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MYSAP

Myanmar Sustainable Aquaculture Programme

MYSAP Inland

Practical training manual

Tilapia breeding and all-male fry production

Implemented by:

WorldFish

DEPARTMENT OF FISHERIES MYANMAR

giz Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH

MYSAP Inland, WorldFish

Practical training – Tilapia breeding and all-male fry production

Tilapia breeding, mass fry production, egg collection & disinfection, hatchery preparation, egg incubation, larvae rearing & shipping, hormone feed preparation & storage, all-male fry production & feed adjustment

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A. Basic tilapia hatchery concepts

What is a hatchery?

A hatchery is a facility for breeding parent (broodstock) fish to produce fertilized fish eggs and fish seed including larvae, fry and fingerlings for stocking into grow-out culture production systems. Broodstock fish are the heart of any hatchery. However good hatchery management is also vital.

Minimum requirements for a tilapia hatchery

- Good centralized location with effective road network and services, including electricity
- Reliable, clean water source
- Well-designed facility
- Quality broodstock (parent fish)
- Effective management control systems documented and implemented
- Good biosecurity measures
- Accessories for packing and shipping of seed (larvae, fry or fingerlings)
- Packing and loading area
- Ponds and cloth or net hapas for spawning, egg incubation and larval rearing system, all-male fry production system and fry and fingerling nursing areas.

Rearing quality broodstock

- Hatchery broodstock should be of known origin and proven quality
- Incoming broodstock should perform well, e.g. better growth, low food conversion ratio (FCR) and show disease resistance
- Incoming broodstock should be tested and free of Tilapia Lake Virus (TiLV), *Streptococcus agalactiae* and *Streptococcus iniae*
- Maintain stock lines in secure and separate facilities
- Replace 25% of the broodstock from within each year
- If possible, replace all broodstock every 24 – 30 months.

Nile tilapia (*Oreochromis niloticus*) must be at least 6 months old (from hatching) to be suitable for breeding. Some strains mature a little later than others and fish of 8 months or older are most suitable. The mature broodstock should not be larger than 100 g in size for egg collection at the start, as smaller fish produce

more eggs per kg and are easier to handle than large fish. Nile tilapia can be spawned continually for 2 years if they are fed a limited diet. However, the more Nile tilapia are fed, the quicker they grow and the shorter their useful life, as they are too big once they get to 500 g.

How to rear new broodstock

Initially maintain the supplied broodstock fry in fine meshed hapas (20 strands per inch [spi] mesh) in fertilized "green water" ponds (use only chemical fertilizer). They can be fed on powdered feed (32% crude protein) 2-3 times per day "ad libitum" and later on small pellets depending on their mouth size. Raising broodstock in hapas will reduce their growth rate, but they must be graded and re-stocked at suitable density every month to ensure good survival. By the time the fish are 4-5 months old, Nile tilapia will only be about 20 g if raised correctly due to stunting.

The broodstock tilapia can then be stocked out in ponds at 6 fish/m^2 for 2 months and fed on pelleted feed twice per day. At this density they will grow fast and will be 80-100 g within 2 months culture period. They can then be harvested, sexed and stocked into spawning hapas.

The reason that the broodstock should initially be raised in hapas is that, contamination from wild tilapia is reduced and raising fish in hapas makes it easy to constrain their growth. They can be maintained like this for well over a year and will not take up too much space. Only when they are needed are they stocked in ponds to get them to 100 g for spawning.

Spawning tilapia broodstock management in breeding hapas

New broodstock should be stocked at a density of approximately 0.6 to 0.7 kg per m^2 of spawning hapa and a sex ratio of 1.5 to 2.0 females per male fish. Stocking a lot more males to females may result in poorly fertilized eggs. New broodstock can be stocked at a lower density, so that as they grow they don't get too dense in the spawning hapas. Stocking too many fish results in inhibited breeding.

Broodstock spawning ponds should be fertilized weekly with 15-15-15 chemical fertilizer (dissolved in water before broadcasting the solution in the pond).

However this is not recommended in the entire pond is covered with shade netting to reduce water temperature.

Spawning broodstock are fed twice per day at about 0.8% body weight per day with floating pellet of 25% crude protein. Feeding more than this will lead to the fish growing too fast and the spawning hapas will get dirty quickly. There will also be more contamination of eggs with faeces. By feeding the tilapia broodstock less, they are encouraged to clear periphyton from the hapas which helps to improve water exchange and dissolved oxygen (DO) levels. Broodstock condition can be assessed during egg collection. If fish are fat and the hapas dirty, feeding should be reduced to as little as 0.6% body weight per day. However if the fish become thin, then increase the feed amount.

