Guidelines for African Catfish and Nile tilapia seed production & hatchery management in Uganda

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1.0 Overview

Seed is one of the basic inputs of any agricultural production activity, yet quality and readily available fish seed is one of the important drivers of the aquaculture industry. In Uganda the aquaculture sector has grown very rapidly in the recent years, with several approaches towards commercialization of this industry. Aquaculture is now seen not only as source of dietary protein but also as a means of generating income through improved productivity and managing aquaculture production as a business venture. This has attracted a new class of farmers; however, issues of fish seed availability and quality are still a major challenge. There is currently one government hatchery and 35 private hatcheries in Uganda, with majority of them operating at small scale level and poorly distributed to ably serve all fish farmers in the country. The private hatcheries face several challenges including: low survival rates (10%), lack of technologies for mass production, poor quality broodstock, cannibalism, poor facilities, among others, leading to very low quality and quantities of fish seed. Currently seed production from hatcheries is estimated at a National average per hatchery per cycle of 15,192 and 9,832 of fingerlings of catfish and tilapia respectively (NaFIRRI, 2010). This kind of production is so low, compared to what is required to cover the 300,000MT fish gap that cannot currently be met by the national fisheries production, due to the dwindling wild fish harvests and low aquaculture production. It is therefore important to find strategies to increase both quality and quantities of fish seed production in the aquaculture sector of the country.

The African catfish is an important food fish species native to Uganda and is found in most of the natural water bodies – swamps, streams, rivers and lakes. The species is highly sought after as a preference in aquaculture in the country because of a number of its desirable attributes that make it attractive for aquaculture development. The species is easy to reproduce, accepts artificial feeds, tolerates high stocking densities, tolerates poor water quality, grows rapidly, its highly sought after in the local, regional and international markets, and its economically viable in pond culture systems - the most common culture system in the country currently (Matsiko & Mwanja, 2007). These attributes have generated a high interest in catfish farming in Uganda, but in spite of this interest, the enterprise has remained largely at subsistence level due to three major constraints - poor quality seed, rampant disease outbreak both in hatcheries and grow-out systems, and poor quality (low protein) feeds (Mwanja, 2007; Nalwanga et al., 2009; Akoll &
Mwanja, 2012). Feeds quality is being addressed through engaging the current fish feeds commercial producers and encouraging more investment in the area to create competition that may lead to improved quality. The poor seed quality is attributed to poor broodstock management, where a few broodstock have been bred over and over for long period of time (Matsiko & Mwanja, 2007). The inbreeding has resulted into poor farm yields with close to 2% fish in grow-out systems turning out to be deformed. The situation has led some farmers in opting for using uncharacterized broodstock collected from the wild whose performance is not known (Mwanja & Mwanja, 2009).

Nile tilapia is the second sought after species for culture in Uganda, the emerging use of more intensive production systems such as cages and tanks for the species grow-out production has greatly increased the demand for quality seed. Production of tilapia seed entails the use of earthen ponds, happas, hatching trays and concrete tanks systems. The quality and quantities produced vary differently within and between hatcheries, but are mostly characterised by low production.

In order to improve the quality and increase the quantity of African catfish and Nile tilapia fry/seed in Uganda and the East African region, in here we provide guidelines developed for best practices in broodstock management and quality seed production of the two species.
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Brood stock or brood fish are parent fish from which fry and fingerlings are produced. The quality and reliable supply of healthy fry and fingerlings having a sound genetic base is largely dependent on the successful stocking and rearing of brood fish. Therefore, brood fish collection, rearing and management are the most important parts of aquaculture activities. Importance of brood fish management is to:

- ensure quality eggs and sperm development
- increase fecundity
- produce stronger and disease free larvae and fry
- spontaneous and timely supply of seeds
- remove the inbreeding problem
- save endangered fish from extinction
- successful aquaculture with high production potentiality

Brood fish/breeders can be collected either from nature/wild or sourced internally and reared from the seeds produced in the hatchery and grow-out in fish farms. Selection of closely related breeders causes inbreeding problem resulting into weak, unhealthy and deformed fish seed as well as declining growth performance. Replacement of brood fish is required due to the declining performance in the hatchery production, brood fish mortality and to maintain genetic diversity. For African catfish, individual female brood fish of weight 0.5–1 kg are preferable because these have a substantial quantity of mature eggs and are easy to manipulate. The same female brood fish can be reconditioned and induced to reproduce artificially every 6-12 weeks without affecting either the quality or quantity of eggs obtained after stripping. For Nile tilapia individuals 150-250g is appropriate as starting broodstock as these can produced between 500 – 1000 eggs per spawning. The brood fish can be induced to reproduce every 5-7 weeks and be used for 15 - 24 months (Lazard & Legendre, 1996). The number of eggs decreases with age and size of broodstock and after 24 months broodstock should be replaced.

Preparation of brood stock development, conditioning and management facilities

Broodstock development and conditioning is done in earthen ponds or concrete tanks, but the tanks are mostly utilized for transition holding especially on the spawning day.
2.1.1 Key considerations:

- **Size/surface area, water depth, volume, and texture of bottom sediments** – The target number of fry/seed determine the number of broodstock and consequently the size of the conditioning facility. Broodstock should be stocked at 2fish/m$^3$ of surface area, with pond depth of 0.75-1m deep end. The texture of sediments should be fine not to harm the brood fish or in case of tanks the surface should be smooth to avoid bleeding by the brood fish from scratching on the walls of the tank by the brood fish.

- **Restraints in the inlet, outlet and the surface** - are important in conditioning catfish in ponds. The restraints are important for preventing intrusion of unknown fish from unknown sources as well as preventing the broodstock from escaping and jumping out.

- **Labeling and identification of a holding facility & its contents** – Ponds/tanks for the different purposes in seed production should be clearly marked/labeled for easier management.

- **Clean environment free from bushes** – this helps in elimination of predators and disease vectors.

- **The supply and drainage systems have to function properly** – This is to enable free flow of water and prevent flooding.

- **Conditioning the pond by liming with 0.1kg/m$^2$ of calcium hydroxide (hydrated lime)** – This is to stabilize pH to optimum level (pH 7-9) recommended for most freshwater fish; to disinfect the pond bottom; and to increase the availability of nutrients that stimulate natural food development in the pond.

