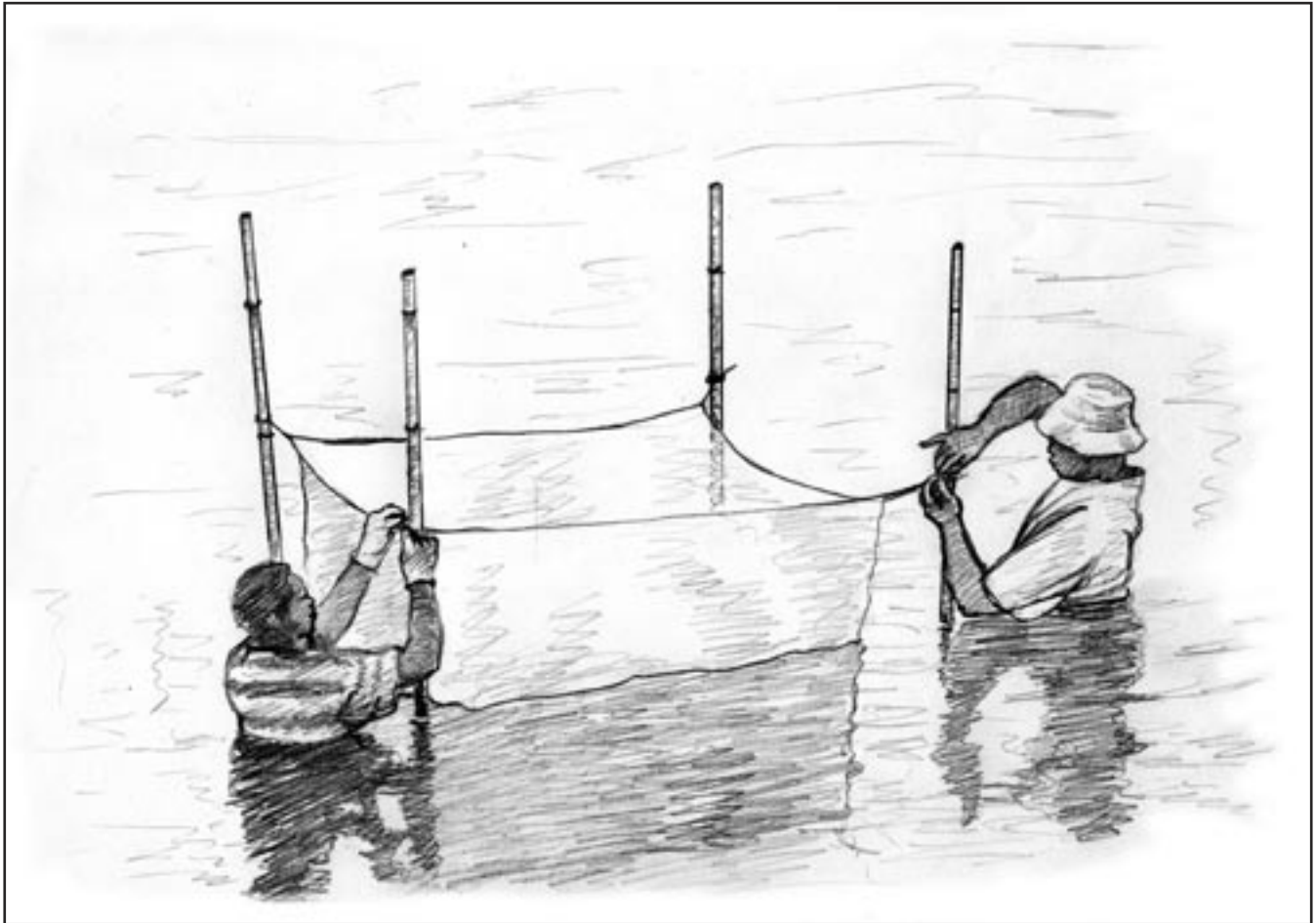
*Example:* A 150m<sup>2</sup> pond will be stocked with 1 breeder per 2m<sup>2</sup> at a 3:1 sex ratio.

$$\begin{aligned} \frac{150\text{m}^2}{2\text{m}^2} &= \text{approx. 75 fish} \\ &= 57 \text{ females and 18 males at 3:1 sex ratio} \end{aligned}$$

## Hapa-based pond method of breeding

A hapa is a fine mesh cage set up in a pond (it looks like a mosquito net turned upside down). The four corners at the top side and four corners at the bottom side of the hapa are tied to bamboo poles or wooden poles (Fig. 11).