Egg collection should ideally be done every 5 days, but in the cold season, egg development is slow and it is better to collect eggs every 6 or 7 days. Most farms worldwide actually collect eggs every 7 days. This ensures that egg collection will always be on the same day and not on a religious holiday. The problem with this, however, is that stage 4 eggs (those that are hatched) will be of mixed age and they will need sorting in the hatchery. Farms do this by using incubators that the hatchlings can swim out of into a collection container. Only older yolk sac fry will begin to swim, whilst younger fry will stay at the bottom of the incubator just like eggs.

Female broodstock can be changed every 5 days and maintained in conditioning hapas (smaller hapas located alongside the spawning hapa) for 10 days. If the temperature is suitable for spawning, this method will result in 20% more eggs, but it means having to sex the fish every harvest and losses of broodstock into the pond are high because fish are being transferred all the time. Often the sexes become mixed up, because the workers have to work quickly with so many broodstock to check and move. If temperature is high and not good for spawning, the egg production using the female exchange and conditioning system will be the same as a system in which there is no resting period for broodstock.

The best and easiest method is to continually stock fish back into the spawning hapa. This way fish need only be sexed once when they are stocked. Weighing and counting the fish of each sex in every hapa is recommended every 2 months

however, as broodstock may be lost or the density of fish per hapa may have become too high, causing reduced egg production.

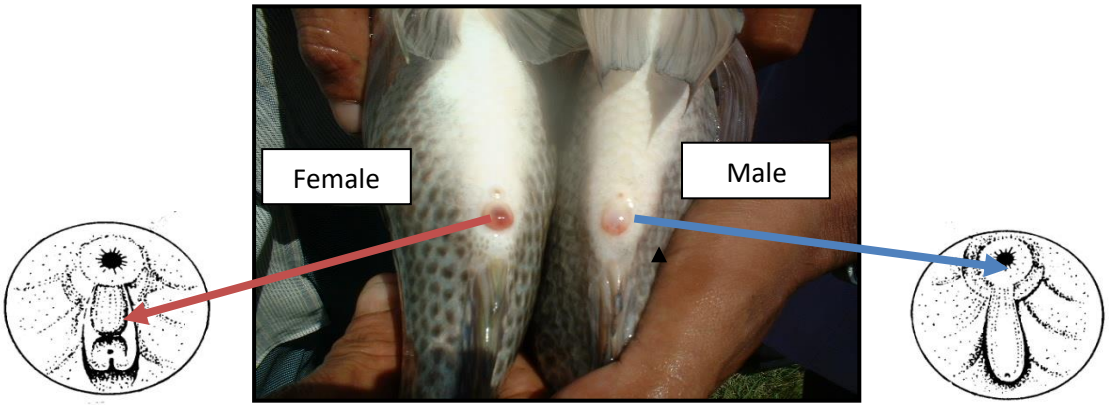
All broodstock ponds should be drained and dried at least once per year.

Key considerations when rearing broodstock tilapia in hapas

- Shade hapas from direct sunlight
- Provide good quality feed in appropriate amounts
- Water showering/spraying and raising pond water level helps to stimulate female tilapia egg production.

Breeding and reproductive biology

- Morphology of tilapia broodstock sex organs:



Female sex organ with 3 openings

- One opening for excreta
- One opening for egg release
- One opening for urine release

Male sex organ with 2 openings

- One opening for excreta
- One opening for urine & milt release

Table 01. Broodstock feed management

Hapa broodstock stocking rate	Pond water colour	Feeding % of body weight	Feeding frequency /day
0.6 – 0.7 kg per m^2	Green	0.8%	2

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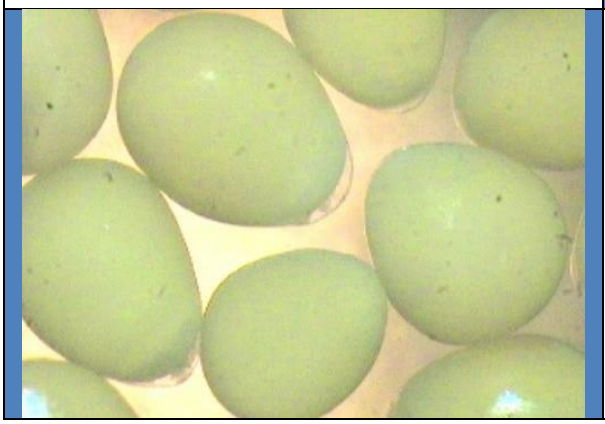
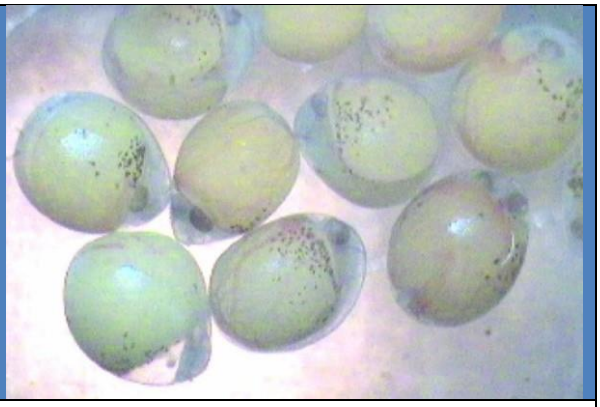
Egg collection and disinfection