- **Monitoring physico-chemical and biological parameters in the system** - Fish perform all their bodily functions in water. Because fish are totally dependent upon water to breathe, feed and grow, excrete wastes, maintain a salt balance, and reproduce, monitoring the physical and chemical qualities of water is critical to good broodstock performance. To a great extent water determines the success or failure of a seed production venture and the whole aquaculture operation.

**Temperature**

After oxygen, water temperature may be the single most important factor affecting the welfare of fish. Fish are cold-blooded organisms and assume approximately the same temperature as their surroundings. The temperature of the water affects the activity, behavior, feeding, growth, and
reproduction of all fishes. Metabolic rates in fish double for each 7°C rise in temperature. Farmed fish in Ugandan are generally categorized into warm water species based on optimal growth temperatures ranging from 24°C to 30°C. So for good broodstock performance the water temperature should be kept in this range or if possible provide the optimal temperatures. For catfish and tilapia the optimal temperatures are 27°C and 29°C respectively.

**Dissolved Gases**

Dissolved gases are those which are in a water solution. An example of gas dissolved in solution is soda water which has large quantities of dissolved carbon dioxide. The most common gases are oxygen, carbon dioxide, nitrogen, and ammonia. Concentrations are measured in parts per million (ppm) or milligrams per liter (mg/l), both units of measure are the same. (One ppm or mg/l is the same as one mg added to 999,999 mgs to total 1,000,000 mgs).

**Oxygen**

Dissolved oxygen (DO) is by far the most important chemical parameter in aquaculture. Low-dissolved oxygen levels are responsible for more fish kills, either directly or indirectly, than all other problems combined. Like humans, fish require oxygen for respiration. The amount of oxygen consumed by the fish is a function of its size, feeding rate, activity level, and temperature. Small fish consume more oxygen than do large fish because of their higher metabolic rate. The amount of oxygen that can be dissolved in water decreases at higher temperatures and decreases with increases in altitudes and salinities. To obtain good growth, fish must be cultured at optimum levels of dissolved oxygen. A good rule of thumb is to maintain DO levels at saturation or at least 5 ppm. Dissolved oxygen levels less than 5 ppm can place undue stress on the fish, and levels less than 2 ppm will result in death. Some warm water species such as catfish are better adapted to withstand occasional low DO levels. Fish are not the only consumers of oxygen in aquaculture systems; bacteria, phytoplankton, and zooplankton consume large quantities of oxygen as well. Decomposition of organic materials (algae, bacteria, and fish wastes) is the single greatest consumer of oxygen in aquaculture systems. Consumption of oxygen by nitrifying bacteria that break down toxic ammonia to non-toxic forms depends on the amount of ammonia entering the system. However, since other bacteria are present in pond and tank culture, a ratio of 6 mgs of oxygen to ≤0.1 mg of ammonia is recommended. Oxygen enters the water primarily through direct diffusion at the air-water interface and through plant photosynthesis. Direct diffusion is relatively insignificant unless there is considerable wind and
wave action. Several forms of mechanical aeration are available to the fish farmer. These include
the following categories: paddlewheels, agitators, vertical sprayers, impellers, airlift pumps,
venturia pumps, liquid oxygen injection and air diffusers.
Mechanical aeration can also increase dissolved oxygen levels. Because of the lack of
photosynthesis in indoor water recirculating systems, mechanical means of aeration is the only
alternative for supplying oxygen to animals cultured in these systems. Oxygen depletions can be
calculated, but predictions can be misleading and should never be substituted for actual
measurements.

*Carbon Dioxide*
Carbon dioxide (CO₂) is commonly found in water from photosynthesis or water sources
originating from limestone bearing rock. Fish can tolerate concentrations of 10 ppm of CO₂
provided dissolved oxygen concentrations are high. Water supporting good fish populations
normally contain less than 5 ppm of free carbon dioxide. In water used for intensive pond fish
culture, carbon dioxide levels may fluctuate from 0 ppm in the afternoon to 5-15 ppm at
daybreak. Excessively high levels of carbon dioxide (greater than 20 ppm) may interfere with the
oxygen utilization by the fish. There are two common ways to remove free carbon dioxide. First,
with well or spring water from limestone bearing rocks, aeration can "blow" off the excess gas.
The second option is to add some type of carbonate buffering material such as calcium carbonate
(CaCO₃) or sodium bicarbonate (Na₂CO₃). Such additions will initially remove all free carbon
dioxide and store it in reserve as bicarbonate and carbonate buffers.

*Nitrogen*
Dissolved gases, especially nitrogen, are usually measured in terms of "percent saturation." Any
value greater than the amount of gas the water normally holds at a given temperature constitutes
super saturation. A gas super saturation level above 110% is usually considered problematic. Gas
bubble disease is a symptom of gas super saturation. The signs of gas bubble disease can vary.
Bubbles may reach the heart or brain, and fish die without any visible external signs. Other
symptoms may be bubbles just under the surface of the skin, in the eyes, or between the fin rays.
Treatment of gas bubble disease involves sufficient aeration to decrease the gas concentration to
saturation or below.

*Ammonia*
Fish excrete ammonia and lesser amounts of urea into the water as wastes. Two forms of ammonia occur in aquaculture systems, ionized and un-ionized. The un-ionized form of ammonia (NH$_3$) is extremely toxic while the ionized form (NH$_4^+$) is not. Both forms are grouped together as "total ammonia." Through biological processes, toxic ammonia can be degraded to harmless nitrates. In natural waters, such as lakes, ammonia may never reach dangerous high levels because of the low densities of fish. But the fish farmer maintains high densities of fish and, therefore, runs the risk of ammonia toxicity. Un-ionized ammonia levels rise as temperature and pH increase.

Toxicity levels for un-ionized ammonia depend on the individual species; however, levels below 0.02 ppm are considered safe. Dangerously high ammonia concentrations are usually limited to water recirculation systems or hauling tanks where water is continually recycled and in pond culture after phytoplankton die-offs. However, the intermediate form of ammonia--nitrite--has been known to occur at toxic levels (brown-blood disease) in fish ponds.

**Buffering Systems**

A buffering system to avoid wide swings in pH is essential in aquaculture. Without some means of storing carbon dioxide released from plant and animal respiration, pH levels may fluctuate in ponds from approximately 4-5 to over 10 during the day. In recirculating systems constant fish respiration can raise carbon dioxide levels high enough to interfere with oxygen intake by fish, in addition to lowering the pH of the water.