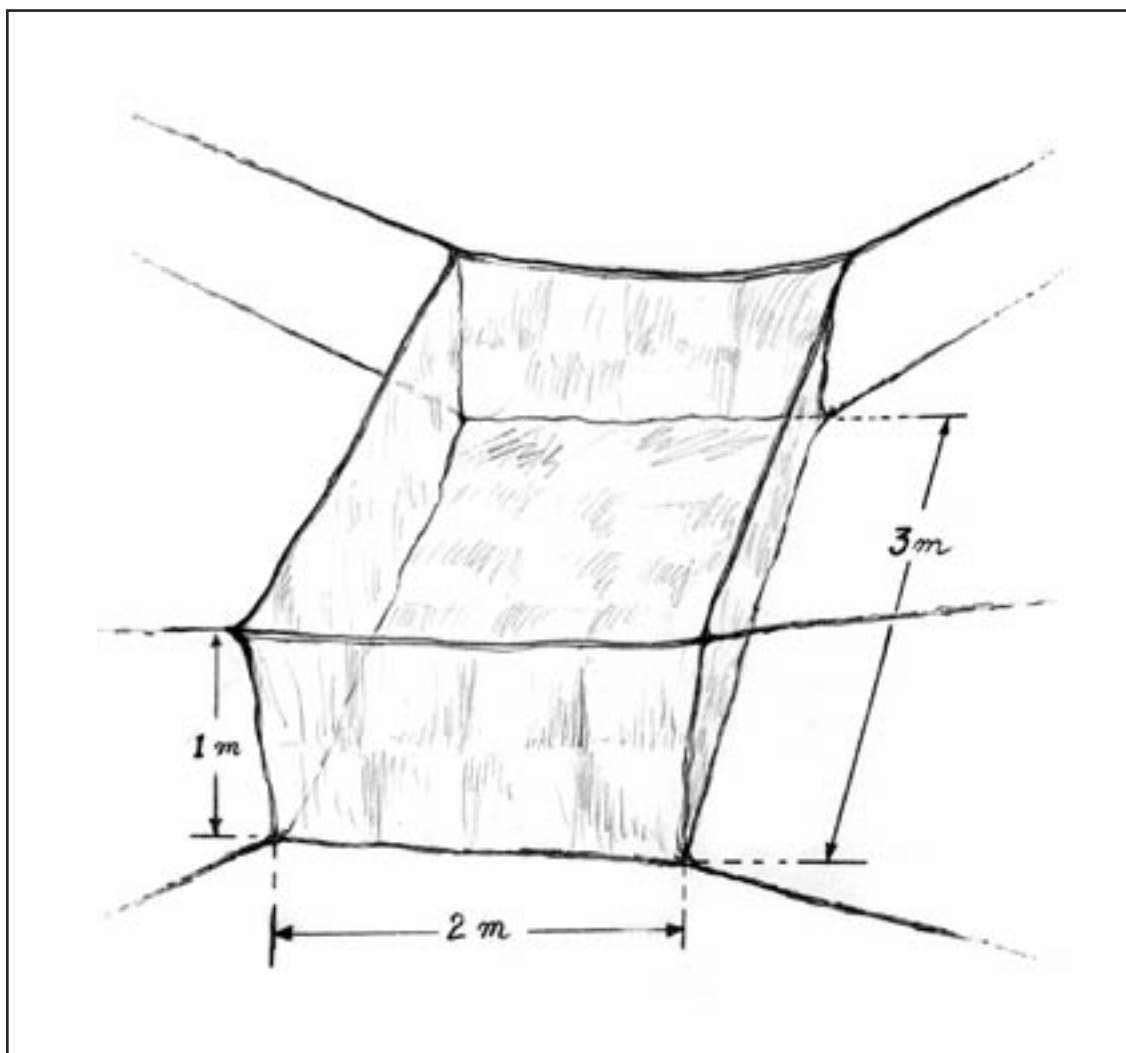
Hapa can be made of many different materials but we recommend hapa made of fine-mesh netting with mesh size similar to mosquito screen. The joints are sewn with nylon thread and machine-stitched to prevent the seams from splitting. The materials can be ordered from fish farming equipment suppliers in Thailand or Taiwan, and the hapa manufactured locally. Alternatively, ready-made hapas can be ordered from these countries through your local Fisheries Department.