Tilapia male and female broodstock are stocked into breeding hapas at a ratio of 1 male to 1.5-2 female tilapia. It normally takes 2 weeks before the tilapia begin to breed after stocking male and female broodstock together in a breeding hapa.

Female fish are mouth brooders and in the wild will collect and incubate the fertilized eggs and later nurse the young fry in their mouth, until the fry are free swimming.

When tilapia eggs are collected every 5 days, they are normally divided into 4 distinct development stages depending on when the female broodstock tilapia spawned.

Stage 1. Un-eyed eggs which are white or yellow in colour (Photo: Nam Sai Farms)	
	Stage 2. Only just eyed eggs now a little darker in colour (Photo: Nam Sai Farms)

Stage 3. Eggs much darker with large eyes large and tail visible and some eggs starting to hatch (Photo: Nam Sai Farms)	
	Stage 4. Yolk sac fry (Photo: Nam Sai Farms)

There may also be some swim-up fry that were missed at the last egg harvest. Discard any fry that have NO yolk sac, as they will compromise sex reversal being of unknown age.

When the tilapia have commenced spawning the broodstock should be checked every 5 days. If there are eggs in the mouth of a female, the eggs should be removed.

Eggs collection protocol

Tilapia eggs are collected from the mouth of the female tilapia broodstock every 5 days and are separated into 4 developmental stages as below:

Stage 1: Freshly laid un-eyed eggs, normally white or light yellow in colour
Stage 2: Just eyed eggs which are darker yellow in color
Stage 3: 2-day old eggs which are darker with visible large eyes and tail, which are starting to hatch
Stage 4: Sac fry.

Any swim-up fry without yolk sacs or bigger fry, left over from a previous egg collection, should be discarded as they are **not** suitable for sex-reversal.

Eggs are removed (shaken) from a female broodstock tilapia mouth

Depending on the egg stage the eggs are put into stage 1, 2, 3 or 4 bowls

Key considerations for egg collection

- Morning is the best time for egg collection
- Eggs must be separated according to their developmental stages
- If egg collection takes a long time, prepare an aquarium air blower
- Avoid physical shock during collection and transportation of the eggs to the hatchery
- If the temperature is high provide shading to reduce over-heating (thermal shock).

Materials required for egg collection

- Different types of scoop net
- Hands gloves
- Smooth bamboo pole or floating PVC pipe to pull under the breeding hapa when crowding the broodstock tilapia to one end to check for eggs
- Rectangle PVC or Bamboo frame for egg holding bowls or a ring setup
- Plastic buckets for moving broodstock females to resting hapas & to move the eggs to the hatchery.

For short distance transportation of eggs to the hatchery an air blower and air stones can be used. Where the tilapia broodstock ponds are a long distance from the hatchery, use a mobile hatchery system located close to the spawning fish should be considered to reduce transport time.

When broodstock ponds are distant from the hatchery, a mobile hatchery can be used (Photo: Nam Sai Farms)

Eggs disinfection process

Disinfection is employed as a common disease management tool in aquaculture. Disinfection is a routine practice in many biosecurity programs.

Disinfection can be used to exclude specific diseases, as well as a routine sanitary measure to reduce disease incidence. The specific reason for using disinfection will determine the disinfection strategy used and how the disinfectant is applied.

The general principles for disinfection of aquaculture facilities involve the application of a chemical treatment at a specific dose rate and for a sufficient time to kill all pathogenic organisms that would otherwise contaminate the fertilized eggs and incubation unit.

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The washing and disinfection procedures should include at least the following stages:

- Removal of solid waste & dirt followed by pre-washing with tap water
- Deep cleaning, washing and sieving
- Disinfection.

