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Guidelines for setting up breeding experiments for small indigenous species (SIS)

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Guidelines for setting up breeding experiments for small indigenous species (SIS)

Authors

Francois Rajts, Ben Belton and Shakuntala Haraksingh Thilsted.

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Contact

WorldFish Communications and Marketing Department, Jalan Batu Maung, Batu Maung, 11960 Bayan Lepas, Penang, Malaysia. Email: worldfishcenter@cgiar.org

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Executive summary

This report describes in detail steps and considerations in the design of hatchery-based breeding trials for small indigenous fish species (SIS) native to South Asia. The aim of the trials is to develop to replicable protocols for the mass production of several micronutrient rich SIS.

Once established and documented, techniques for mass production of SIS seed will be disseminated widely to hatcheries in the region to support the commercialization and scaling up of nutrition sensitive SIS aquaculture.

The report provides detailed coverage of a wide range of important topics, including hatchery selection, water supply, pond layout and management, hatchery and incubator design, water quality requirements, predator management, induced breeding techniques, feeding, nursing, and transportation.

The report also includes annexes covering topics such as protocols for setting up feeding experiments and experimental design, forms for daily record keeping, fish sampling, and water quality management, incubator design specifications, and a fish transportation checklist.

As such, the report will serve as an invaluable resource to anyone interested in establishing fish breeding experiments for new species or involved in the management or operation of a freshwater fish hatchery.

1. Introduction

WorldFish is implementing a GIZ-supported project called "Taking Nutrition-Sensitive Carp-SIS Polyculture Technology to Scale", in Odisha and Assam states in India. Techniques have been successfully developed for farming SIS in polyculture with carps, and in rice fields, but a lack of readily available SIS seed produced by hatcheries is the key technical bottleneck inhibiting the scaling up of nutrition sensitive aquaculture. The project therefore aims to develop induced breeding techniques for several micronutrient rich small indigenous fish species (SIS).

Six SIS - Mola carplet (*Amblypharyngodon mola*), Pool barb (*Puntius sophore*), Flying barb (*Esomus danrica*), Dhela (*Rohtee cotio*); Banded gourami (*Cholisa fasciata*); and Koi (*Anabas testudineus*) have been selected for potential inclusion in induced breeding trials, to be implemented by partner hatcheries as part of the project.

This report was prepared to provide a set of guidelines for the development of induced breeding techniques for these target species, and for use more widely by anyone interested in breeding small fish species or operating a freshwater fish hatchery.

The report gives detailed recommendations on topics including:

- Site selection criteria.
- Water supply and water quality requirements.
- Pond and hatchery layout and design.
- Broodstock and nursery pond management.
- Development of breeding techniques, and alternative breeding methods, with prioritization given to environmental stimulation.
- Feed composition trials using locally available ingredients.
- Transportation guidelines for fish seed, breeders, and market-sized fish.

The report also includes annexes on protocols for setting up feeding experiments and experimental design, forms for daily record keeping, fish sampling, and water quality management, incubator design specifications, and a fish transportation checklist.

Nest of *Colisa fasciata*, a micronutrient rich SIS candidate for breeding trials.

2. Site selection criteria

For site selection, existing hatcheries are preferable. Selecting appropriate sites is a prerequisite for success of any planned induced breeding trials. The most important factors for site selection are as follows:

- The hatchery and the ponds must be operational.
- The hatchery/landowner/partner and staff must be experienced and reliable. Considering the continuous nature of hatchery operations, it is crucial to have devoted staff. One batch of a hatchery operation generally lasts at least 1 week, and supervision is necessary 24 hours a day, 7 days a week. For this reason, the hatchery must have sufficient staff in place for rotating shifts to maintain operations 24 hours every day, as well as a room for staff on night duty.
- For trial operations, the owner must provide staff with hatchery and fishing equipment, one full set of breeding tanks or devices, and at least four small ponds of about 200 m^2.
- The land or farm must be free of any legal complications.
- The site must be close to the landowner's home for security reasons.
- The farm must be equipped with sanitary toilets.
- The highest experienced flood level must not exceed the top of the pond dikes or the floor of the hatchery and pump house, stores, etc.
- The hatchery must have a consistent, full-time supply of electricity. In addition, it must also have a standby generator as well as a standby diesel pump for hatchery water supply, in case of electricity failure.
- The hatchery will preferably have a water supply from a borehole. The quality of the water must be suitable for use in the hatchery use, with parameters pH (7–8.5), iron (<0.5 ppm) and total dissolved solids (TDS) (<2000 mg/L). High TDS is preferable to stimulate gonadal development and breeding by manipulating the environment, such as using rainwater for sudden dilution of the water.
- It must have an aeration tower to aerate the water before it reaches the storage tank.
- It should have an additional supply of runoff rainwater from small streams, ditches or an old *bundh* (bundh is a traditional breeding system in India, using a rainwater collection pond).
- The hatchery must have space to install additional tanks and incubators to prevent interference with the main hatchery operations. Installing additional devices may also be necessary.
- It should have available land in the likely event that small ponds need to be built. It would require about 1500 m^2 for broodstock, SIS nurseries and feeding trials.
- Soil quality must be suitable for pond construction.
- Existing ponds must retain water throughout the year. If not, an additional water supply will be necessary.
- It should have a water area of at least 1000 m^2.
- Gravity is the preferred method for water supply and drainage of the ponds.
- It should be possible to dry the pond, so the bottom of the pond should be above the water table.
- The trials are intended to develop SIS culture in Southeast Asia, so the owner should allow the project to conduct training courses on trial results in his hatchery and pond site.
- The location of the farm and connecting roads must allow for demonstration activities and easy marketing of farm products.
- Regarding biosecurity, although hazardous chemicals are either used in very small quantities or not at all, effluents from the hatchery should not be released directly into natural water bodies. If possible, they should be released into ponds, where they should remain for no less than 1 week. In the absence of a borehole, if water is recycled for use in the hatchery, there should be at least two consecutive ponds used to treat the water before it is reused. There should be no fish the second pond, and the growth of dense aquatic vegetation should be allowed to ensure clean water. There must also be fencing around the farm to prevent the entrance of predators and disease-carrying fish, such as walking catfish, climbing perch and frogs.

A summary of the criteria is presented in Annex 5 to facilitate evaluation during the selection process of farms.

3. Breeder requirements and design of broodstock ponds

The aim of the trials is to develop and disseminate large-scale seed production technology of SIS to other hatcheries. Plans for reproduction include at least five species. This would require large numbers of ponds, which may not be easily available. For this reason, alternative methods are also proposed.

All ponds should have a water depth of 1–1.5 m and controlled water inlets and outlets. Gravity is the preferred method for water inflow and drainage. Grass needs to be planted to protect ponds slopes from erosion, and trees should be far enough from the pond to allow full sunlight and wind. Ponds containing climbing perch should have fencing all around to prevent them from migrating during rains.

3.1. Broodstock ponds and breeder requirements

For species in which males are smaller than females, such as mola, darkina and dhela, 20 kg of female breeders and 10 kg of male breeders are required. The breeders need to be kept separated by species in earthen ponds during the off-season and further separated by sex during the breeding season. Spent fish require a further separate pond for quick recovery and preparation for additional breeding.

In a 1000 m^2 carp broodstock pond, the usual number of breeders, at an average weight of 2.5 kg, is 60–80 fish and 150–200 kg. This represents a stocking density of 0.06–0.08 fish per m^2 and 150–200 g/m^2. When conducting a pilot breeding trial of SIS, the stocking density should be no higher than 100–150 g/m^2. Because SIS are small, the tendency is to stock at higher density, but this increases the risk of spreading parasites or diseases and increases food competition. A low density would ensure better fish growth and health as well as pond conditions. Since the nutritional requirements of SIS are not well known, it is especially important to have an adequate amount of natural food available in the pond for the fish. Further investigation is necessary to find out the possible upper limits of the stocking densities of SIS breeders.

The following are the ideal conditions for each SIS under actual trials:

- One pond of 200 m^2 for breeders.
- One pond of 100 m^2 for spent breeders.
- One pond of 100 m^2 for male breeders, if possible, to separate the breeders by sex.

3.2. Maturation ponds

Also called "ante ponds", these ponds are used to prepare breeders for induced breeding over 1–2 weeks. In these small ponds, flushing and temperature control are easier than in the larger broodstock ponds. For this, there should be four ponds with a 50 m^2 water area. Two sources of water are needed: one from a borehole and the other from rainwater runoff. The width of the ponds should be about half of the length so that a greenhouse-type covering can be installed (Plate 1), as ponds require full exposure to sunlight all day long. The water depth should be about 0.8 m.