**Fig. 11 Hapa net set up in a pond**

The hapa size, design and mesh-size depend on its purpose. For fry and fingerling production, 1mm mesh is used. For conditioning and holding of broodstock fish, a hapa with bigger mesh size (B-net) is used.

A hapa measuring 3m x 3m x 1.5m is a suitable size for fry and fingerling production. Alternatively, hapas can be made to suit the circumstances, for example 3m x 2m x 1m (as shown in Fig. 12). The sides of the hapa, when installed, should extend about 40cm above the water surface, to prevent fish jumping out.



**Fig. 12 Typical hapa net dimensions**

The hapa method of fingerling production allows higher output per unit area than the pond method, and produces more consistent and uniform size of fingerlings. The pond does not have to be drained and prepared repeatedly for stocking between each breeding cycle.

The hapa method is the most efficient and economical of the three methods shown in this manual. It does, however, require more management effort. Adequate water exchange is needed, and use of supplementary feed is essential. Hapa are affected by strong winds or storms, and the hapa advertise to thieves exactly where to come and steal fish from.

**Steps involved in the hapa method of fingerling production are as follows:**

1. Prepare the pond for breeding. Drain the pond. Spread lime uniformly over the pond bottom at 100–300g per m<sup>2</sup> (depending on soil acidity). Ensure that no predators are present.
2. Spread chicken manure over the pond bottom at 1000–2000kg per ha to stimulate plankton growth.
3. Fill the pond with water to a depth of 100–120cm, ensuring that the inlet and outlet are screened properly (see Fig. 4). Place chicken manure in “teabags” (hanging sacks, see Fig. 8) in the pond to continue fertilizing the pond. Use about 2000kg per ha per month.
4. Before setting up the hapa, check to make sure it is not damaged. Install the hapa in your pond by tying the top and bottom corners onto bamboo or wooden poles staked into the mud. The poles should be positioned so that when the hapa is installed each panel is stretched out.

5. Set up the hapa in rows, if more than one is being used, with space in between each one to allow water movement between them.
6. Condition male and female breeders in separate hapas (B-net) for at least 10–12 days.
7. Whenever the mesh of the hapa becomes clogged with algae, scrub the outside with a scrubbing brush.
8. Transfer ready to spawn (RS) females (see Table 2) and similar sized active-ripe males into the breeding hapa, at a stocking density of 4–5 broodstock per m<sup>2</sup> in a sex ratio anywhere from 3:1 to 7:1 (female:male).
9. Feed the fish in the breeding hapa twice daily with supplementary feed at 3–5% of body weight daily.
10. Collect fry with fine-mesh scoop nets (Fig. 13), starting 10–14 days after stocking the breeding hapa, place them gently into water-filled containers, count them and then transfer them to the nursery hapa, pond or tank.
11. Feed the fry in the nursery pond with a powdered mash feed at 40% of body weight daily, reducing to 10–20% per day when they reach fingerling size. Divide the daily food ration into 3–4 feedings a day.
12. After fry are collected the breeders are transferred into separate B-net hapas for conditioning again.



**Fig. 13** *Fine-mesh scoop net for handling fry and fingerlings*

### Worked example for producing 8000 fingerlings using the hapa method

Your target is to produce 8000 fingerlings for a grow-out pond. How many hapa and broodstock, will you need?

One hapa is  $3\text{m} \times 3\text{m} = 9\text{m}^2$ . At 4 breeders per  $\text{m}^2$ , this is 36 fish per hapa. At a 3:1 sex ratio, this will be 27 females and 9 males. Each female will produce a minimum of 200 fry, so each hapa can yield  $200 \times 27 = 5400$  fry.

To produce 8000 fry, you will need two hapa, 54 female fish, and 18 male fish.

### Tank method of breeding

Tilapia fry production in various types of tanks (Fig. 14) has been practised in several Pacific Island countries, where there is not much space available for ponds or where soils are too sandy for pond construction.