There are three different disinfection chemicals that are commonly used to disinfect tilapia eggs. The preferred chemical disinfection option will depend on the local context.

Egg disinfection with 40% formalin.

- Add 2 ml formalin/ litre of water for 3 minutes for stage 1 eggs
- Add 2 ml formalin/ litre of water for 2 minutes for stage 2 & 3 eggs
- Add 2 ml formalin/ litre of water for 1 minute for stage 4 eggs
- After dipping in formalin wash thoroughly with clean water to remove the formalin from the eggs.

Egg disinfection with potassium permanganate ($KMnO_4$)

- Add 15 mg potassium permanganate/ litre of water
- Dip the stage 1, 2, 3, and 4 eggs in the solution for 10 minutes
- After dipping in $KMnO_4$ wash thoroughly with clean water to remove the $KMnO_4$ solution.

Eggs disinfection with Acriflavin

- Mix 2 ml of Acriflavin solution per litre of water
- Dip the stage 1, 2, 3 and 4 stage eggs for 30 seconds
- After dipping in Acriflavin wash thoroughly with clean water to remove the Acriflavin solution.

After proper disinfection wash the eggs repeatedly with clean tap water. Thereafter weigh the eggs on a digital balance.
Stage 1 and 2 eggs should be transferred to a hatching jar. 400-500 g of stage 1 and 2 eggs can be placed in a 6-8 litre hatching jar.

Stage 3 and 4 eggs should be transferred to a hatching tray. 200 g of stage 3 and 4 eggs can be placed in each hatching tray.

Key considerations for egg disinfection

- Any chemical disinfectant used to treat a specific condition in fish eggs must be effective
- Secondly treatment using the chemical disinfectant should cause no harm. While this is obvious, it is often overlooked
- Precautions have to be taken to ensure the safety of any staff working with chemical disinfectants and disposal of waste chemical disinfectant has to be done in a manner to protect the surrounding land and water environment.

Reasons for disinfection treatment

- The primary reason for disinfecting eggs is to remove fungus and other disease pathogens that may attack the eggs during incubation and reduce the number that successfully hatch
- To clean the eggs before moving or transporting the eggs from one location to another. This reduces the likelihood of transferring disease pathogens and reduces the load of particularly bacterial pathogens which can negatively impact on egg hatching success
- There are a few disease pathogens however which occur inside fish eggs and which cannot be treated by disinfection.

Washing eggs with clean water. A scoop net is used to sieve out coarse dirt particles

Weighing the tilapia eggs before transfer to the incubation and hatching jars or trays

Materials required

- Disinfection chemicals as stated above
- Digital measuring scale
- Different types of beaker or measuring cylinder and plastic 5 ml syringe
- Different types of soft, fine, scoop net
- Coarse net sieve, plastic buckets, and bowls.

B. Hatchery preparation, egg incubation, larvae rearing and shipping

At least 5 days before it is expected that tilapia in the breeding hapas will produce eggs that will be collected, all the materials and equipment in the hatchery including all trays, jars and hatching tanks should be properly cleaned with clean ground water from a tubewell, ideally from a deep tubewell. After a thorough cleaning all the bio-filter tanks and the hatching tanks should be filled with ground water from a tubewell.

Four days before egg collection apply the disinfecting chemical TIMSEN® to the overhead reservoir at a dose rate of 0.5 g per 1000 litres of water.

One day before egg collection apply 0.2 g of salt per liter water in the overhead tank. For example an overhead tank with 1,000 litres of water will require 200 g of salt. Every 15 days if tilapia egg production is still on-going, apply the same dose rate of salt to the overhead tank to prevent fungal infestation of the system.

On the same day when salt is added to the overhead tank apply 2 g of pro-biotic per 1,000 litres of water volume in the bio-filter tank. This will kick-start the organisms in the bio-filter which will break down harmful nitrogenous waste products into non-harmful products.

When needed, top up the overhead reservoir tank with fresh, clean tubewell water to replenish water lost due to evaporation.

At a commercial hatchery like Nam Sai all water is recirculated and separate incubator jar and tray systems are used. Incubator systems incorporate a slow sand filter to remove bacteria, whilst tray systems only have gravel filters. The reason for this is that eggs are extremely sensitive to bacterial levels and dead eggs are food for bacteria. All dead eggs have been removed by the time they are transferred to trays and sac-fry are much less sensitive to bacterial levels. Salinity is kept at 4 parts per thousand (ppt) in incubator systems and 7 ppt in tray systems primarily to prevent *Trichodina* infection.