3.3. Breeding ponds

These ponds are specifically made for spawning using mainly environmental stimulation to simulate natural flooding in a floodplain. Prior to the development of induced breeding techniques in hatcheries, this type of pond, called a *dubich* pond, was used in Europe for common carp breeding. The bottom of the pond has a deeper ditch-type area and a higher flat part, which is covered with grass or other vegetation. The higher flat part has about a 1% slope toward the deeper part of the pond. Screens protect both inlet and outlet structures, and the preferred water supply comes from both a borehole and surface water.

3.4. Ponds for spent fish

These are similar to a broodstock pond, except they have a smaller water area and are about half the size. Following breeding operations, breeders are kept there for recovery over a few weeks.

3.5. Nursery ponds

A high number of small nursery ponds is preferable to allow at least three replications for each treatment, as well as for demonstration purposes. This can be arranged by involving professional nursery operators and/or partner nongovernmental organizations with access to homestead ponds.

A high number of ponds is likely not available, so feeding trials can be done using hapas. The advantage of conducting trials in hapas is that all treatments with all replications are done in the same pond under the same water quality. However, for the trials to be done properly, the hapas must be maintained and cleaned to allow proper water circulation. The protocol for feed comparison trials in hapas is presented in Annex 1.

3.6. Alternative arrangements

If it is not possible to use the structures listed in sections 3.1-3.5 due to a shortage of ponds, using existing small ponds or ditches will be necessary. This may require digging up the earth and constructing a water supply and drainage structures. The minimum size of these ponds is 50 m^2 each, the same as breeder preparation ponds. If broodstock ponds cannot be established, breeders should be procured a few weeks before induced breeding and then kept for breeding preparation in these small ponds.

Plate 1. Solar heating of a broodstock pond in Vietnam.

4. Water quality

Fish physiology, including appetite, growth, metabolism, oxygen consumption, gonadal development, migration for breeding, breeding, and immune function, depends on the physicochemical characteristics of the water. For induced breeding trials of SIS, the water quality parameters should fall within the following acceptable ranges (Table 1).

Substance	Desired concentration
Oxygen (O_2)	5–15 mg/L
Hydrogen (H^+)	pH 7–9
Ammonium (NH_4)	0.2–2 mg/L
Ammonia (NH_3)	< 0.1 mg/L
Nitrate (NO_3)	0.2–10 mg/L
Nitrite (NO_2)	< 0.3 mg/L
Hydrogen sulfide (H_2S)	0 mg/L
Carbon dioxide (CO_2)	1–10 mg/L
Calcium (Ca^{++})	5–100 mg/L
Magnesium (Mg^{++})	65–100 mg/L
Iron (Fe^{++}+ Fe^{+++})	0.05–0.5 mg/L
Bicarbonate (HCO_3^-)	50–300 mg/L
Carbonate (CO_3^{--})	0–20 mg/L
Chloride (Cl^-)	1–100 mg/L

Source: Extracted from Boyd 1998a.

Table 1. Acceptable range of dissolved inorganic substances in pond waters.

5. Water supply

5.1. Water supply to ponds

Ideally, ponds should have two sources of water: one from a borehole and the other from surface water.

5.1.1. Borehole water

The benefit of using underground water is to avoid unwanted organisms and diseases from entering the pond. Another advantage is that it has a higher hardness than surface waters. The stimulating effect on the gonadal development of breeders is stronger when the broodstock pond is initially filled with hard water from a borehole. The pondwater can then be diluted later, when the breeding season is approaching, using collected runoff rainwater from a river, stream, or ditch.

5.1.2. Surface water

Using water from dams or streams at higher elevations than ponds saves energy because it avoids having to pump the water. It also offers the security of knowing water is always available. However, one major drawback is that water from a natural source can carry pathogens and parasites that are harmful to fish. For biosecurity reasons, it is worthwhile to install a storage pond before the water is used to fill the broodstock ponds. The storage pond should not have fish and the water should remain there at least 3 days. Ideally, the pond should be at a slightly higher elevation than the other ponds so that the cleaned water can flow into them using only gravity.

5.2. Water supply to hatchery

5.2.1. Borehole water

Ideally, the hatchery would get its water supply from a borehole because the water is free of pathogens and the temperature is constant. As detailed in section 7.3., it is recommended that the hatchery use an aeration tower before the water reaches the overhead storage tank. For SIS, the optimal water temperature in a hatchery for fast development of embryos and best hatching rates is 28°C–29°C. In Assam State, however, the water temperature from boreholes is likely much lower. At lower temperatures, mortalities in the hatchery would be higher because of reduced immune function to fend off fungal and bacterial infections. Because of this, it may be necessary to cover the pond or concrete tank with a small plastic sheet, like you would for a greenhouse, so that the water is heated using solar energy.

In some cases, water from boreholes has a dissolved iron content that exceeds 0.5 ppm. Another factor to consider is that iron is frequently accompanied by dissolved toxic arsenic. In this case, either a new borehole should be tested to find an iron-free layer of water (under strict supervision) or the water should be pumped into a storage pond, where the iron and arsenic would settle on the bottom through oxidation. To ensure quick oxidation, the water should be pumped into the storage pond through an aeration tower. The water should remain in the storage pond for at least 3 days, without being refilled, before it is used in the hatchery.

From April to June, the water can overheat in the storage pond, so it is necessary to maintain floating water hyacinth on half of the surface of the pond surface and use ponds about 2 m deep to prevent overheating. Another option is to use filtration devices to remove the previously oxidized and coagulated molecules of iron oxide. However, this is expensive and requires intensive maintenance, considering the high volume of water used in the hatchery.

5.2.2. Surface water

If a borehole is not available, surface water can be used instead. The volume of water in this type of pond should last at least 1 week for hatchery operations. Gravity is the preferred method for filling the pond, and the inlet should be covered with a mosquito net to prevent any harmful organisms from entering it. The drawbacks of using surface water are that the growth of plankton cannot be prevented and that copepods will damage fish eggs and larvae if they get into the hatchery. To prevent the entrance of zooplankton, it is necessary to install a 250 micron mesh hapa under the aeration tower inside the hatchery's elevated water reservoir. In addition, the hapa and the whole systems of tanks, pipes and incubators must be cleaned and disinfected following every batch of operation. Disinfection can be done using commercial Clorox (5% active chlorine) at 200 ppm free chlorine concentration (2 L of Chlorox in 500 L of water). The temperature of the water in this pond can be controlled to some extent as described in section 8.1.2.

6. Structural requirements for hatcheries

6.1. Hatchery building

The hatchery building should be situated close to the breeder ponds at a sufficiently high elevation to avoid flooding, and the floor must have a 0.5% slope toward a drain to prevent the accumulation of stagnant water. Generally, hatcheries must be equipped with the following:

- An elevated water reservoir.
- Holding and treatment tanks for breeders.
- Incubators for eggs and larvae.
- A small office room with an adjacent storeroom for storing scientific equipment, hormones, disinfectant and other hatchery equipment.
- Sanitary facilities.
- A restroom for staff, including wardrobes for work clothing.
- An outside machine room adjacent to the main hatchery building.

6.2. Elevated water reservoir

The elevated reservoir should be made with a cement concrete tank built on pillars. To ensure adequate water pressure for incubators and tanks, the bottom of the elevated water reservoir should be situated at least 2 m above the top of the incubators and tanks. An excessively high reservoir will increase the cost of pumping the water. The capacity of the reservoir should be large enough to ensure at least 4 hours of continuous gravity flow for full hatchery operations without the need for refilling.

Generally, a 4-inch diameter, 12 hp pump is needed to supply water to the reservoir. An electric pump with a standby diesel engine is also recommended. During hatchery operation, non-stop water supply must be ensured, as a few minutes disruption of continuous water supply can be lethal to eggs and larvae. An automatic floating switch connected with the electrical pump and a battery-driven alarm system placed in the elevated reservoir will greatly improve the security of water supply.

6.3. Aeration tower

The aeration tower is placed inside the elevated reservoir. Inflowing water passes through the tower before reaching the reservoir to remove toxic gases such as carbon dioxide, ammonium, and hydrogen sulfide from the water. It also dissolves oxygen, which guarantees close to 100% saturation of oxygen in the water supply. The effectiveness of the aeration tower is influenced mainly by the size of the droplets produced, the distance over which they fall, the temperature of water, the atmospheric pressure, the initial concentrations of dissolved gases, and the physico-chemical characteristics of the water. The saturation point of oxygen in freshwater at different temperatures is shown in Annex 7. An example of evolution of dissolved gases in water passing through six stages of the water tower in a hatchery is shown in Figure 1.