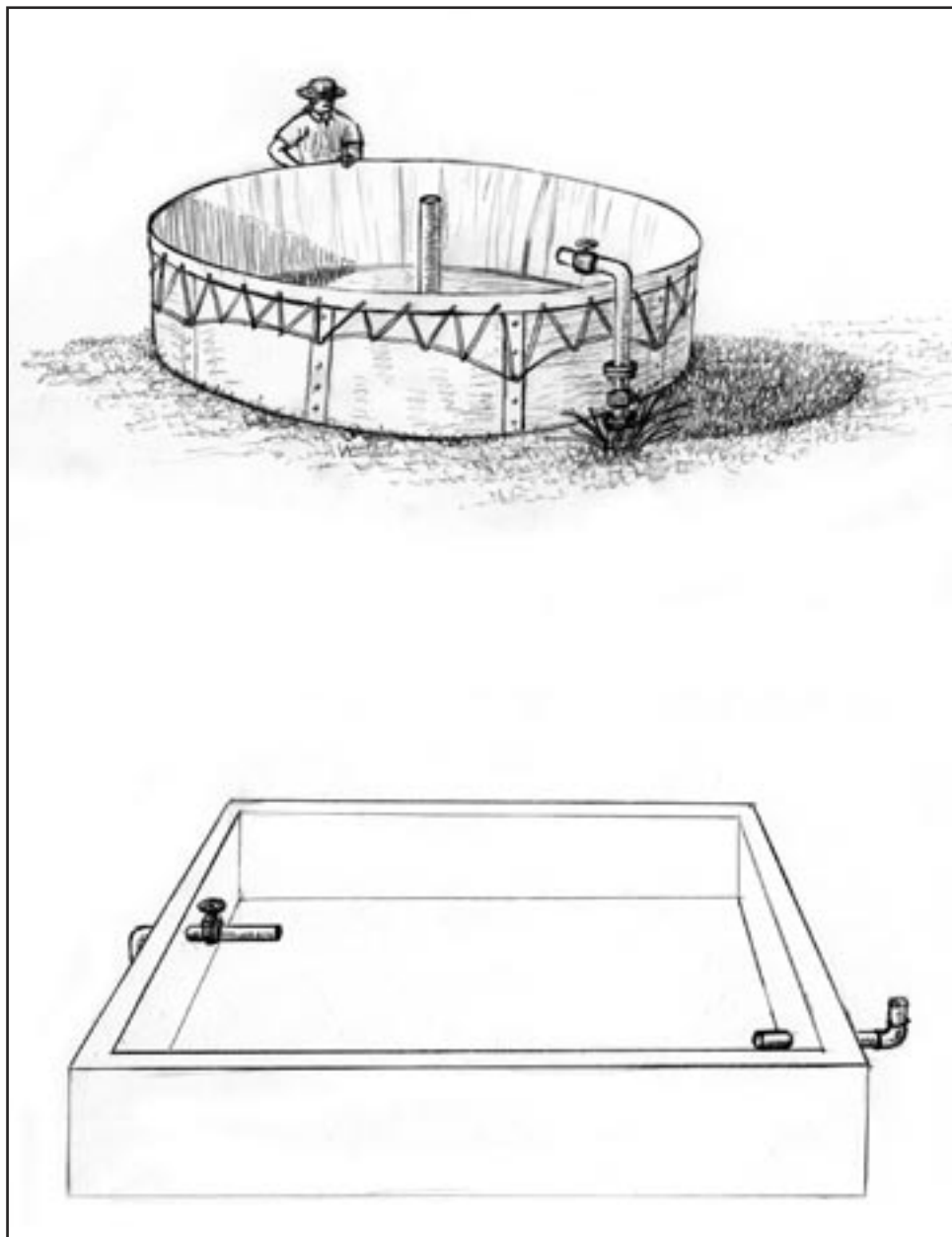


Fig. 14 Two types of tanks suitable for fry production: knock-down tank with plastic liner (above), and cement tank (below)



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In the tank method, the number of fish to be stocked will be limited by the volume of water in the tank. Moreover, to maintain the oxygen level in the tank, either water has to be flowed constantly through the tank or aerated (air bubbled through the water using blowers or compressors). This makes the tank method more expensive to operate, compared with the other two methods.

### **Steps involved in the tank method of fingerling production are as follows.**

1. Sufficient tanks should be available for males and females to be held separately for conditioning before spawning. Tank water depth can be in the range of 50–80cm.
2. Condition the fish for at least 10–12 days.
3. Transfer RS female fish of 150–300g size to the breeding tank along with similar sized males at a stocking density of 7–14 breeders per m<sup>2</sup> at a female to male sex ratio in the range from 3:1 to 7:1.
4. Collect fry using a fine-mesh scoop-net, starting 10–14 days after stocking, and transfer them gently in water-filled containers to the nursery tank or pond or hapa.
5. Collect all fry after 21–25 days, and transfer the breeders into separate conditioning tanks again. Alternatively, remove the breeders and leave the fry in the breeding tank till fingerling size.
6. Feed the fry in the nursery tank with powdered mash feed at 40% of body weight daily at first and reducing to 10–20% at fingerling size. Divide the daily food ration (calculated as described above) into 3–4 feedings a day.
7. Alternatively, when sufficient fry are present the broodstock can be removed and the breeding tank can then become the nursery tank. Do not wait too long before removing broodstock, or cannibalism by adult fish will reduce the number of fry.