Nam Sai does NOT add any chemicals to the water. Most hatcheries harvest eggs regularly to reduce the workload. If they only harvest eggs every 5 days,

then there will be a lot of work on that one day. The system described in which new tubewell water is added to the system at the start of the season and treated with TIMSEN, 4 days prior to eggs entering the recirculation system should however be fine.

There is no need for a bio-filter in the hatchery, as fish do not excrete ammonia in any measurable levels before feeding commences.

Egg incubation for different egg stages

After disinfection and proper measurement of the collected egg weight, the eggs should be placed into jars/jugs or trays for hatching as appropriate depending on the egg development stage.

In a well-designed hatchery, all egg stages are first placed into incubator jars/jugs before later transfer to trays. The reason for this is that it is much easier to manage in terms of getting the right water flow and removing any dead eggs.

Far fewer eggs can be incubated in a tray and they need constant work using a tooth brush to remove the dead eggs that stick to the side of the tray. The eggs should be incubated in incubator jars/jugs as long as possible, but will have to be transferred to trays, when they will swim out of the incubator jar if kept there too long.

Stage 1 – keep in incubators for 4 nights

Stage 2 – keep in incubators for 3 nights

Stage 3 – keep in incubators for 2 nights

Stage 4 – keep in incubators for 1 night.

The amount of eggs put in an incubator jar will depend on its size. Large incubator jars/jug (8 inch) can take up to 2.5 kg of eggs. At the correct stocking density, a tray can only be used for 200 g of eggs.

The mean size of eggs should be calculated by weighing 2 samples of 200 eggs with a balance to 2 decimal figures. This is important in order to calculate egg numbers and survival of eggs during incubation.

- Stage 1 are placed into incubation jars/jugs until they hatch. Water flow should be relatively slow within the jar/jug to prevent physical damage of the eggs.
- Hatching trays have a faster water flow, as later stage eggs are fully hardened and more robust.

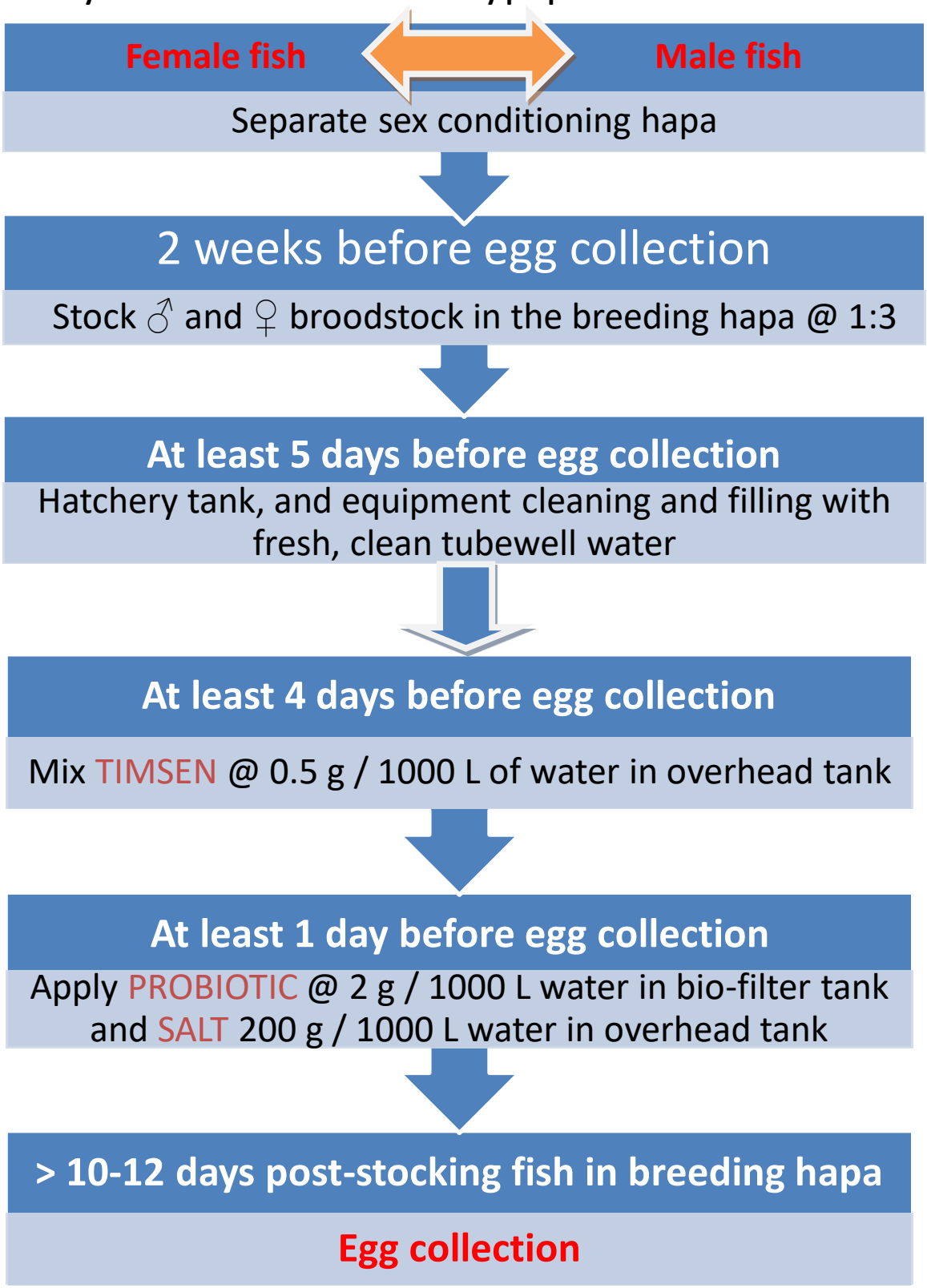
Stage 1 and 2 fish eggs stocked into jars for incubation