**pH**

The quantity of hydrogen ions (H+) in water will determine if it is acidic or basic. The scale for measuring the degree of acidity is called the pH scale, which ranges from 1 to 14. A value of 7 is considered neutral, neither acidic nor basic; values below 7 are considered acidic; above 7, basic. The acceptable range for fish culture is normally between pH 6.5-9.0.
Figure 1: showing a pH scale (source: www.thepondcompany.com/part-1-testing-your-pond-water-water-testing)

Alkalinity

Alkalinity is the capacity of water to neutralize acids without an increase in pH. This parameter is a measure of the bases, bicarbonates (HCO$_3^-$), carbonates (CO$_3^{2-}$) and, in rare instances, hydroxide (OH$^-$). Total alkalinity is the sum of the carbonate and bicarbonate alkalinitities. Some waters may contain only bicarbonate alkalinity and no carbonate alkalinity.

The carbonate buffering system is important to the fish farmer regardless of the production method used. In pond production, where photosynthesis is the primary natural source of oxygen, carbonates and bicarbonates are storage area for surplus carbon dioxide. By storing carbon dioxide in the buffering system, it is never a limiting factor that could reduce photosynthesis, and in turn, reduce oxygen production. Also, by storing carbon dioxide, the buffering system prevents wide daily pH fluctuations. Without a buffering system, free carbon dioxide will form large amounts of a weak acid (carbonic acid) that may potentially decrease the night-time pH level to 4.5. During peak periods of photosynthesis, most of the free carbon dioxide will be consumed by the phytoplankton and, as a result, drive the pH levels above 10. As discussed, fish grow within a narrow range of pH values and either of the above extremes will be lethal to them. It is recommended that the fish farmer maintain total alkalinity values of at least 20 ppm for
catfish production. Higher alkalinities of at least 80-100 ppm are suggested for hybrid striped bass. For water supplies that have naturally low alkalinities, agriculture lime can be added to increase the buffering capacity of the water.

**Hardness**

Water hardness is similar to alkalinity but represents different measurements. Hardness is chiefly a measure of calcium and magnesium, but other ions such as aluminum, iron, manganese, strontium, zinc, and hydrogen ions are also included. When the hardness level is equal to the combined carbonate and bicarbonate alkalinity, it is referred to as carbonate hardness. Hardness values greater than the sum of the carbonate and bicarbonate alkalinity are referred to as non-carbonated hardness. Hardness values of at least 20 ppm should be maintained for optimum growth of aquatic organisms. Low-hardness levels can be increased with the addition of ground agriculture lime.

**Other Metals and Gases**

Other metals such as iron and sodium, and gases, such as hydrogen sulfide, may sometimes present special problems to the fish farmer. Most complications arising from these can be prevented by properly pre-treating the water prior to adding it to ponds or tanks. The range of treatments may be as simple as aeration, which removes hydrogen sulfide gas, to the expensive use of iron removal units. Normally iron will precipitate out of solution upon exposure to adequate concentrations of oxygen at a pH greater than 7.0.

*Figure 2:* Multi-parameter water quality probe for measuring most of the above mentioned water quality parameters.
2.1.2 Stocking broodstock for conditioning

Brood stock for conditioning are carefully selected from the overall population system basing on the following criteria: body conformity (shape, thickness, colour, and deformities), size and age, gonad conditions (mature, ripe or immature) and belly size (bulging belly of females indicate readiness). The female African catfish has a fully developed ovary which contains “ripe” eggs the whole year round, if kept in ponds and once the water temperature remains above 22°C. The eggs of a “ripe” female African catfish make up 15-20% of the body weight (i.e. a “ripe” female of 1 kg having about 150-200 g of “ripe” eggs) (FAO, 1996). The oocyte development decreases once the temperature drops below 22°C. In general the testis of a male African catfish is fully developed at an age of 8 to 12 months once they reach a weight of approximately 200 g (FAO, 1996). The stocking rate is 2 fish/m², about 1 kg body weight/m³ in ordinary conditioning pond where there is no aeration.

2.1.3 Feeding & feeding management:

The nutritional status of brood fish is one of the most important factors affecting fry production. The nutrition of fry destined to be used as broodstock is as important as its later nutrition during breeding. A well balanced compounded diet containing all the essential nutrient requirements, particularly the amino-acids, vitamins and minerals is a prerequisite for proper gonadal development. In immature fish a high-protein well-balanced diet is essential during the laying down of germinal tissue which contains the future egg stock. Similarly, egg development during breeding requires high quality and quantities of proteins and lipids. If food is restricted during this period the proportion of eggs finally maturing and ovulating will be reduced, resulting in lowered fry production. Further, in mouth-brooding species females deprive themselves of food during oral rearing and only feed for the short periods between the end of oral rearing and the next spawning cycle. Therefore, during the periods of feeding, abundant food of high quality needs to be provided to replenish lost energy and supply nutrients for the next spawning. The quality and quantity of broodstock diet will vary with the type of seed production system. For tilapia to reduce costs and provide a well-balanced diet, ponds may be fertilized to increase natural food production. Brood fish diet may then be supplemented with diets containing 20–30% protein. If clear water systems are used, a complete well-balanced diet must be provided.
For good fry yields brood fish should be fed on diets containing 35–45% protein. For catfish use feeds with crude protein ranging from 45-55% and less fat content. Feeding has to be done at least twice a day between 10am to 5pm daily. Employ stimulants such as noise or hitting the ground frequently from the usual feeding point. Sinking feeds are better for catfish broodstock because of benthic nature at that stage. Conditioning process takes at least three months of intensive management to produce the best results for both catfish and tilapia. The quality of the protein is also important. Fish meal is the best source of high quality protein but is expensive. For tilapia, applying chicken manure weekly at 200-250 kg DM (dry matter)/ha and supplementing it with urea and triple super phosphate (TSP) at 28 kg N/ha/week and 7 kg P/ha/week and stocking rate of 2 fish/m² will be sufficient. Similar yields are obtained solely with inorganic nutrients if alkalinity, a source of carbon, is adequate.