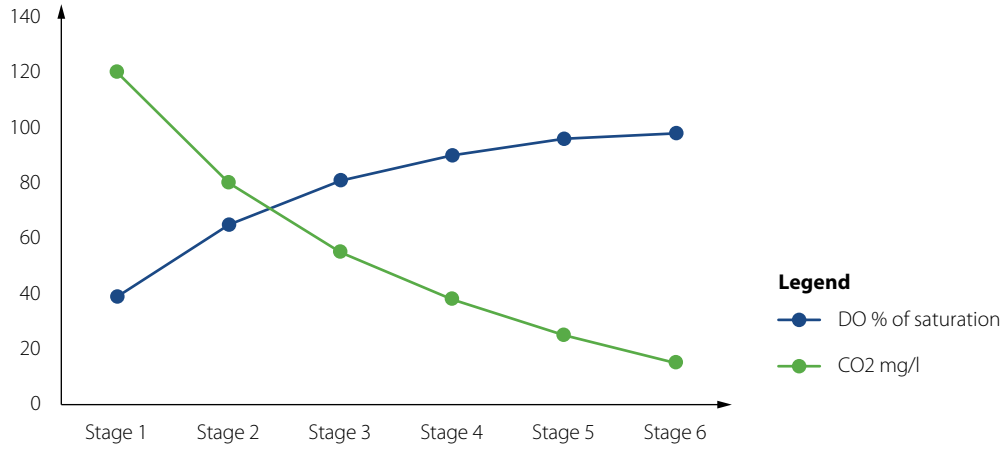


Figure 1. Changes in the concentration of dissolved gases in water passing through a six-stage aeration tower in a hatchery in Bangladesh.

Without an aerator, the water pumped from the borehole into the hatchery reservoir will have insufficient dissolved oxygen (DO) saturation and a high level of harmful gases. This will decrease the hatching rate and survival rate of larvae and hatchlings and cause eggs to prematurely hatch, resulting in high mortalities, including during transportation to nursery farms.

The aeration tower must have at least four to five perforated GI sheets, with a 5 cm high border, that are each placed under one another 40–50 cm apart. The number of holes on each sheet should be adapted to the capacity of the water supply. One hole with a 10 mm diameter is enough for 3 L of water flow per minute. The advantage of using this tool is that it automatically aerates the water before it reaches the reservoir so that additional aeration is not required (Plate 2). A diagram illustrating the construction of an aeration tower is shown in Annex 6.

6.4. Breeder treatment tanks

Tanks must have rounded corners to prevent injuries among fish. The size of the tanks generally varies between 5 and 20 m^2, with a depth of 0.75–1 m. The water depth must be maintained at minimum to avoid breeders from jumping out of the tank. In some cases, it is necessary to cover the tank with a net if the fish are jumping out.

These tanks can be also used for trials on induced breeding of SIS, either by keeping a hapa inside the tank with a substratum or using a hapa alone for species that have non-adhesive or slightly adhesive eggs (Plate 3). Breeders are kept in the hapa for about 6 hours before administering hormones.

Plate 3. A hapa kept in a concrete tank for SIS breeding trials.

Plate 2. Aeration tower in a small hatchery in Sierra Leone (left) and an aeration tower (designed by F. Rajts) for a large hatchery in Bangladesh with high levels of carbon dioxide (right).

The water should be supplied in two ways. One is a simple ball valve from where the water falls directly into the tank for quick filling. The second is a long perforated pipe that is used to spray water, like heavy rain. The bottom of the tank has a 0.5% slope toward the outlet, which is made from a PVC elbow and is situated vertically on the bottom.

A common mistake is placing the outlet horizontally in the wall of the tank. Having a vertical outlet on the bottom makes it easier to empty the tank quickly for cleaning and to maintain a removable standpipe to regulate the water depth.

To avoid small fish from escaping, the standpipe should be perforated and the water level in the tank should be regulated using a turndown pipe that is situated outside the tank. Because the filtering area of the perforated pipe is relatively small, it is better to keep small fish in a hapa in the tank (Plate 4).

In the case of continuous water exchange, the rate of water flow should be 1 L per minute for each kilogram of breeders kept in the tank. Aeration from compressed air or using oxygen reduces the rate of water exchange, which saves energy and water.

Most hatcheries have a series of indoor and outdoor breeder treatment tanks, which can be used for induced breeding trials of SIS that have adhesive eggs (Plate 5).

6.5. Spawning tank

A spawning tank is a large round tank with water injectors that circulate the water, and a circular perforated water inlet pipe that simulates heavy rain. The centrally placed outlet facilitates harvesting of fertilized eggs, which are collected from a hapa that is placed in the collection chamber. Circulating the water and spraying water on the surface of the tank stimulates spawning among the hormone-injected breeders. Due to stimulation in the spawning tank, some species can breed without hormone treatment. Stripping of eggs and milt is not required. This method is safer for breeders, and less labor intensive.

The disadvantage of a spawning tank is that it must be large. It requires high water consumption, which is necessary to stimulate breeders and allow them to move freely. Regardless of the number of breeders, the same amount of water is needed to ensure constant water movement. Another problem is that only one species can be reproduced at a time, whereas nursery operators need to spawn several species at once, which requires the species to be separated. Mixing closely related species in a spawning tank is not advisable, because this will produce a variable percentage of hybrids and result in a mixture of species with unknown proportions. Spawning tanks could be used for SIS breeding trials, and for species with

Plate 4. Alternative use of a breeder tank, where spawn or fry can be kept in a hapa during trials.

Plate 5. Outdoor concrete tank for use in SIS breeding trials.

adhesive eggs, by providing a substrate for egg attachment and using minimal water exchange.

6.6. Zug jar incubator

Zug jars are useful because they are small and allow for egg and larval incubation of various SIS to be done simultaneously or different treatments of the same species. They can be made locally and at minimal cost (Plate 6).

6.7. Circular incubators

Circular incubators are used for incubating large quantities of eggs or larvae, particularly during peak breeding season. The water enters through injectors and circulates horizontally between the inner and outer ring wall. Because the contents are continuously mixed in the incubator, metabolic products and pathogens are only partially eliminated. By comparison, funnel-type incubators can do this much more efficiently. The required speed of water flow is 30 cm/second to keep the eggs in motion, so the same quantity of water is needed for incubating a few eggs or many of them. Water holding capacity varies up to 10 m^3. The maximum egg density in the circular incubator is 500 eggs per L.

Using circular incubator tanks or spawning tanks for SIS breeding trials could be important because installing a water shower can stimulate spawning and it lessens the difficulties in handling delicate breeders, which weigh only few grams. Using synthetic substrate would be necessary for species that have adhesive eggs.

6.8. Cylindro-conical incubator

A cylindro-conical incubator, also called a funnel-type incubator, has a funnel-shaped bottom and a cylindered wall, similar to Zug jars but much larger (Annex 2.). Generally, eggs or larvae from one or a few pair of breeders are kept there. Its water holding capacity is about 300 L, with an egg density of up to 3000/L. The water inlet is at the bottom, and water flows to the outlet at the top. A filter net made from synthetic cloth prevents eggs and larvae from escaping, and a weighted rubber ball is placed at the bottom to disperse the water upward.

The required rate of waterflow can be adjusted to the species, temperature, the DO level measured at the outlet, the density of eggs or larvae and their development stage, but generally it is 2%–10% per minute of the incubator's total capacity. If the DO is less than 50% of the saturation point, as measured at the outlet of incubator (>4 mg/L at 27°C), then the waterflow needs to be increased.

An analysis of circular incubators with special attention to SIS breeding is presented in Annex 3.

Plate 6. Zug jars used for carp genetic improvement trials in Bangladesh.

7. Requirements and management of broodstock ponds

7.1. Broodstock pond management

Managing broodstock ponds entails making sure that the water quality, feeding rates and density are similar to natural conditions.

To habituate the fish to handling, ponds should be netted every 7–10 days. Breeders should be held in the net for few minutes and then splashed with clean water before releasing them back into the pond. This is necessary to reduce stress and injuries when breeders are collected and transported to a hatchery or another pond.

The pond should be kept clean from debris and excess aquatic weeds. Overdevelopment of filamentous algae, which grows fast in fertilized shallow and transparent water, is dangerous for small fish because it affects the growth of phytoplankton. Once established, removal is not easy.

A Secchi disk should be used to monitor water transparency, which should be maintained between 20 and 25 cm from the beginning of the culture period at a water depth of no less than 1 m. Raking the pond bottom regularly to increase turbidity will reduce the amount of light that penetrates to bottom, which will help remove and limit the growth of filamentous alga. Also, grass carp fingerlings can be stocked for biological control.