#### **Worked example for producing 50,000 fingerlings using the tank method**

Your target is to produce 50,000 fingerlings using the tank method. You have 5 circular tanks with surface area of 25m<sup>2</sup> and sides 1.2m high, with air-bubblers in each. You have 167 fish available of size 200–400g. You have found that 92 are female and 75 are male. How many breeding cycles will you need?

Your tanks can each hold 100 fish at 4 per m<sup>2</sup>. At a 3:1 sex ratio that is 75 females and 25 males.

Use two tanks to hold the female and male fish separately and condition them. After 10–12 days, select the 75 best females and place them in a third tank with 25 similarly sized males.

As fry appear, scoop them out and transfer them into the fourth and fifth tanks, which will be used as nursery tanks. Feed them a powdered mash feed until 2–3g size, then transfer them to grow-out ponds.

After about 21 days take the breeders out of the breeding tank, separate the males and females to condition them for a new breeding cycle.

Each breeding cycle takes about 21 days, followed by 10–12 days for fish to condition for spawning again.

Each cycle should produce a minimum of 200 fry x 75 females = 15,000 fingerlings.

To produce 50,000 fingerlings, 2 to 3 breeding cycles will be necessary.

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## Nursery ponds

Fry that are collected from breeding ponds, tank, or hapa can be reared intensively to fingerling size in an earthen nursery pond. Compared with direct introduction of fry to grow-out ponds, using a nursery pond has the advantage of more rapid growth of fingerlings before stocking. The other advantage of using a nursery pond, compared with leaving fry and broodstock together longer in breeding ponds, tanks or hapa, is avoidance of cannibalism by parent fish.

### Characteristics of a good nursery pond are as follows:

- sufficient water supply
- good water quality
- convenient water-filling and drainage arrangements
- rectangular shape
- area of 400–600m<sup>2</sup>
- water depth of 60–120cm
- pond dykes that are solid and watertight
- even, smooth bottom
- little silt
- no aquatic weeds
- plenty of sunlight

### Nursery pond preparation

First, excessive silt and weeds are removed and pond dykes are repaired. At least 7–10 days before stocking with fry, dry ponds should be treated to eliminate any predatory fish or other pests. Common pond treatments are quicklime, hydrated lime, teaseed cake (all imported products), or rotenone (derris roots, which can be available in your area). When applying quicklime or poison, appropriate clothing should be worn (Fig. 15).

**Quicklime:** An application rate of 1kg of quicklime for every 10m<sup>2</sup> of pond area can be used for established ponds, but can be increased to 1kg per 5m<sup>2</sup> for new ponds. When quicklime (CaO) absorbs water, it transforms into calcium hydroxide Ca(OH)<sub>2</sub>, which raises pH and draws oxygen from the water. The effect of quicklime is to kill animals and plants, and any pathogenic bacteria.

**Teaseed cake:** This is a residue of the fruit of a plant (*Camellia sasanqua* or *Camellia semiserrata*) after the fruit oil is extracted. The by-product contains saponin, which is a poison to fish. At a concentration of 10ppm, saponin causes fish to die in a few hours. The usual dosage is 1kg per 15m<sup>2</sup> of pond area. Teaseed cake in powder form is first soaked in water until dissolved, and this water is then evenly spread over the pond.

**Rotenone:** This is extracted from the roots of a plant (*Derris uliginosa* or *Derris elliptica*). The extracted solution contains about 25% rotenone, which is a poison to fish. The recommended rotenone concentration for pond clearing is about 1.3kg/660m<sup>2</sup> of pond area. The rotenone solution is first diluted 10–15 times with water, and this water is then evenly spread over the almost empty fish pond.

Once no pests or unwanted fish remain in the pond, add lime to counteract any acidity in pond soil. After 2–3 days, apply fertilizer, usually chicken manure at 1000–2000kg/ha. The nursery pond can then be filled with water. A shallow water level of about 50cm is preferred for fry-rearing because shallower water is more easily warmed by sunshine during the day, promoting plankton growth. Fry will grow more quickly if the water is warmer. As the fry become fingerlings, the water depth can be increased to provide more space and better water quality for these larger fish.