Figure 3: Showing powder fish feeds
Figure 4: Showing pellet fish feeds of 1.5mm

Figure 5: Showing pellet fish feeds of 2.5 mm
Figure 6: Showing pellet fish feeds of 5.0 mm

Figure 7: Showing feeding fish in a pond by broadcasting method (a lot of feed is lost using this method)
2.2 *African catfish spawning process*

In captivity the African catfish does not spawn spontaneously since the environmental factors such as the rise in water level and inundation of shallow areas do not occur on the fish farms. Since the early seventies several techniques have been developed (with or without hormone treatment) for the artificial reproduction of the African catfish (FAO, 1996). The various activities involved in the process of artificial propagation of the African catfish include: selection of brood fish from nature or from fish ponds, rearing of brood fish, inducing final maturation and ovulation with hormone treatment, procurement of ripe eggs by stripping, procurement of milt by dissection of a male donor, artificial fertilization, incubation and hatching of eggs, and rearing of larvae and fry. When the spawners are ready (running males and females), a spawning program can be organized and the following preliminary activities have to be considered: targeted number of fingerlings, available space (hatching, nursing and grow-out facilities), Weaning and nursery feeds (larval weaning, fry and fingerling rearing).

Use the predetermined target number of fingerlings to calculate number of broodstock to be used in the spawning programme. The number of females which should be treated with hormone for the production of larvae depends on the following characteristics:

a. Body weight of females. Female of 500 – 1000g are easy to manipulate and have a high absolute fecundity.
b. Stripping percentage. This is defined as the weight of stripped eggs x 100/body weight. Taking into account a security margin a stripping percentage of 10% may be used for calculations using “hatchery” females (year round) and 15% for “pond” females (during the reproductive season)
c. Number of eggs per gram. This number varies from 600 – 900. Use the average of 750 eggs/g for the calculations.
d. Hatchability - This is defined as the percentage of incubated eggs which develop into normal viable larvae. Hatchability ranging from 70 to 80% is normal under standard conditions. But for calculations use a mean of 50% hatchability.
e. Survival rate. Survival rate from larvae up to fingerlings of 1g is about 70%.
f. Production capacity of hatchery – This number of fingerlings of a particular fish species raised annually in a hatchery. For moderate well managed hatchery is expected to raise at least 500,000 fingerlings.

Based on these production data, the number of females required for propagation can be calculated as follows:

- Targeted number of fingerlings = 100,000
- Number of viable larvae = 100,000 x 100/70 (70% survival) = 143,000 larvae
- Number of eggs incubated = 143,000 x100/50 (hatchability of 50%) = 286,000 eggs
- Weight of eggs 286,000/750 = 382g
- Weight of females (stripping percentage 10% = 100g eggs/kg fish) 382/100 = 3.82 kg
- Number of females (500g) 3.82/0.5 ~ 8 females

2.2.1 Organizing the hatchery for spawning

Prepare a checklist of materials and requirements; check the worthiness of the hatching, nursery and holding tanks; check status of the aeration system; check status of the thermoregulatory system, alternative power sources; HEP, solar and thermo generators and even fuel wood; work out the water budget and ensure constant supply of clean water in required quantity throughout the hatching and nursery period; clean and disinfect all contact points including work tops, containers, hatching and broodstock holding tanks; test the system at least a day before the beginning of the spawning; and organize enough manpower depending on the scale of operation.
Figure 8: Digital weighing scale (for estimating total weight of eggs)

Figure 9: Aeration system (for artificial delivery of oxygen to the incubation/nursing system)
Figure 10: Fish bucket (for internal movement of brood fish)

Figure 11: Scoop net (for handling of brood fish during harvesting and spawning process)
**Figure 12:** Bowel and Sieve for use in holding and fertilization of the eggs

**Figure 13:** Dissection kit in opening the belly of the fish to access the male gonads that provide the milt and opening the heads to access the pituitary glands.
Figure 14: Thermoregulatory rods for setting the required water temperature during eggs hatching and larvae/fry nursing.

Figure 1: Thermoregulatory system used for controlling temperature of the water used in the fish hatchery
2.2.2 Harvesting and holding broodstock on the spawning day

When indoor hatchery unit is prepared, the right number of male and female brood fish is selected from the lot in the conditioning pond using the right procedure and facilities. This is done through the following procedures: reducing water level in conditioning pond, harvesting brooders using broodstock scoop net into plastic buckets, weighing females to determine the male biomass to use for induction and fertilization. Sex ratio F/M = 1:2kgs (1kg each for induction and fertilization), stocking a known weight and number of brood fish in resting tanks at least 6hrs prior to induction, stock separate sexes to avoid brood fish hurting each other and delay during the induction time, and keeping fresh clean water flow through system and covering with nets to avoid brood fish jumping out of holding containers/tanks. Ensure the tanks are smooth enough not to bruise the selected stock.

2.2.2.1 Sexing and criteria for selection of broodstock

Artificial reproduction starts with the selection of females from broodstock ponds, after which they are transferred to a holding tank within a hatchery. Ideally, brood fish weigh between 300-800 g, with larger fish being difficult to handle and often resulting in substantial egg losses prior to stripping. In general, mature females are selected according to the following criteria: A well distended, swollen abdomen from which ripe eggs can be obtained by slightly pressing the abdomen toward the genital papilla. Ripe eggs are generally uniform in size and an experienced hatchery operator can see the nucleus as a small dark point in the centre of the egg and a good percentage of Oocytes with nucleus at the periphery is good for predicting fertilization rate. For male broodstock there is only one criterion: they should be larger than 200 g and not less than 7 months old. The males are more elongated with protruding genital papillae and no gametes on gentle thump pressure along the belly.
2.2.3 Inducing females with pituitary hormones and procurement of milt

Use natural, on-spot extraction from catfish by sacrifice or synthetic already made for use. The operation requirements for pituitary extraction include: hand towel, syringes, strong hand knife/hacksaw, harmer, masticating rod & motor, physiology saline solution, dissecting kit, protective gear for personnel & work top.