7.1.1. Procurement of breeders

Because of high fishing pressure on water bodies, including almost total dewatering of beels for irrigation and fishing purposes, only a limited number of small fish survive into the next breeding season. This can also happen after harvesting ponds, where only a few small fish remain for seeding the next season, either intentionally or accidentally. This can lead to reduced genetic variation of the population in small water bodies and may result in negative genetic selection for small fish, as well as inbreeding depression and genetic drift.

Breeders should be procured from different sources of the same water shed and then mixed in the broodstock pond to ensure that genetic variation is maximized. Breeders need to be stocked separately by origin for observation over 2 weeks followed by a health check to avoid introducing diseases or parasites into the farm.

7.1.2. Temperature

Through evolution, each fish species has adapted to the temperature and other physicochemical characteristics and biological conditions of the ecosystem where they have evolved. This includes seasonal changes. Temperature influences factors such as fish survival, metabolic activity, appetite, digestion, gonadal development and immune system response to pathogens. Fish intentionally select optimal thermal conditions, called the final temperature preference (FTP) (Golovanov 2006).

Diseased fish will stay close to the surface during daytime where the water temperature is higher, which activates their immune system more effectively. In shallow water, however, the temperature can become excessively high, which affects the well-being of the fish.

Most SIS and some carps breed in shallow water, but they prefer to breed during heavy rains and cloudy weather. This helps prevent fertilized eggs from dying because of excess heating of shallow waters from sunlight. Metabolic activity of fish, including oxygen consumption, carbon dioxide and ammonia secretion, drops in cold water, which is why cool water is preferred during transportation.

Breeding ponds used for SIS trials should include an area with a minimum of 1 m depth where fish can escape when the surface water becomes too hot. SIS in tropical habitats do have a range of slightly different requirements, but have an optimal FTP of about 28°C. To accelerate gonadal development during low temperatures, a greenhouse-type covering can be applied to SIS ponds, as shown in Plate 1.

Thermal stratification is a harmful phenomenon in the concerned regions. The bottom layers of the pond can become hypoxic or anoxic from thermal stratification because the hot, upper oxygen-rich layer of water is less dense and remains close to the surface (Wurts 2013). In addition, plant nutrients for phytoplankton growth remain in the bottom layers, where not enough light can penetrate for photosynthesis. This promotes anaerobic conditions that form toxic gases at the bottom of the pond.

Finally, accumulated anoxic water and toxic gases from the pond bottom can be suddenly mixed with the upper layers during weather related drops in temperature and storms, resulting in fish mortality. This also can happen when starting mechanical aeration after a long period. For this reason, the first time an aerator is operated after a long period should be on a sunny day in the early afternoon, when the upper layer of the pondwater is saturated with oxygen.

7.1.3. Total dissolved solids

The origin of TDS can be explained by the hydrological cycle. Water is constantly evaporating from the surface of fresh waters, seas, wet soils and through evapotranspiration. Under favorable conditions, this creates clouds, which can lead to rain or snow. This means that rainwater is almost equivalent to distilled water, except for some elements dissolved from the atmosphere, as well as carbon dioxide.

Dissolved carbon dioxide reacts with water, forming weak acid, which is why the pH of rainwater is slightly acidic. Acidic rainwater dissolves minerals such as calcium, magnesium, iron and sodium, as well as sulphates, during contact with the soil. As the duration of water contact with the soil grows, the level of dissolved material increases, because of the additional amount of carbon dioxide dissolved from the presence of this gas in underground layers, the temperature during contact, the quality of soil layers through which the material percolates and the depth of aquifer (pressure).

Seasonal variation of TDS in surface waters is explained by how the water reaches the surface. During heavy monsoon rains, most water cannot be absorbed and flows above the soil until it reaches surface waters. As a result, surface waters have less TDS during the monsoon season than the dry season, when water mostly comes from springs. In stagnant waters, TDS also increases because of evaporation.

Most SIS are sensitive to these changes in water quality. Rain and dilution of the water causes accelerated gonadal development, which can result in spawning. The main effect of TDS concentration on fish is related to osmotic pressure, which is higher at increased concentrations of TDS. TDS concentration is related to the electrical conductivity of water (Boyd 2020) so a conductivity meter can be used to quickly assess TDS, making it a practical tool for use in SIS broodstock management.

7.1.4. Dissolved oxygen

Managing DO levels is one of the most important tasks in SIS breeding trials. Low DO must be prevented at all costs. The ponds should get sunlight all day to allow for the growth of phytoplankton, which is the most important natural source of DO in pond water. However, phytoplankton is also the highest oxygen consumer during night, so using a mechanical aerator is necessary to ensure adequate DO levels in the morning.

7.1.4.1. Dissolved oxygen in nature

In natural conditions, DO levels vary. They are higher in the afternoon and lower in early morning, particularly in stagnant waters, due to photosynthesis and the effects of respiration. In flowing waters, daily DO fluctuation is minimal because the water is constantly agitated.

During monsoons or freshly flooded floodplains, DO levels are initially constantly high because there is a lack of oxygen consumers, specifically plankton, and fish density is low and organic matter on the bottom has not yet begun to decompose. This is the ideal moment for SIS to spawn, because there is a constant level of high DO, fewer pathogens and few predators at that time.

About 1 week later, the offspring are able to swim and feed. DO begins to fluctuate at this time, but the fry can migrate to another area

of the pond to escape low DO levels and can find plenty of small food, such as paramecium, rotifers and nauplius of copepods, which develop at the same time as the offspring. This is likely one of the reasons for the strong influence of DO on final gonadal development and the spawning of many fish, including SIS.

In broodstock ponds, fluctuation of DO levels is similar to that in natural stagnant water bodies, but the amplitude of minimum and maximum levels is more significant. This is due to higher photosynthesis and respiration from pond fertilization, which results in high phytoplankton densities, feeding rates, temperatures and fish densities.

The minimum DO level should not fall below 50% of saturation (4 mg/L at 27°C). This can be achieved by using an aeration device, either a paddlewheel or a venturi, twice a day. This enhances oxygen production and storage in pondwater during afternoon hours, while maintaining suitable DO levels at night and early morning.

7.1.4.1.1. Aeration during afternoon hours

Due to thermal stratification, some oxygen escapes from the oversaturated surface layer into the atmosphere, while the layers below remain poor in DO. Using an aerator for 1 or 2 hours during the afternoon will eliminate water stratification and mix the oxygen-rich upper water with poorly oxygenated deeper layers. This optimizes the total amount of DO reserve for the night, which improves the water quality in the bottom layers. At the same time, this also promotes photosynthesis in phytoplankton. The level of free carbon dioxide can be a limiting factor for photosynthesis. Generally, free carbon dioxide is exhausted from the heated upper layer of pondwater during the afternoon from phytoplankton activity. Mixing the carbon dioxide-rich bottom layers with the surface layer will promote phytoplankton growth, resulting in higher natural food production for SIS. Early morning operation for aerators lasts from about midnight to 09:00, depending on the actual DO levels.