Stage 3 and 4 fish eggs stocked into trays for incubation

Key considerations for egg incubation

- Ensure that all tanks, pipes, fittings, hatching jars and trays have all been properly disinfected with either $KMnO_4$ or TIMSEN powder.
- Be sure that the hatching jars, trays and pipe fittings are all properly placed and connected.
- Every 2-3 hours until hatching starts, clean the fine mesh outlets at the side of the incubation trays with a tooth brush to remove any trapped dead eggs.
- Ensure that the system has a continuous water flow.
- Be careful of any air trapped in the pipes which may cause blow-back of water under pressure.
- During hatching remove egg shells by siphoning and adjust water flow as appropriate.
- 2-3 days after hatching, and immediately when the egg yolk sac has been absorbed the swim-up fry need to be transferred out of the hatchery and stocked into sex-reversal treatment (SRT) nursing hapas.
- Carefully follow the protocol given below to transfer the swim-up fry from the hatchery to the sex-reversal treatment (SRT) nursery hapas.

Activity flow chart for brood & hatchery preparation:



Transfer process for swim-up fry

Immediately the egg yolk sac is absorbed the swim-up fry must be moved directly and stocked into a sex-reversal treatment (SRT) nursing hapa which has been set up in advance in a green water nursery pond. The fry should be fed hormone treated feed five times per day for 24 hours.

Fry should be transferred and stocked at 6,000 swim-up fry equivalent to 60 g of swim-up fry per m^2 of nursing hapa.

Young broodstock tilapia have small eggs and old broodstock large eggs. Ideally each batch of SU fry should be sampled. 2 samples of 100 SU fry can be weighed with a balance to 2 decimal figures to do this. The exact number of SU fry can then be stocked per hapa. This is very important to getting close to 100% male fish.

Key considerations for SRT and hapa nursing

- Check daily that the hapa is tightly (top and bottom) tied to the bamboo poles and that the fry are feeding well.
- Transfer the swim-up fry from the hatchery to SRT nursing hapa in the mid- to late-afternoon when the dissolved oxygen level will be higher.
- If the SRT nursing hapa is dirty change it for a clean hapa.
- If the pond does not have an outflow pipe to control the water level in the pond, during heavy rainfall events raise the hapa in the pond, if needed, to make sure the fry do not escape with the rising water level.
- Preventing heat stress by providing shading over the hapa if necessary.
- Putting bird netting over SRT nursing hapas.
- After 21 days of treatment grade the all-male fry with a 5 mm mesh grader.
- If you have a rush order for large all-male fry, after 21 days reduce the stocking density to 1,000 fry per m^2 of nursing hapa.

C. Hormone treated feed preparation and storage

Why hormone treated feed required?

During courtship displays, mature male tilapia waste a lot of energy to build their breeding nests, displaying and protecting their territory. Female tilapia expend a lot of energy on egg production, and incubating eggs and protecting their larvae, which is not used for their growth. If breeding is stopped this wasted energy is used for tilapia growth. Additionally male tilapia grow about 20-30% faster than female tilapia. For this reason male tilapia fry are preferred for stocking in grow-out tilapia production systems.

The most commonly used commercial method for tilapia sex-reversal is to feed the tilapia fry with feed treated with 17-α methyl testosterone hormone from egg yolk absorption for 21 days at a dose of 60 ppm. This is equivalent to adding 60 mg of hormone to 1 kg of feed.