**Fig. 15 Application of quicklime. When applying quicklime or other poisons, appropriate protective clothing should be worn**

## **Nursery pond daily maintenance**

The condition of the pond and the behaviour of fish should be observed twice daily, morning and afternoon. Water colour (for example greenness) and fry activities (including surfacing behaviour) should be noted to determine the amount of feed and manure to be administered, and whether fresh water should be added. If the fry gasp for air at the water surface in early morning (behaviour called “piping”) and continue to surface after sunrise, the dissolved oxygen content is too low and fresh water should be added into the pond to wash out some of the “green” colour.

Turbidity or water “greenness” or transparency can be measured by a Secchi disc (Fig. 16). Turbidity (and colour) is due to the abundance of plankton or suspended clay in the pond water.

A Secchi disc base can be made from a white ice cream container lid, 20cm in diameter, painted with alternate black and white quadrants, and nailed onto the end of a metre-long stick marked with a centimetre scale.

This disc is held underwater, and where it just disappears from view, the depth is read off the marked scale. If the disc disappears at a depth of 20cm or less (average of several readings), the water is too turbid or too green with plankton, and clean water should be flowed into the pond to wash some of it out. A Secchi disc reading of 30–45cm indicates good water condition.



**Fig. 16 A Secchi disc. The water depth at which the black-and-white disc just disappears from view underwater can be read off the centimetre scale drawn on the handle**

Management of a nursery pond, or any pond, involves a daily routine of tasks including checking for the following:

- pond water level (at least 40cm)
- activities of fish (actively feeding? gasping at surface?)
- water inlet and outlet, ensure mesh screens are in place
- general colour of pond water (Secchi value should be 30cm or more)
- growth of aquatic weeds, erosion, damaged water lines
- floating dead fish

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## Record keeping

Hatchery production records are important for good management of stocks. They can provide detailed information about the stocks, inputs and outputs of the operation and can help to identify any problems that occur. It is recommended that records be maintained in a logbook and where possible in a computerised database.

If good hatchery records are made available to researchers, they can compare the efficiency and profitability of different techniques and performance of breeders. This information then helps the hatchery operators to choose techniques and breeders that are most appropriate and profitable for a particular site. There are many forms of hatchery records.

### Hatchery records provide the information necessary to:

- compare the profitability of various production techniques
- compare the productivity of breeders
- compare the productivity of major inputs with alternatives
- improve efficiency of the operation

### What data should you record?

A logbook or record of each breeding cycle should be kept using a “Breeding and Fry Collection” form. Use the form to record items such as the tank, pond or hapa number, sex of fish, number of breeders stocked, dates of stocking, date of fry or fingerling collection, number of fry or fingerlings, body weight and origin of the broodstock used, date of transfer to nursery pond, date of fingerling harvest and the income from fingerling sales (if applicable). In the remarks column, record any mortalities, water parameters, name of farm where the fingerlings are supplied or sold to. Hatchery operators are advised to design their own form applicable at their site. A sample is given below.

### BREEDING AND FRY COLLECTION

\_\_\_\_\_ Generation

Hapa Tank Pond No.	Sex	Number stocked	Date stocked	Date of harvest	Number of fry	Av. body weight breeders	Nursery Hapa No.	Remarks

### Why is it useful to record this data?

These records allow you to check on your management routine, compare breeding cycles and see the performance through the year. For example, you will be able to see the effects of changes in your management, uncover any problems and look for solutions:

- If one harvest is less than others, was there some change that made it worse?
- If one harvest is more, what improvements were made?
- Did a cold season (low temperature) result in a lower number of fry?
- Know how many fish to expect at different times of the year.
- Low survival might be due to bad water quality or other factors.
- Low harvest of fry may mean someone is stealing your fish or fingerlings.
- Low harvest of fry may mean some of your fish are not conditioned or are escaping.
- Low harvest of fry may mean you have predators in the pond.

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## Packing and transportation of fingerlings

There are two basic systems for transporting fry, fingerlings and broodstock fish — the closed system and the open system. The closed system is a sealed container where requirements for survival are self-contained, for example a sealed plastic bag partly filled with water and oxygen. The open system consists of water-filled containers in which the requirements for survival are fully or partially supplied from outside sources, for example a small tank with an aerator stone for long distance delivery of fingerlings or without a aerator stone for short distance (10–15 minutes) delivery. Of the two systems, there are three methods usually practised in the Pacific. The three methods are described in the Box.