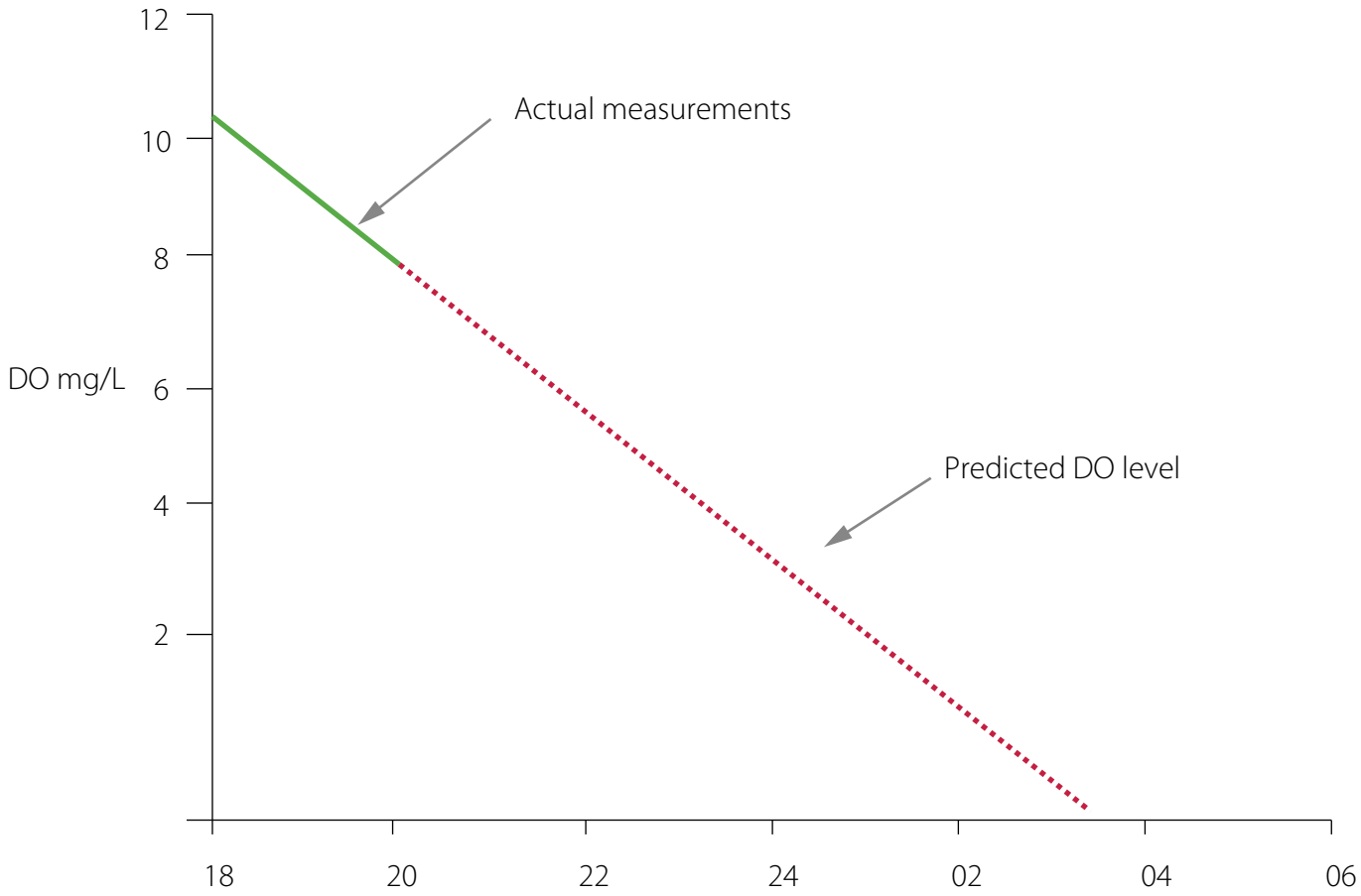
During sudden weather changes from sunny days to cloudy weather, hypoxia can occur in pondwater. For this, continuous aeration is needed to prevent fish from dying. Following phytoplankton die-off, photosynthesis drops, which results in a minimal evening DO reserve in pondwater and can result in further mortality among all organisms breathing oxygen over the next several days. In addition, decomposing dead biomass produces carbon dioxide and other toxic gases.

High levels of carbon dioxide negatively affect the ability of fish to absorb and carry oxygen in their blood. To reduce the level of carbon dioxide, previously diluted hydrated lime must be spread evenly on the pond surface at a rate of 20–50 kg/ha following previous tests on its effect on pH. In waters with poor alkalinity, the pH may increase excessively. Fish can survive better at lower DO levels when carbon dioxide is reduced in the water. Hydrated lime reacts with carbon dioxide by forming calcium bicarbonate, which improves water quality in soft waters by increasing alkalinity and hardness.

Following a phytoplankton die-off, fish can be seen gasping in the morning because the fast-decomposing organic matter removes oxygen from the water. As an emergency measure, 0.6 ppm of potassium permanganate must be applied to the pondwater. This is a strong chemical oxidizing agent which – at this dose - reacts with part of decomposing organic matter including bacteria. Thus, the rate of oxygen consumption by decomposition is temporarily reduced, which allows time to save the fish. However, aeration should continue until new phytoplankton develops and DO levels stabilize.

The amount of oxygen required to ensure a safe level of DO during the night can be estimated by determining the rate of oxygen consumption after sunset. Two samplings made at 2-hour intervals will reveal if DO is declining (Figure 2). Using these results, the required amount of mechanical oxygen input can be estimated by preparing an oxygen budget (Table 1). They can also be used to predict potential low DO situations about 6–7 hours in advance, which helps with programming aeration. The suggested start time for mechanical aeration is when the water reaches about 50% of DO saturation.

The oxygen input capacity of aerators is called the Standard Aeration Efficiency (SAE), according to Boyd 1998b. This is an average



Source: After Boyd et al.1978.

Figure 2. Predicting DO levels (an example of an extreme case).

Budget component	Oxygen available or required (kg/ha/night)
Available oxygen: Diffusion from the air	10
Available oxygen: Water column at dusk	100
Oxygen demand for respiration: Fish	60
Oxygen demand for respiration: Organisms in sediment	42
Oxygen demand for respiration: Water column (plankton)	120
Total oxygen demand for respiration	222
Total available oxygen	110
Oxygen required from mechanical aeration	112

Source: Boyd 1998a.

Table 2. Typical night-time oxygen budget for a 1 ha intensive channel catfish pond.

value, because the efficiency is inversely related to the actual DO level and the actual oxygen consumption in the pond during operation.

A venturi aerator is recommended for ponds equal or deeper than 1.5 m, because this type of aerator mixes water from the bottom of the pond, while a paddlewheel aerator only stirs the surface layers. This was demonstrated under WorldFish's Aquaculture for Income and Nutrition project in 2012. The estimated SAE was 0.45 kg of O_2/kWh (Rajts and Shelley 2020). The speed of water circulation measured on the shoreline of a 1300 m_2 pond was 12 cm per second when a locally made 4 hp venturi aerator was used. The use of aerators in broodstock ponds significantly improved water quality and made possible year-round reproduction of several fish species in carp hatcheries of Jessore in Bangladesh (Zahidur Rahman, personal communication, 2020.

The paddlewheel aerator has a SAE around 1 kg O_2/kwh. It is a surface aerator, so the bottom layers are not well mixed and could remain hypoxic. If operated during daytime, when the DO concentration of the surface layer is oversaturated, the paddlewheel releases excess oxygen into the atmosphere.

7.1.5. Changing water quality by flushing the pond

Flushing the pond by adding fresh water is another stimulating factor for gonadal development. The best method is to flush the pond using soft water, by collecting runoff rainwater or pumping it from small streams, ditches or an old *bundh*. However, flushing a broodstock pond too vigorously when breeders are ready to breed can result in undesirable spawning.

7.1.6. Water current

Creating a water current has a stimulating effect on gonadal development. Apart from improving DO levels, using pond aerators can also simulate conditions in rivers, including water current and reducing thermal stratification.

7.1.7. Feeding breeders

In the Gangetic Delta, before habitats were damaged and indigenous fish overexploited, breeders able to find ample amounts of feed while migrating to feeding grounds on inundated floodplains. This resulted in good growth, improved condition, and initial development of gametes for the next year's breeding. This has all changed because of habitat destruction and overexploitation. With the reduction of floods from October to December, a huge number of fish are being concentrated in remaining waters, such as beels, haors and rivers. Due to the high density of fish, the availability of food has declined.

Under natural conditions, breeders have had to use their energy reserves for survival and for further development of gametes up to the dormant stage, when they are ready for breeding. When the breeding season (April–June) arrives, migration to breeding grounds further reduces the energy reserve of the fish. Eventually, the fat is eliminated from the fish altogether. This means that excess fat does not jeopardize ovulation.

In improperly managed hatcheries, poor results of induced breeding can be frequently explained by excess fat from overfeeding breeders. This results from low stocking densities and excess feeding immediately before and during the breeding season. Therefore, feeding and fertilizing rates need to follow the natural conditions described above. The idea is to feed the spent breeders at about 3% of bodyweight per day from June to November, then gradually reduce the rate to 0.5% until breeding.

7.1.8. Breeder conditioning for handling and transportation

Handling breeders is generally a delicate operation, but injuries and losses are rather easily prevented during usual hatchery operations with large, domesticated breeders. However, SIS breeders to be used for induced breeding trials are not yet domesticated and are smaller in size and very sensitive to handling, so special attention should be paid to preparing them for induced breeding (section 14.1.). Before transferring the breeders to the preparation pond or other ponds, they should be starved by stopping supplementary feeding at least 1 day prior to transfer.

8. Maturation and spent fish pond management

8.1. Maturation pond management

In trials of induced breeding outside the natural breeding season, gonadal development for spawning stage can be achieved in small maturation ponds. It is easier to stimulate maturation of selected breeders in small ponds than in a large broodstock pond, which involves the whole breeder population. Small ponds make it easier to make changes to stimulating factors such as photoperiod, temperature, TDS, salinity, hardness and alkalinity.