The hormone is first dissolved in at least 95% ethyl alcohol solution, which acts as a solvent and dissolves the hormone, which does not dissolve in water. The prepared standard hormone stock solution can be stored for 6 months in a fridge at 4^0 Celsius. The standard hormone stock solution is then mixed into high quality powdered fish meal or a commercial grade high quality powdered nursing feed, with the addition of vitamin C. Vitamin C is more important if sex-reversal is being conducted in clear water ponds, or in shaded conditions without sunlight. It can be omitted if sex-reversal is conducted in hapas in green water ponds.

Conducted correctly the 21 day sex-reversal process should yield fry which are at least 99% male.

Hormone mixing feed preparation protocol

The following materials required are required when preparing hormone treated feed for tilapia sex-reversal:

- Surgical latex gloves and mask
- Best quality hormone, ethyl alcohol and vitamin C
- Hormone mixing machine or liquid sprayer
- Electronic digital weighing scale
- Glass measuring beakers

- Glass bottles for storage 1.5 or 2 litre
- Plastic 5 ml syringe
- Clean plastic bowls and/ or buckets
- Plastic bags or plastic Tupperware boxes for storage of hormone treated feed
- Permanent marker pens for labelling bottles and storage bags and containers.

17-α methyl testosterone hormone stock solution preparation

- Put on latex gloves and a face mask before working with the hormone
- Carefully weigh out 0.5 g (or 500 mg) of 17-α methyl testosterone hormone
- Measure out 1 litre or 1,000 ml (or cc) of at least 95% ethyl alcohol into a suitable glass container with a top
- Dissolve 0.5 g (or 500 mg) of hormone into the 1 litre (1,000 cc or ml) of at least 95% ethyl alcohol
- Put the top on the container and shake the mixture for several minutes until all the hormone is fully dissolved in the ethyl alcohol
- The standard stock solution bottle should be carefully labelled and put in a fridge at 4^0 - 6^0 Celsius. Kept cool and away from direct sunlight the standard stock solution can be stored for up to 6 months (180 days).

To prepare 1 kg of hormone treated feed for tilapia fry sex-reversal the following ingredients are needed:

- 60 mg of 17-α methyl testosterone hormone
- 10 g of vitamin C powder – can be omitted if sex-reversal is conducted in green water ponds
- 240 ml (cc) of 99% ethyl alcohol.

Preparing hormone treated feed for tilapia sex-reversal

- Put on latex gloves and a face mask before working with the hormone
- Measure 120 ml of, previously prepared, 17-α methyl testosterone hormone stock solution into a glass beaker
- Measure 120 ml of fresh 99% ethyl alcohol into another glass beaker
- All the 120 of stock solution to the 120 ml of 99% ethyl alcohol

- Weigh 10 g of vitamin C powder
- Add the vitamin C powder to the 240 ml of solution
- Put a lid on the container and mix the solution carefully until the vitamin C has been completely dissolved
- When dissolved pour the liquid into a container with a sprayer nozzle
- Tip 1 kg of powdered fish meal or commercial grade high quality powdered nursing feed into a clean plastic bowl
- The hormone solution can be mixed evenly with the feed either by mixing machine or by hand manually
- If done by hand, spray the feed with the hormone solution a little at a time, until the feed colour darkens on the surface and then mix the feed carefully by hand (latex gloves must be worn)
- Keeping spraying and mixing until all the hormone solution has been added
- After all the hormone solution has been sprayed over the feed and it has been thoroughly mixed by hand, then pass the treated feed through a fine meshed net to sieve the feed and to ensure that there are no lumps within the feed
- Dry the treated hormone feed at room temperature or under a slow moving fan
- When dry the feed can either be used immediately or the hormone treated feed can be kept in a labelled, sealed plastic bag or plastic box and can be stored in a refrigerator at 4^0 - 6^0 Celsius for up to 21 days.

Key considerations when preparing hormone treated feed

- Wear latex gloves and a face mask when working with hormone powder or hormone treated feed
- Be sure each feed particle has been soaked by alcohol
- Mix the hormone into the feed slowly and thoroughly
- Feed must be properly air dried to evaporate off all the ethyl alcohol, to reduce moisture and humidity levels and to prevent attack by fungus when stored for longer periods
- Pack small amounts of hormone treated feed, sufficient for daily use

- If the hormone treated feed is going to be used within 72 hours, keep the hormone in a cool place and out of direct sun where rodents and insects cannot enter
- For longer storage keep the hormone powder, the hormone stock solution, the hormone treated feed and the vitamin C powder in a refrigerator at 4^0 - 6^0 Celsius
- The hormone powder when refrigerated at 4^0 - 6^0 Celsius will last until the expiry date on the packet
- The hormone stock solution will last for 6 months (180 days) when kept in a refrigerator
- The hormone treated feed, if properly air dried, should last for at least 21 days when refrigerated
- If any fungus grows on the hormone prepared feed, then it has not been dried and stored correctly. Throw this feed out and do NOT use.