2.2.3.1 Pituitary hormone extract - A male donor to female recipient average weight is 1:1kg. (one male donor per kg of female broodstock). Preparation of the pituitary gland solution and calculation of quantity of hormone solution to be injected involves use of the following calculation:

- Preparation of pituitary gland solution:
  - Number of females 08 females
  - Total weight (08x 0.5kg) 4.0 kg
  - Dosage 4 mg/kg body weight
  - Quantity of pituitary powder/solution 4.0 kg x 4 mg/kg = 16 mg
  - Quantity of solvent (1 mg/0.5kg body wgt) 16 mg x 1 mg/0.5kg body wgt = 8 ml
  - Solvent is made of
    - 10% glycerine (8 ml x 10/100) 0.8 ml glycerine
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- 90% salt solution (8 ml x 90/100) 7.2 ml salt solution

Calculation of hormone solution to be injected:
- Concentration of pituitary gland solution
  (16 mg/8 ml) 2 mg/l
- Weight of female 720 g (0.720 kg)
- Required quantity of pituitary gland
  (4 mg/kg x 0.720) 2.88 mg
- Required quantity of hormone solution
  (2.88 mg/ 2 mg/ml) 1.44 ml

2.2.3.2 Procurement of pituitary - Kill and decapitate the donors. Put the head upside down and cut the lower jaw away. The pituitary located inside skull. Wash the skull with clean water to eliminate blood clot and then open the palate mouth with a pair of pincers or hacksaw. The pituitary is a pinky-like organ, situated on the ventral side of the brain. Collect the pituitaries with a pair of forceps and put them in a mortar containing 2 ml physiology solution (9 g NaCl/l clean/distilled water). Masticate the glands & inject the suspension as soon as possible. Dosage 2 mls/kg weight female. While inducing, carefully hold the female brooder in a moist towel while covering the eyes.

Figure 3: Showing the open head of a catfish and location of pituitary gland indicated by the pointed pen
2.2.3.3 Post induction treatment - Stock the induced females in quiet conditioning tanks at least one female fish per container. Temperature difference between conditioning and propagation facilities should not be >1°C, raised gradually to an optimum level of 27°C. Very higher temperature may over speed ovulation leading to poor quality eggs & low fertilization. Keep a flow through system of temperature regulated water at rate not >5 litres/hr. Keep adequate water level and cover the conditioning tank with a small mesh size net to avoid escape. Keep the room dark after setting the system and leave the induced stock rest awaiting ovulation and stripping.

**Figure 18**: Indoor hatchery facility at ARDC-Kajjansi

**Figure 4**: Tank for nursing African catfish larvae/fry
2.2.3.4 *Preparation for stripping* - Make sure all the facilities are available immediately inducing is done to avoid late preparation. Arrange for enough manpower to man fertilize the following morning. At 27 °C, ovulation may occur between 8 to 12 hours. Better two people, one holding the tail while the other the head region and stripping. Hold fish with moist towel and cover the eyes of the fish while stripping. Either strip first or strip in milt solution (dry or wet fertilization).

![Figure 20](image1.png) **Figure 20:** Crashing male tests to produce milt for use in egg fertilization

![Figure 21](image2.png) **Figure 21:** Stripping a ready female catfish to obtain eggs
2.2.3.5 Collection of milt - Kill a male (average weight 1 – 0.5kg) - About one hour before stripping, the sperm must be obtained from a male spawner. Milt is obtained by sacrificing the male and dissecting the testis. Open the body without damaging the inner organs and entirely remove the two yellow-pink/cream testes without squeezing them. Dry the testis with a piece of filter paper and then make some small incisions into the lobes of the testis. Milt is then easily squeezed out and collected into a vial or small bottle. In this way, several droplets of milt can be obtained, after which the milt is diluted with physiological salt solution (0.6–0.7% NaCl). It is essential to avoid any contact with water otherwise the sperm will lose fertilization activity. The milt solution can be stored in a refrigerator for one or two days without affecting its activity.
Figure 5: Exposed testes of the African catfish

Figure 24: Making incisions into the testes during the collection of milt
2.2.3.6 **Checking sperm viability** - To check the sperm viability put droplet of sperm solution on microscope glass slide and add a droplet of water. Observe the motility of the spermatozoids with microscope (magnification 100X). If they are actively moving for 30 second, the sperm will be of good quality.

2.2.3.7 **Determination of egg production** - There are a number of methods used to determine egg production, which include the following:

i. **Area count method** – In this method the total eggs produced is determined by counting eggs in gazetted sub samples of the overall quantity spread on the tray. Number of eggs is calculated as a function of the total number of eggs/mm3 x overall area of the tray.

ii. **Gravimetric estimation** - is based on weighing the eggs. After the eggs have been liberated from the ovarian tissues, they are thoroughly washed and spread on blotting paper to dry in air. The total number of eggs is then weighed and random samples of about 500 eggs are counted out and weighed. The total number of eggs in the ovaries is then obtained from the equation $F = nG/g$ where $F$ = fecundity, $n$ = number of eggs in the subsample, $G$ = total weight of the ovaries, $g$ = weight of the subsample in the same units. Or if Gilson's fluid is not available it is possible to estimate fecundity using this method by weighing both ovaries and then taking subsamples which are weighed. The eggs are teased out with a pair of needles and counted. At least three subsamples should be taken from each ovary, one from the anterior, one from the middle and one from the posterior. The method is tedious and less accurate than if it had been possible to use Gilson's fluid. Its great advantage is that it does not require large volumes of an expensive fluid and can be used in the field. It is particularly useful for fish with low fecundity and large eggs.

iii. **Volumetric estimation** – After separation in Gilson's fluid the cleaned eggs are put in a measuring cylinder and made up to a known volume with water. Subsamples are then taken by shaking the container until all the eggs are evenly distributed through the water, a subsample of known volume withdrawn with a Stempel pipette, and the number of eggs in the subsample counted. The fecundity is then $F = nV/v$ where $n$ = number of eggs in the subsample, $V$ = volume to which the total number of eggs is made up and $v$ = volume of the subsample.
**2.2.3.8 Determination of fertilization rate** - The fertilization rate is determined as percentage ratio of the embryo to the total number of eggs produced. i.e., B/AX100, where A is total number of eggs produced & B, total number of fertilized eggs.

**2.2.3.9 Determination of hatchability** - Hatchability is estimated as percentage ratio of hatched larvae to the total number of fertilized eggs /embryo.

**2.2.4 Management of incubation process**

**2.2.4.1 Egg treatment** – this involves weighing, prophylaxis, acclimatization, introduction to the hatching trays, and maintenance of water quality. Fertilized eggs are incubated in running water in troughs containing small trays. These trays have a perforated bottom (diameter of the holes: 1.2–1.5 mm) which can also be made of mosquito netting. The incubator is filled with clean, well oxygenated water, free of plankton organisms. The eggs are spread homogeneously in one single layer in the incubation tray. These trays are made in such a way that the eggs are continuously oxygenated by circulating water. About 100–150 g eggs can be incubated in a trough containing about 80–100 l of water. If no incubation trays are available, fertilized eggs can be placed directly on one half of the bottom of the trough towards the outlet. Once the fertilized egg comes into contact with water, it starts to swell and becomes sticky. The stickiness is strongest after 30–60 sec and disappears with time towards the end of the incubation period. Therefore, incubation must be started promptly after fertilization, at the most 60 sec after adding water to the egg mass.