In maturation ponds, the sexes are stocked separately to eliminate the risk of spontaneous unwanted breeding. For sorting breeders by sexes, an adjustable fish grader should be used first, followed by manual sexing. The best females of most species will remain on the screen of the grader. They should be fed no more than 0.5% of their bodyweight per day, and feeding should be stopped 48 hours prior to shifting the breeders to a hatchery or into breeding ponds.

As much as possible, the feed needs to be adapted to the nutritional requirement of the species, with particular attention paid to essential amino acids, fatty acids and vitamins. Nets should be used for conditioning every 4–5 days to reduce stress during induced breeding operations (section 8.1.7.). Breeders should be measured and carried in water, at all times.

8.2. Spent fish pond management

During spawning, breeders lose energy and become exhausted, and some become injured from handling. Small fish, particularly SIS, should be carried in aerated water, otherwise serious injuries may occur.

Fish should be disinfected during transfer from breeding ponds or tanks to the pond for spent fish. The transfer time should not exceed a few minutes, so the pond for the spent fish should be located close to the hatchery or breeding pond. To avoid additional handling, spent fish can be disinfected upon arrival at the pond by quickly mixing the disinfectant in the same water. Sensitivity to disinfectant varies among SIS. Trialling is necessary to identify the best tolerable doses of chemicals for each species concerned.

Daily feeding rates in spent fish ponds should be 3% of weight, and the feed should be high in protein and energy.

9. Development of breeding techniques, and alternative breeding methods

Induced breeding is a technique to produce fish seed by inducing gonadal maturation. This is followed by the release of eggs and sperm, called spawning. It can be done in two ways: (1) by environmental management to achieve spontaneous spawning; (2) by environmental manipulation combined with hormone-induced breeding.

For all types of trials, having well-conditioned breeders in broodstock ponds is fundamental (Section 8.1.). Readiness of breeders can be checked from their external appearance, though this is not always satisfactory. Sampling gonads and observation using a microscope allows better estimation of readiness. The motility of sperm and position of the nucleus (germinal vesicle) can be easily determined using a microscope. In a ripe egg, the nucleus has already migrated to the periphery of egg, while in an immature egg it is found at the center (Rottmann et al. 1991).

Monitoring is required. This means keeping records of all interventions, including sources of procurement, quarantine, environmental manipulation, the physicochemical characteristics of water, hormone used (if any), and induced breeding data. Random sampling of broodstock should be done to record condition factors, such as gonadosomatic index (GSI) and sex ratio. The results of each trial in hatchery and nursery ponds should also be recorded, for monitoring and possible improvement during later trials.

9.1. Breeding trials using environmental stimulation alone

For breeding trials, avoiding the use of hormone for inducing ovulation and spawning under controlled conditions is preferable. Success depends on the degree to which the environmental manipulation process mimics conditions of the natural ecosystem where the species reproduces. Some of the known factors that stimulate breeding are temperature, photoperiod, substrate, water depth and flow, turbidity, hardness, DO, pH and TDS, as well as the presence of the opposite sex following initial separation of sexes.

9.1.1. Trials in breeding ponds for species with adhesive eggs

In a standard breeding pond the frequency of breeding operations is limited by the fact that the fry produced cannot be harvested before about 2 weeks, and the number of fry produced is limited by the size of the pond. This is the case for most SIS that lay demersal adhesive eggs. More breeders can be used and the frequency of breeding operations increased if artificial grass or a locally made substrate from synthetic fibers is used. The fertilized eggs along with the substratum would be collected and incubated under controlled conditions in the hatchery, either on the substratum or separated.

The following is a breeding operation plan for the breeding pond:

- Dry the soil on the breeding ground.
- Cover the breeding ground with artificial grass or a locally made substratum made from synthetic fiber.
- Fill only the ditch of the breeding pond with hard water, if possible.
- Stock matured breeders from preparation ponds at a rate of 200 g/m^2 and at a sex ratio of 1:1 or even 2:1 male/female.
- Maintain a constant adequate level of DO.
- Do not feed breeders in the ditch.
- Add soft water, if possible, to flood the breeding ground 20–30 cm in the afternoon on the second day after stocking.
- Maintain a nonstop water shower and a slight water current.
- Observe likely spawning the next morning.

- Carefully collect the egg-laden substratum.
- Disinfect eggs by trying different authorized disinfectants according to the tolerated concentrations and duration of application of each species, at the development stage of the embryos.
- Transport eggs to the hatchery for incubation.
- Harvest breeders and, if possible, separate them by sex. Some species become colored during breeding period. For example *P. sophore* males have an orange-red coloration on their lateral line.
- Record the rate of spent females.
- Transport breeders to ponds for holding spent fish.

9.1.2. Trials in breeding ponds for scattered breeders with floating eggs

A few SIS, such as climbing perch (*Anabas* spp.) and *Gudusia chapra*, have floating eggs and abandon them once they are fertilized. Under natural conditions, the eggs are carried along the water current or the movement of the water from the wind until they reach shallow stagnant waters in inundated floodplains, where conditions for nursing are favorable. In breeding ponds, similar to what happens in nature, some of the eggs are lost if the wind drives them to the shoreline or if the water level drops. Using plastic protective fencing, as shown in Plate 13, prevents the loss of eggs and facilitates the collection of eggs that accumulate on one side of the pond because of the wind, similar to what happens with artemia cysts. The accumulated eggs can be collected and then incubated in funnel-type incubators.

9.1.3. Trials in tanks for scattered breeders with non-adhesive demersal eggs

Some SIS, such as danios and some barbs, have demersal, non-adhesive eggs. These species eat the fertilized eggs. To breed these species in concrete tanks, use of a double hapa is suggested. The first hapa should be shallow and serve to hold breeders, with a mesh size adapted to the size of the breeders. Laid eggs fall into the finer meshed second hapa below, which is deeper. As a result, breeders and eggs can be removed separately once spawning is completed. Eggs can then be incubated in funnel-type incubators in hatcheries following cleaning by adapting the size of the filter (Plate 10) and through disinfection.

9.1.4. Trials in concrete outdoor breeding tanks

A similar technique can be used in existing concrete outdoor tanks, which are designed for fish seed farms for fry production, though some minor modifications and repairs would be necessary.

In addition, concrete breeding ponds could be used for induced spawning of SIS that have demersal or floating non-adhesive eggs. Because the conditions are controlled, the eggs can be collected and then incubated in a hatchery. Cleaning the eggs can be done using perforated plastic trays or a bucket, depending on the quantity. Plate 8 shows a farm-made device used to clean collected eggs.

9.1.5. Trials using Chinese hatchery tanks

Chinese spawning tanks, as shown in Plate 7, can be used for induced spawning of any SIS, particularly those with non-adhesive eggs. Water is circulated using water injectors on the bottom, and the current concentrates the eggs to the central outlet and directs them into a hapa placed in the collection chamber, where the eggs are collected. Water is sprayed from a circular pipe all around the tank to simulate rainfall. Collected eggs can then be placed in circular or other incubators.

Because the spawning tank is so large, and to create the necessary speed of the water current, water consumption for this type of tank very high. It is recommended to do trials in this type of tank only when induced breeding has proven successful in small units.

9.1.6. Trials using indoor tanks

Indoor or outdoor tanks used for treating breeders or holding spawn prior to dispatching can also be used for induced breeding of SIS. Hapas can be kept in the tank to handle breeders and for easy collection of non-adhesive or slightly adhesive eggs.

Adhesive eggs stick together and can be damaged by a lack of oxygen and infection by pathogens. Breeding SIS with adhesive demersal eggs in these hapas could be trialled, following similar techniques as are used for European catfish. This

requires removing sticky layers of the eggs after partial development of the embryos, when oxygen consumption of the eggs is low (Woynarovich and Horvath 1980). Eggs attached to the hapa can be collected using a similar method and then kept in funnel-type incubators, which ensure better survival.

9.2. Induced breeding trials using hormones in combination with environmental manipulation

Hormone-induced breeding is widely practiced for cultured fish species. The best environmental conditions prior to the spawning stage are the same as those for induced breeding using only environmental manipulation. Hormones used for this purpose are either gonadotropins, which act directly on gonads, or GnRH analogues, which act on the hypophysis, with or without a dopamine antagonist, such as Domperidon.

Gonadotropins are species-specific proteins and may result in adverse effects on the injected fish by activating the immune system, as well as spreading disease through pituitary gland extract. Mortality may also occur if fish that are not fully matured are injected with gonadotropin hormones, because these act directly on the ovaries. Partial ovulation occurs if the development stage of the eggs is not yet synchronized in the ovaries. This frequently results in death of breeders. Generally, two injections are required, which means more handling is needed, so the risk of injuries and infections is higher. The doses of hormones applied for induced breeding vary by species, so trials should be done to establish the proper doses for each SIS concerned.