### Three methods of transporting fish in the Pacific

#### Transporting fish in household containers

This method is ideal for movement of fish from one pond to another in the same farm or to a neighbouring farm. The container or bucket should be covered with a lid to keep the water cool by preventing direct sunlight from reaching it.

The number of fish per container depends upon the size of fish (fry or fingerling), the volume of the container, the temperature and the transportation times. For example, with fish size of 1–5g and transportation time of 30 minutes at a temperature of 25°C, you can carry 8–12 fish per litre of water. If the transportation time is longer than 30 minutes and the temperature higher than 25°C, change the water every 30 minutes.

#### Transporting fish in oxygenated bags

Water is poured into the bags (double bag) and fish are transferred into bags. The bags are pressed above the water to remove atmospheric air, oxygen is added, and the bags are tied with a rubber band. The plastic bag is placed inside another sack or in a wooden box or container for ease of carrying. With oxygenated bags, the fish density can be twice as much as when buckets are used.

#### Transporting fish in tanks (in trucks)

The tanks are made of fibreglass, or plastic-lined frames/boxes, or plastic containers. The containers have an open top. The size of container used depends on availability, but a recommended size is 1m<sup>3</sup>. The open side is covered with plastic or a sack and tied around with a rope. This cover protects the water from splashing and also from exposure to direct sun. The container should be filled with water to 70% of its volume and where possible fitted with an aerator. Higher densities of fish can be transported, compared to other fish transportation modes.

Care should be taken to minimize forward and backward movement of water when the vehicle is in motion as this affects fish and may even kill the fish.

If the hatchery is located a long distance by road from the grow-out ponds, or located on another island and fingerlings need to be sent by airfreight, then the fingerlings ready for pond-stocking will need to be carefully prepared and packaged for transportation.

During transportation a large number of small fish are going to be crowded together into a small volume of water. Many could die before they reach their destination, unless they are packed properly. The following steps may be followed:

1. Harvest fingerlings from ponds or nurseries and put them into hapas set up in clean tanks, or directly into tanks.
2. Leave the fingerlings for two to three days to recover from handling stress.
3. Ensure sufficient aeration or a continuous flow of water to provide oxygen.