The incubation in perforated trays aides the separation of healthy larvae from egg remnants and spoiled eggs automatically. The viable larvae seek shelter by passing through the perforated bottom of the tray by actively swimming, leaving behind deformed larvae, dead eggs and empty shells. The trays are removed as soon as hatching is complete and normal larvae have gathered under the incubation trays.

**2.2.4.2 Separation of larvae from egg shells and spoiled eggs** - Catfish larva is, like all fish larvae, very different from adult fish. Most of its main organs such as barbells, mouth, gut, gills etc. are not yet developed. The yolk sac contains high quality reserve food for growth and development during the larval stage. The weight of hatchlings is about 1.0–1.5mg, and its total length is about 4mm. They will gather in dark places on the bottom of the trough Larval. In the
case of incubation in perforated incubation trays the separation of healthy larvae from egg remnants and spoiled eggs take place automatically. Only viable larvae, looking for shelter, pass through the perforated bottom of the tray by actively swimming, leaving behind deformed larvae, dead eggs and empty shells. The trays are removed as soon as hatching is complete and normal larvae have gathered under the incubation trays. In the case of incubation in trays with mosquito netting the separation is less complete because most of the deformed larvae fall into the trough due to too big mesh of the mosquito netting. Even small fertilized eggs will pass this type of trays. The crippled larvae are siphoned off on the second day after hatching. The first day after hatching the “swimming” capacity of larvae is not yet well developed, and viable larvae will be wasted by too early siphoning. When fertilized eggs have been placed directly on the bottom of the incubation device, separation is obtained by covering the eggs - free part of the incubator. The healthy larvae will swim into the shadowed part under the cover and cluster at the edges of the tank. Egg shells, dead eggs, and deformed larvae are removed by siphoning.

2.2.5 Larval rearing
The technology employed for mass rearing of larvae and fry in indoor facilities is the flow-through technique. This technique is based on the following principles:

i. inflowing water ensures water quality requirements

ii. inflowing water replaces the “used” water permanently

iii. out flowing water removes the accumulated metabolites and feed remnants

iv. Fish are concentrated in a relatively small, “easy to control” area.

Larval rearing involves the following processes: weaning of larvae on live feed, weaning larvae on Artemia replacer, preparation of Artemia for larval weaning, administration of Artemia and storage of Artemia nauplii. The water level in the larval tank can be adjusted by changing the position of the stand pipe or turn-down pipe. A fine mesh (≤ 1.0 mm) screen is placed diagonally just anterior to the water outlet. The screen should be cleaned several times a day (removal of accumulated waste matter) to prevent over-flowing of the rearing device and loss of young fish. The screen can be cleaned automatically by installing an air stone under the diagonally placed screen.
2.2.5.1 Larval nursing stocking density - After hatching, the rearing tank as described earlier may contain about 45,000 to 70,000 larvae. The recommended water level in the larval rearing device is about 12 to 15 cm which corresponds with about 100 to 120 l water and a stocking density of about 375 to 700 larvae per litre.

2.2.5.2 Water flow rate/Oxygen - Larvae need highly oxygenated environment, preferably air saturated. It is advisable that the dissolved oxygen level does not fall under $\geq 5$ mg/l. This can generally be obtained with a water flow rate of about 3–5 l/min. Catfish larvae, which gather on the tank bottom, beat their tails unceasingly. This would force the water around their body to move in order to ensure sufficient oxygenation. A very high water flow rate, which may press the larvae against the filtering surface, should be avoided. The dissolved oxygen content of the outflowing water must be measured at least once every day.

2.2.5.3 Temperature - The optimum temperature for rearing of catfish larvae and young fish is about 30°C. Too low ($< 22^\circ$C) and too high ($> 36^\circ$C) temperature, retarding larval development, considerably should be avoided.

2.2.5.4 Water quality - Clean, highly oxygenated water free of parasites and predators is a necessity for rearing of young fish stages under hatchery conditions. The larval rearing facility must be cleaned (removal of incubation remnants, dead or deformed larvae and waste matter) once or twice daily by careful siphoning.

2.2.5.5 Health management and hygiene – Provide adequate hygiene in the hatchery. In addition to proper hygiene, prophylactic treatments (bathing) to prevent disease outbreak should be applied once a day. Expensive antibiotics such as oxytetracycline, neomycin, streptomycin, chloramphenicol and other chemical bactericides such as sulfonamides are not advised for prophylactic use. They should only be employed for therapeutic treatment in the case of emergency. Yolk sac oedema due to bacterial contamination of fertilized eggs may sometimes occur. In the case, bathing with 50 ppm oxytetracycline for 1 hour should be done during 4 to 6 days. From then on, it is advisable to disinfect eggs before incubation with iodine solution (25 ppm Wescodyne or Betadine for 5 min.) to prevent vertical contamination (contamination of eggs by brood fish). Contamination by pathogens can be avoided by using boiled water for incubation of eggs (stagnant water technique).
2.2.5.6 Production of fresh water zooplankton for catfish larval feeding - Preparation of the culture medium - The most critical factor for successful nursing of the African catfish is the availability of zooplankton during the first week after the ponds are stocked with hatchlings as they feed only on live food. Live feed is provided through well fertilized ponds. Filling of ponds and fertilizing with dry chicken manure at a rate of 50kg/100 m$^2$ one week before stocking can provide a nice phytoplankton bloom prior to stocking. Feeding of the catfish must start immediately after stocking and the following feeding rates are recommended in case a composed feed is used (with fish meal as animal protein source) as follows:

- First week – 0.50 kg/100 m$^2$/day
- Second week - 0.75 kg/100 m$^2$/day
- Third week - 1.00 kg/100 m$^2$/day
- Fourth week - 1.25 kg/100 m$^2$/day
- Fifth week - 1.50 kg/100 m$^2$/day

2.2.5.7 Preparation of Artemia nauplii – Origin eggs - dried brine shrimp eggs of cysts (Artemia salina) are collected in high saline pools and shallow lakes. They can be obtained from commercial companies on the market. The quality of the dried eggs varied enormously from source to source, and between different batches from the same source.