Plate 7. Chinese hatchery unit in private farm of Assam.

The use of synthetic GnRHa analogues is preferred because they act at higher levels in the hormonal cascade and are not highly species-specific. By stimulating the hypophysis to release the fish's own gonadotropin hormone, the problems created by using gonadotropin do not arise. Generally, a single dose is enough to result in ovulation. Moreover, mortality caused by not releasing premature eggs following GnRHa application rarely occurs. Repeated breeding in the same season is currently done using GnRHa-based products. The dose of Ovaprim varies from 0.3 to 0.5 ml/kg for female fish, while half that dose is applied for males.

9.2.1. Method of hormone administration

Mature breeders are selected from a nearby situated broodstock maturation pond and then carried to the hatchery tanks about 6 hours prior to hormone administration. Continuous waterflow is needed to maintain good water quality conditions. The hormone is generally injected into the body cavity at the base of the pelvic or pectoral fins. Considering that most SIS to be tried are small, the injection should be done with the finest needle possible and under sedation. Pheromones released by the injected specimen could stimulate other non-injected ones as well, so partial treatment of breeders should be attempted. The latency period following a single dose of Ovaprim or Flash is highly variable, depending on the species, readiness, and temperature.

For the safety of sensitive SIS, sedating breeders is recommended during hormone administration and stripping. Clove oil is a natural product that is proven for use in sedation. It is produced in India and costs much less than other expensive chemicals used for this purpose. The dose is 30–60 ppm for mild sedation, though the concentration varies by species

and temperature. A trial for safe concentrations should be done for each species concerned.

9.2.2. Method of egg collection and incubation

Induced spawning can be done in two ways: either by letting the breeders spawn themselves, or through stripping. The first is recommended because it is safer for breeders as well as hatchery staff. The second is applied when environmental manipulation does not lead to spawning. Eggs are collected and kept in incubators. For demersal adhesive eggs there are several possible modes of egg incubation. For species that have only slightly adhesive eggs, hapas can be used for spawning the breeders, after which eggs can be collected for incubation in funnel-type incubators.

For stripping, the treated breeders should be observed for 1 hour before the expected spawning time. Stripping should be started when spawning begins in the tank. Stripped eggs are fertilized with collected milt and treated to remove the sticky layers. Without neutralizing the sticky layers, eggs will stick to every hard surface, or to each other. The rolling eggs can then be incubated in Zug jars or other funnel-type devices. This method provides good gas exchange for every egg, unlike non-treated eggs which tend to coagulate, frequently causing the inner layer of eggs to die.

Non-adhesive eggs can be stripped or collected from spawning devices or structures and placed in incubators. Eggs collected from breeding ponds or tanks are often mixed with debris. This can be filtered out using a perforated plastic bowl adapted to the diameter of the eggs (Plate 8).

Collected eggs require disinfection. Various chemicals can be used, including acriflavine, formalin, methylene blue and iodine. The concentration and duration of disinfection varies according to the chemical, duration, species, and development stage, so this must be determined during trials.

The use of chemicals during incubation can be minimized by disinfecting the whole water supply system before each batch in hatchery operations. Water supplied from ponds requires more disinfection than water from a borehole, which is almost sterile. Low temperatures may promote the development of pathogens and

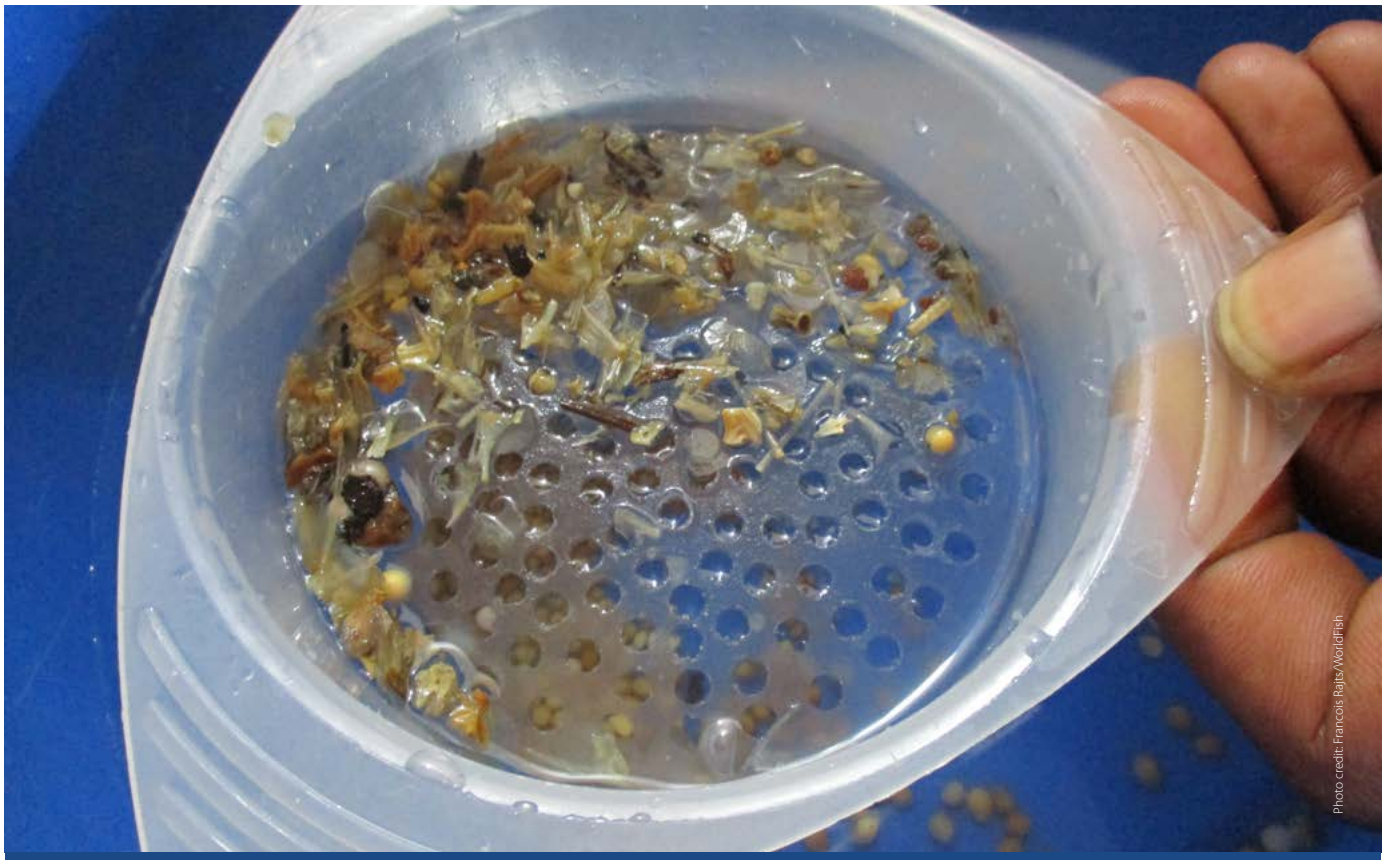


Plate 8. A locally made filter for cleaning collected demersal non-adhesive eggs.

make the incubation period longer, while overly high temperatures can harm the larvae and result in deformities or even death.

The incubation of fertilized eggs lasts about 1–3 days, and larval incubation about the same. The larvae should remain in the incubator until the air bladder is filled and until the larvae reach the first feeding stage. (For required waterflow, water quality and densities refer to sections 6 and 7.)

Initially, fertilized eggs are kept in funnel-shaped or circular incubators until the larvae are ready to feed. Using circular tanks for egg incubation is better for larger quantities of eggs. The use of chemicals during incubation can generally be avoided by disinfecting the whole water supply system beforehand.

9.2.3. First feeding of larvae

Generally, larvae start to feed on the second or third day following hatching, depending on the temperature and the species. At this point, they are called hatchlings. First feeding is applied in incubators. This is necessary for survival until the hatchlings become acclimatized and able to find food in the nursery pond. The best food for them is live or frozen zooplankton.

Hatchlings have a poorly developed digestive tube and only a few enzymes, and enzyme activity increases in step with the development of the whole digestive system (Yufera et al. 1998). When feeding on zooplankton, enzymes from the prey ensure optimal digestion. Artemia nauplii are too large for first feeding, except for some predator species. Cultured rotifers or filtered live zooplankton are the best choice for first feeding of hatchlings. In the absence of zooplankton, boiled egg yolks can also be used, but a micro-encapsulated whole egg (Plate 9) is recommended because its nutritional content is better balanced than an egg yolk alone (Chow 1980).