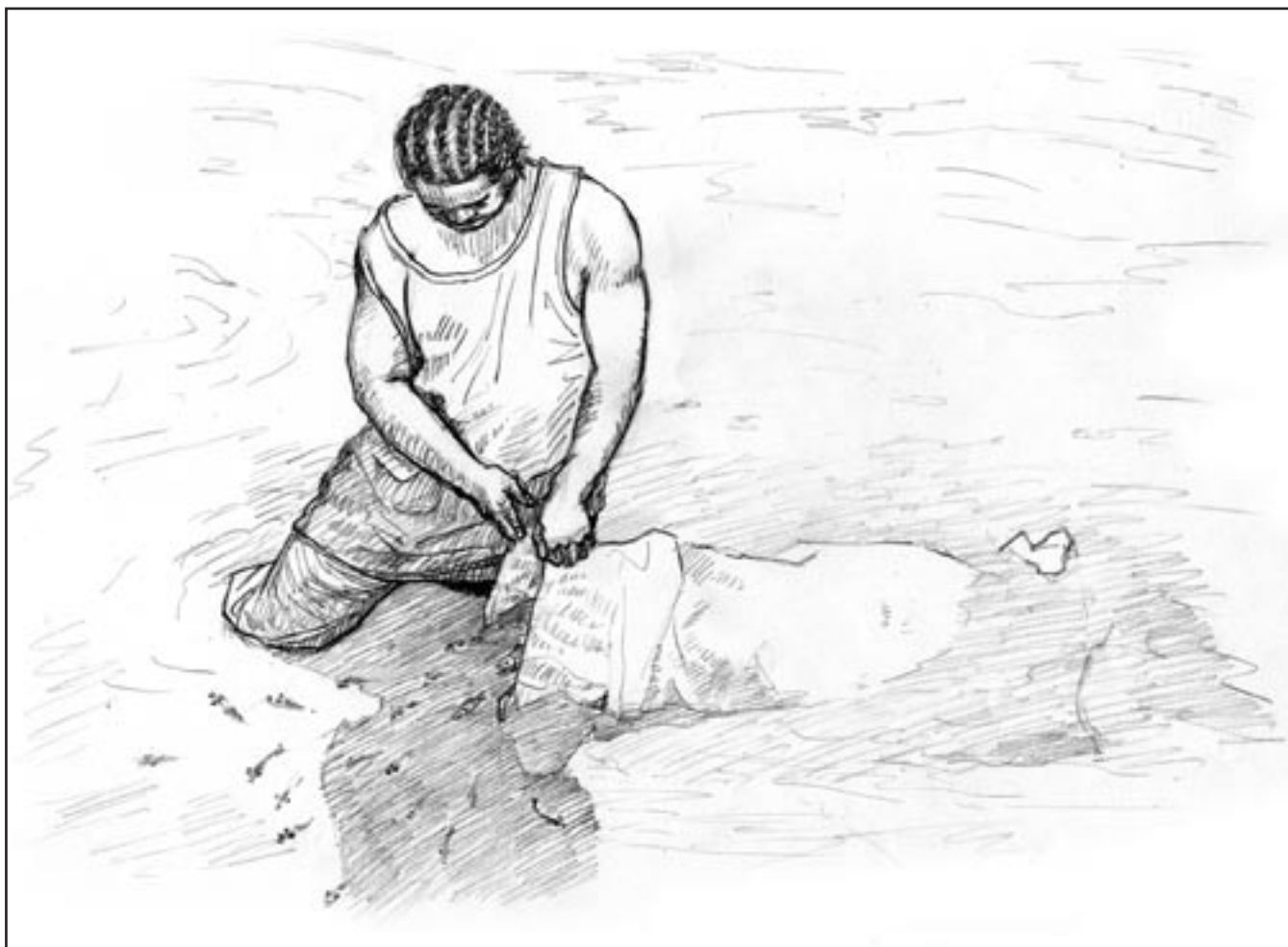
4. The sides and bottoms of tanks should be smooth, so fish do not get damaged when scooping them out.
5. They can be fed for the first two days, but not in the 24-hour period before transport. This gives the fish time to empty their guts of faeces, so they will not foul their water during transport.
6. Avoid overcrowding in the hapa or tank. If fingerlings are all gulping air at the surface, reduce the number of fingerlings in the unit or increase aeration.
7. Use large, strong clear plastic bags for fish-packing that can hold up to 10L of water when only 1/3 full (the rest will be filled with oxygen). Use clean water.
8. Use two bags, one inside the other, as insurance against damage to the plastic.
9. Pack 300–500 fingerlings per bag for short journeys (up to 6 hours) or 200–300 per bag for long journeys (up to 18 hours). A general rule is to pack 600g of fish for every 10 litres of water for a journey of 8–10 hours. One method is to weigh the fish in water in a container on an electronic balance and keep adding fish until you have added 600g. Another method is to estimate average body weight by weighing a sample of fish and dividing the weight by the number of fish in the sample. Then count out into the bag the number of fish needed to make the total weight required for each bag (for example, for an average body weight of 2g you should count out 300 fish).
10. Insert a hose from an oxygen cylinder in to the bag, hold the neck closed around the hose, and fill the bag up with oxygen (see Fig. 17).
11. Withdraw the hose, then twist and double back the neck of the bag, using strong rubber bands to hold it closed and gas-tight.
12. The plastic bags should be fitted into styrofoam boxes or any suitable box.
13. Add an ice-pack to each box before taping up its lid. Cooler temperature will reduce the fishes' demand for oxygen. Avoid direct sunlight or conditions where bag water temperature could rise above 30°C.



**Fig. 17** *Packing fish for transport to ponds*

On arrival at the pond site, care should be taken to acclimatize the fingerlings to the temperature of the pond water by floating the transportation bags in the pond for 15–20 minutes before opening them. Sudden changes in temperature of more than 5°C can harm the fish.

When you open the bags, allow in some pond water to mix 50:50 with the transport water. Sudden changes in water quality can also be harmful, so the fish need time to adjust to their new water conditions. After another 2–3 minutes, tip the bag on its side and allow the fish to swim out (see illustration in Fig. 18). Do not tip them out all at once, but rather let them swim out by themselves. This will leave any dead ones remaining in the bag, so they can be counted and the mortality of fish during transportation can be ascertained.



**Fig. 18 Releasing fry into the pond. Let the fish swim out by themselves, so any dead ones left behind can be counted**

## References

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