Decapsulation - Decapsulation of Artemia eggs is preferable for the following reasons:

i. increase in hatching percentage
ii. decrease the incubation time (less than 24hrs at 25°C)
iii. disinfection of eggs
iv. No separation required between eggs shell and nauplii
v. Catfish digest un-hatched decapsulated eggs in addition to nauplii. (No wastage of non-hatched eggs)

Artemia eggs are decapsulated by treating them with a commercial hypochlorite or bleach. Hydrate 300 g of cysts in 5 l of fresh water with vigorous aeration for one hour, filter and wash the hydrated eggs on a 150 micron screen, add the eggs to 4 l commercial or prepared hypochlorite solution in which 40 g sodium carbonate has been dissolved, keep the eggs in suspension by continuously stirring, after 7–10 minutes, when the eggs have become orange in colour filter through a 150 micron screen, wash thoroughly several times to eliminate all
hypochlorite remnants until there is no more smell of chlorine, stir well and allow cysts to settle, siphon off unbleached eggs and debris from the surface, and then filter off the decapsulated eggs with the fine mesh screen. Decapsulated eggs can be either incubated by sea water or preserved in a saturated salt solution (300g NaCl per litre).

**Incubation** - Decapsulated eggs are incubated in funnel-incubators. A 15 l incubator contains up to 100 g eggs. The technique consist of: dissolve 375 g clean kitchen salt (NaCl) in 15 litres clean water, add 30 ml of 0.5 m sodium carbonate (Na₂CO₃) solution to buffer the incubation solution, aerate vigorously and adding 100 g of decapsulated eggs. Maintain adequate aeration and proper illumination (tube light) at ambient temperature, for 24–48 hours. At a water temperature of about 24–26°C, the nauplii hatch in 20 to 24hrs. The Artemia solution may be fed directly to the fish with a 50 or 100 ml pipet (the Artemia incubation water is not harmful for catfish and may be added to the rearing tanks). Sometimes, it is convenient to separate unhatched eggs and nauplii. This can be done by stopping aeration. The unhatched eggs settle first, followed by the nauplii. The two organisms can be siphoned off separately using a pipet or small rubber hose.

### 2.2.6 Enterprise budget for a medium scale commercial catfish hatchery operation in Uganda

<table>
<thead>
<tr>
<th>ITEM DETAILS</th>
<th>UNIT</th>
<th>QUANTITY</th>
<th>RATE</th>
<th>TOTAL</th>
</tr>
</thead>
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<td>Preliminaries</td>
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<td></td>
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<td>2,120,000</td>
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<tr>
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<td>Month</td>
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<td>Broodstock (1kg female: 2kg male)</td>
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<td>Larval feed &amp; disinfectants</td>
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<tr>
<td>Tax &amp; banking</td>
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<tr>
<td>--------------------------------</td>
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</tr>
<tr>
<td>Total variable cost (TVC)</td>
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<td>Net Returns Above TVC</td>
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<td>Total fixed cost</td>
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<td>Total cost (TC)</td>
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<td>Above TC</td>
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<tr>
<td>Net Returns Above TC in 3months period</td>
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</table>

### 2.3. Nile tilapia seed production

#### 2.3.1 Broodstock development

**2.3.1.1 Rearing facilities** - the three most commonly used facilities include: hapa net cages installed in ponds, concrete tanks, and earthen ponds. Hapa net cages are made from net material of mesh size 0.5 mm. The hapas are submerged at a depth of at least 1.0-1.5 m, leaving an allowance of 0.5-1.0 m of the net above the water surface. The hapas may or may not be covered. Concrete tanks are constructed in series and of rectangular shape. Each compartment is designed with a catch basin occupying about 15-20% of the floor area with depth of about 10-12 cm. This serves to collect both the breeders and fry during harvest. Water is drained through a removable PVC (polyvinylchloride) stand pipe, which also maintains the water level at 50-75 cm. Earthen ponds with rectangular shape having an area of 100-320 m² per pond. Each pond is provided with water inlet and outlet, both of which are protected with a net barricade. Water level in the pond is maintained at a depth of at least 70 cm. Choice of facility will depend on available resources and the demand for fingerlings.

**2.3.1.2 Broodstock selection** – Nile tilapia with weights ranging from 100-250 g are used for breeding. The stocking density varies according to rearing facility used. In hapas, the breeders are stocked at 5 females/m³. In concrete tanks, a density of 4 females/m³ is used without aeration and 6 females/m³ with aeration. A lower density of 2 females/m³ is used in earthen ponds. The
sex ratio for all types of breeding facility is 1:2-3 male to female. The broodstock are fed daily with formulated dry pellets with 40% dietary crude protein. The feed is given twice daily at 3.0% of the biomass, once in the morning and once in the afternoon.

Tilapia broodstock are selected from the wild and from different grow-out systems like pens, cages, and ponds at BFS and from other government and private institutions. Broodstock from fishpens and earthen ponds are collected by seining. Harvested fish are held in a more convenient container like small cage or tank with aeration for the selection process. From the cage or tank, fish are directly transferred to the transport containers. At least one day before transport, the broodstock are temporarily held in tanks with aeration for conditioning.

Sex is determined during the selection process, by examining the genital papillae located near the anal vent. Males have pointed papillae with one opening, the urinogenital, while females have rounder papillae with two openings, the urinogenital and the ovipository. In general, males are larger than females of the same age.

2.3.2 Systems for producing tilapia fingerlings

A number of systems are used for producing tilapia fry and fingerlings with varying production capacities. The production capacities are affected by many variables including environmental factors such as temperature and water quality, the management practices and skills of the producer, fish health and others. These systems include:

2.3.2.1 Single grow-out pond - This system is the simplest and requires only one pond. Fingerlings are stocked in the pond and cultured for a full production cycle. Some reproduction occurs during this time and the resulting fingerlings are restocked into the same pond for grow-out after the food fish are harvested. Fingerling holding facilities are required while the grow-out pond is being prepared for restocking. One production cycle ranges from 4 to 6 months. Numbers of fry and fingerlings produced in this system are low because of crowding and cannibalism. Estimated production is roughly 2000 – 5000 fingerlings per production cycle of 4 - 6 months, mostly subsistence level farming.