Measuring and mixing hormone stock solution when preparing hormone treated feed

D. Sex-reversal treatment (SRT) feed application, feed adjustment and fry grading

At a commercial tilapia seed production like Nam Sai where swim up fry are stocked at 6,000 per m^2, equivalent to 60 g per m^2, feeding is conducted five (05) times per day at 08.00, 09.30, 11.30, 13.30 and 16.30 hours at the following rates:

Days 1-5: 15.6 g per day

Days 6-10: 32.6 g per day

Days 11-15: 54 g per day

Days 16-21: 89 g per day

In the winter (from 1st Dec to 31st January for Nam Sai) the sex-reversal period is increased to 25 days sex, using the same amount of total feed, but with less fed at each meal.

Applying feed to a sex-reversal nursery hapa

Fry grading

Grading is done with a 5 mm grader after sex reversal is finished and fry are transferred to 40 m^2 nursery hapas. Small fry are stocked at 50,000 per hapa while large fry are stocked at 60,000-70,000 in the same 40 m^2 sized hapa. This is because the large fry are sold after 3 days, while the small fry are sold after 5-7 days.

After sex-reversal the nursery feed consists of a finely sieved powdered feed materials mixed to give a 32% crude protein. Using more vegetable protein, such as soybean meal, will lead to less ammonia build up in transportation bags when the fry are shipped. It is also cheaper than fish meal.

The feeding rate after sex-reversal in the nursery should be 15-20% body weight per day divided into 4 feeds.

The importance of grading tilapia fry

- Gives even sized fish which can all eat sufficient food
- Grading fish reduces cannibalism and gives better fish survival
- There are less undersized or stunted small fish at harvest
- Less competition during feeding, greater feeding efficiency and a lower food conversion ratio (FCR)
- Better accuracy of stock numbers for monitoring.

Fry graders (3 mm and 5 mm)

Grading tilapia fry in a pond

Key grading considerations

- Morning is the best time for fry grading
- If fishes appear stressed, delay the grading by one day
- Try to minimize stirring up the water when grading as dissolved oxygen levels will go down
- Do the grading as quickly as possible to minimize fry stress.

Implemented by:

E. Broodstock conditioning (optional)

There are several options for exchanging females from the breeding hapas after they give up eggs.

Conditioning is a short resting period for both of male and female broodstock. Typically the following tilapia conditioning process is as shown in **Table 02** below.

Table 02. Broodstock conditioning protocol

Fish sex	Stocking density (fish/m^2 of hapa)	Resting period (days)	Feed %	Feeding frequency per day
Female	6-8	21 days	2-3	2
Male	8-10	21 days	1.5-2	2

Male broodstock in the breeding hapa can be changed every 3 weeks and can also be kept in single sex male resting or conditioning hapa for 21 days.

During resting and conditioning male & female broodstock tilapia must be kept separately.

The simplest option is just to return females that have given up eggs into the breeding hapa and not to have a resting period. This way fish only need to be sexed once at the beginning of the breeding season when they are stocked.

A second option is to transfer each female that has given up eggs to a single sex female resting or conditioning hapa for 3 weeks and to replace each removed female in the breeding hapa with a rested female.

A third option is to change all female broodstock every 5 days and to rest them in a nearby conditioning hapa (smaller hapas located alongside the spawning hapa) for 10 days. If the water temperature is optimal this method will result in 20% more eggs.

However it means having to sex the fish every harvest and some broodstock fish are likely to be dropped into the pond when the fish are sexed and moved from

breeding hapa to nearby resting hapa. In addition when staff work quickly to check and move the broodstock there is a greater risk that the sexes will be mixed up. When water temperature is high and not optimal for spawning, the female exchange and conditioning system produces the same egg production as a system where the broodstock are not rested.

For the above reasons the easiest method, which many tilapia hatcheries follow is to continually stock female tilapia broodstock back into the spawning hapa.

However weighing and counting the fish of each sex in every hapa is recommended every 2 months, as broodstock may be lost or the weight of fish per hapa may have become too high, causing lower egg production.

All broodstock ponds should be drained and dried at least once per year.