2.3.2.2 Reproduction pond - this system employs a separate pond for reproduction. Brood fish averaging 100 - 200 g are stocked in this pond for spawning. Their fry grow into fingerlings weighing from 1 to 15 g. Continuous partial harvesting (once every two weeks) of fingerlings with nets of mesh sizes ranging from 6 to 12 mm, depending on fingerling size desired,
beginning 5 to 7 weeks after stocking the brood fish. Fingerlings are transferred to other facilities for culture to larger sizes. The reproduction pond is drained, prepared and restocked with brood fish every 6 to 8 months. A one pond operation is possible. Fingerlings obtained from this system are more uniform in age and size than fingerlings produced using the single grow-out pond. Partial harvesting results in increased fingerling production and growth due to reduced cannibalism and over crowding. This system is estimated to produce an average of 3000 fry of 1 g per 100 m² every two weeks can be used for commercial fingerling production.

2.3.2.3 Multiple ponds - The objective of this system is to produce 20 g male fingerlings in nursery ponds. The multiple pond system requires at least 2 ponds. A reproduction pond produces 1 to 2 g mixed-sex fingerlings which are harvested and stocked into a nursery pond for culture to approximately 20 g. They are then harvested and sorted by sex. Males are used in monosex tilapia culture where food fish of at least 200 g are preferred by the market. This system is designed for commercial operations with high fingerling requirements where control of reproduction in grow-out ponds is desirable, and for specialized markets where the additional expense of producing fast growing, all-male fish is justified. Two to three production cycles per year are possible. This system is estimated to produce an average of 3000 fry of 1 g per 100 m² every two weeks and during the nursery phase – 400 fingerlings of 20 g all-male fingerlings per 100 m² per 9 weeks

2.3.2.4 Net enclosures or hapas - Brood fish are stocked into net enclosures called hapas for reproduction. Fry are collected and transferred to other hapas, ponds or tanks for further culture into fingerlings or food fish. Complete removal of fry from the breeding hapa eliminates cannibalism by parent fish and siblings. Fry are concentrated in a small area so maximum recovery rates are achieved. Total fry production per unit area is much higher than previous systems. Hapas may be moved and set up in a variety of locations, but are especially well suited to lakes and ponds. Continuous production is possible. This system is estimated to produce an average of 1000 fry per 4 m² hapa per week with continuous production possible

2.3.2.5 Tanks - Tank production of tilapia fry and fingerlings is practical where space for ponds is limited or expensive to develop. Cement tanks are common, but other materials, such as fiberglass or plastic lined pools, may be used. Greater control over water management and routine maintenance is possible than with other systems. Fish may be easily collected with dip-
nets or a small seine, and well-built tanks can last a lifetime. Continuous production is possible. Fry yields per unit area are higher than all the reproduction systems described except for net enclosures. This system is estimated to produce an average of 6000 - 8000 fry per 8 m² tank per month with continuous production possible.

2.3.3 Sex reversed tilapia fry production by hormonal treatment

2.3.3.1 Feed and Feeding Schedule - A special formulated feed enriched with protein and vitamin E, which enhances the gonad maturation in tilapias, is fed to the brood stock. Feed is applied by weight at the rate of 4 % total body weight of tilapia two times daily at morning and afternoon. This is supplemented with regular manuring chicken droppings or cow dung done at 15 days interval at the rate of 5kg / decimal and inorganic fertilization done with urea and TSP at the rate of 100 g / decimal and 80 g / decimal respectively to stimulate the growth of plankton. Procedure of monosex.

2.3.3.2 - Tilapia fry production egg collection and incubation – check adult females' mouths for eggs every seven days. Place the collected eggs or fry in buckets and transfer to incubation jars or aquaria/tank respectively at the hatchery. The fertilized eggs in the incubation jars start hatching after 72 hours of stocking. Install water flow through in the jars from the bottom to the surface leading to continuous movement of the eggs inside the jar. The hatchlings after absorption of their yolk-sac should swim out of the incubation jars with the water flow and are collected and taken to nursing tanks/ponds.

2.3.3.3 Larval rearing in Tanks/ponds – A rectangular tank (8 x 4 x 2) m or pond 100 - 200 m² is used for rearing the hatchlings/larvae. These kind of facilities can carry up to 40000 fry. Hatchlings are fed with 17 alpha-methyltestosterone mixed with powdered feed immediately after absorption of their yolk sac. The hormone is mixed at the rate of 60 mg / kg. Larvae are fed six times daily to apparent satiation at the rate of 100 % by body weight. Provide additional oxygen to the tank through aeration using motored aerators. Rear the larvae for 3 days before transferring to the hapas set in a pond.

2.3.3.4 Hormone feed preparation - Dissolve 60 mg of 17 alpha-methyltestosterone (MT) in 1 L of ethanol, this amount is fit for 1 kg of food then blend the food with the solution. Dry the food in a shade and keep it in cool and dry place and later store in a fridge. Use a new portion every
week and use the right texture of feed considering the mouth size of the larvae. Feed the larvae to satiation. Use gloves and where possible wear face mask to protect the eyes. Success can be measured in 5g size.

2.3.4 Key considerations during Nile tilapia fingerling production

Culture facilities require sufficient, good quality water free of harmful chemical substances; all facilities should be cleaned and maintained on a routine basis. Hapas require periodic scrubbing to remove organisms and debris which clog the netting and prevent water circulation; ponds and tanks should be built where they will not flood; pond inlets and outlets should be screened to keep out predators; ponds should be exposed to sunlight so that adequate plankton can be produced as natural food; reproduction and nursery ponds should be dried after each production cycle to eliminate small tilapia, wild fish or other undesirable organisms; ponds and tanks for commercial fingerling production should be completely drainable and have catch basins.
### 2.3.5 Enterprise budget for Nile tilapia hatchery operation using the reproduction pond (total area = 1000 m³) system for one year

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<tr>
<th>Item</th>
<th>Description</th>
<th>Unit</th>
<th>Unit price (UGX)</th>
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<td>Facilities</td>
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<tr>
<td><strong>Total Fixed Costs (TFC)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>800,000</td>
</tr>
<tr>
<td><strong>TOTAL COSTS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>70,599,360</td>
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<tr>
<td><strong>Net returns above TC</strong></td>
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<td>15,800,640</td>
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3.0 References