After eating egg yolk, healthy larvae will have a yellow belly. That is an indication that they can then be stocked in nursery ponds. Development of enzyme production parallels the progress of the digestive tube development of the fry, which it lasts about 1 month, depending on multiple factors.

Plate 9. Feeding hatchlings with a micro-encapsulated whole egg.

10. Nursery pond management

10.1. Nursery pond preparation

During the initial days of the trials, fry require plenty of small zooplankton because their mouths are small and they are not able to catch fast prey. This type of zooplankton consists of protozoans and rotifers. About 3–4 ml of zooplankton per 100 L of pondwater should suffice (Kumar 1992).

The density of zooplankton is measured by collecting water samples from several places all around the pond, using a bucket and a plankton net. Strong sunlight, low DO and high winds can modify the depth and the side of the pond where zooplankton may concentrate. Zooplankton can be killed and then collected using either formalin or alcohol, and the volume of settled zooplankton can be identified using a measuring tube (Plate 10.).

If the density is not satisfactory, stocked hatchlings must be fed immediately by pouring micro-encapsulated egg all around the pond at 2-hour intervals. At 6–7 days after stocking, commercially manufactured or homemade formulated feed should be applied at least four times daily.

Plate 10. Measuring zooplankton levels in a pond.

For carp species, pre-stocking management includes draining the pond, then cleaning, liming, drying, refilling gradually, applying inorganic fertilizers and organic manure, and controlling predators. Applying dried grass all around the shallow areas of the pond is particularly helpful to develop paramecium and rotifer populations, which are the best source of food for the small mouths of hatchlings. The decomposing grass can be removed after 10 days.

Fry are sensitive to predation from copepods and some insects and insect larvae, such as backswimmers and dragon fly larvae. Liming with powdered active hydrated lime on wet soil, followed by drying the pond bottom, will eliminate most predators during pond preparation. However, it takes just one night for flying insects to colonize the pond again when it is refilled and fertilized. Without controlling them, most of the stock of fry could be lost. In the past, these predators were controlled using insecticide. Nowadays the use of these chemicals is prohibited, except for ornamental fish. Alternative methods of predator control are listed in sections 12.1.1. to 12.1.3.

10.1.1. Control of copepods

To prevent predator copepods at the time of stocking, the pond should be filled no sooner than 3–4 days earlier. Rotifer eggs hatch first from the mud. This population develops first, and copepods a few days later. At this time, fry can feed on rotifers and will be strong enough to escape from copepods when they appear later in the pond. Fry survival will be poor if the pond is filled earlier than 4 days before stocking or if copepods are introduced with the newly filled water. Because of this, refilling the pond should be done from a borehole, or surface water should be filtered using a 100 micron net. Double filtering, as shown in Plate 11, is the preferred method. This will prevent the entrance of copepods, including at the nauplius stage. In new ponds, mud from old nursery ponds could help provide "resting" eggs of zooplankton.

10.1.2. Controlling predatory insects

During the night, backswimmers and other predator insects fly in search of zooplankton-rich waters. Before stocking the nursery pond, netting with a mosquito net mesh should be applied several times to remove backswimmers, as well as other predatory insects and their larvae. A small pond can be covered with a mosquito net before refilling to prevent backswimmers from invading, and dragonflies and other predators from laying eggs.

10.1.3. Controlling frogs and tadpoles

If frogs are allowed to breed in a prepared nursery pond, the tadpoles will compete for food with the fry in the pond. Simple fencing around the pond can reduce the entrance of frogs, though they can sometimes jump over the fence. Rajts (2016) placed plastic sheet fencing along the shoreline of a pond inside the water. This prevented frogs from entering, except the open area between the fencing and the water shoreline. The frogs bred in the narrow area outside the water area, but neither the frogs nor the tadpoles could enter the protected area.

Plate 12 shows the difference in water color inside and outside of the protected area. Extreme turbidity in the outside area resulted from the activity by tens of thousands of tadpoles searching for food. Frog eggs can be collected daily from nursery pond, preferably in the morning, as frogs breed during the night. Checking for frog eggs should continue daily throughout the entire nursing period. Collected frog eggs from the shoreline of nursery pond can help to sustain endangered frog species, if liberated in beels.

10.2. Stocking

First feeding hatchlings should be stocked in the nursery pond. The stocking rate depends on the productivity of the pond, the fertilizer used, the feeding techniques applied, and the species stocked, as well as the targeted size of fry to be produced. Carp hatchlings are usually stocked at 100–500 hatchlings per m^2, but the suggested stocking rates of SIS are initially no more than 100/m^2, though this may be modified following the results of the trials.

Plate 11. Filtering inflow water to fill a nursery pond.

Plate 12. Unwanted frog breeding successfully restricted to the separated shoreline.

Hatchlings cannot support sudden changes in water quality, so stocking hatchlings in the nursery pond should be done carefully. If the hatchlings are carried in plastic bags containing pure oxygen, there is high risk of mortalities. Sudden release into normal pondwater from the plastic bags can result in gas embolism and mortalities a few hours later. The risk is even higher if the temperature of the pond is higher than the oversaturated water of the plastic bags. If transportation takes too long, the accumulated metabolic wastes of ammonia and carbon dioxide can affect the fish. To prevent this from happening, the following steps are necessary during stocking:

- Releasing hatchlings in late morning when the DO levels are already high, to allow them to feed all day and get stronger.
- Open the plastic bag and carefully transfer the water with the fish into a large plastic basin.
- Wait until the fish come to surface, which indicates that the DO level has dropped.
- Slowly add pondwater into the plastic basin to mix the water.
- Wait a few minutes and observe the hatchlings, adding more water if necessary.
- Make sure the temperature of the water in the basin is the same as in the pond.

10.3. Post-stocking management

Post stocking management consists of controlling water quality, maintaining the zooplankton population, supplementary feeding and harvesting. The growth rate and time necessary for nursing fry must be determined through trials for each SIS.

10.3.1. Maintaining good water quality

In nursery ponds, water quality deteriorates easily if excess fertilizer is applied. It is recommended to start nursing at about half of the water depth in the pond and add 5–6 cm of fresh water daily until the pond is filled to a maximum depth of 1.2 m at the deepest point. The pond should be exposed to sunlight and wind all day. Using a paddlewheel aerator is recommended to ensure constant high DO concentrations and to avoid hypoxic conditions in the lower water layers.

10.3.2. Natural food management

Natural food includes plankton, detritus, bacteria, worms, insects, snails, aquatic plants, etc. For nursery operations, the main natural food is plankton. Small fry generally do not search for food on the bottom, but rather prey on zooplankton in the water column. Plankton that are established during pre-stocking (mainly rotifers) and at the beginning of post-stocking (mainly crustaceans)

Plate 13. Releasing hatchlings in a nursery pond.

should be maintained by controlling the density, and adjusting the intensity of fertilization.

Maintaining a zooplankton density of about 3 ml per 100 L of water is necessary for adequate nutrition of fry (section 8.2.3). Supplementary feeding helps, but cannot fully replace natural food at an affordable cost. Food for zooplankton should be ensured constantly by maintaining an appropriate phytoplankton density. Ideally, phytoplankton density should be 25 cm, which can be measured using a Secchi disk. Readings should be carried out around noon, when sunlight is penetrating the water almost vertically.

Once the phytoplankton population is exhausted, it is too late to restart pond fertilization because without food the zooplankton population will decline. Restarting fertilizing would take a few days to effect phytoplankton density, so by the time the phytoplankton population is increased, the growing fry will have eaten most or the entire zooplankton population. Once zooplankton are eliminated, the fry should be harvested. Generally, the nursing period should last no more than 3–4 weeks for fast growing species, because it is difficult to maintain the zooplankton population beyond this period at usual stocking densities of fry.

Daily fertilization is best to maintain high phytoplankton and zooplankton levels. Depending on phytoplankton density, approximately 2 g of urea and 1 g of TSP should be applied per square meter every week. In addition, fermented organic fertilizer such as mustard oil cake (MOC) should be sprayed evenly on the surface at a rate of 1.2 g of dry matter per square meter every day. Since urea dissolves instantly, it can be mixed with the fermented organic fertilizer.

Granulated TSP sinks into the mud, which decreases its effectiveness, so the weekly dose should be kept in a bag that is suspended below the water surface in the middle of the pond. This way it can dissolve slowly each day. The material of the bag should be such that its releases the fertilizer dose over a period of 1 week. If the total ammonia nitrogen (TAN) is more than 1 ppm, the morning DO is less than 3 ppm or a Secchi disk reading is less than 25 cm, then fertilization should be stopped.

Although phytoplankton is the main source of DO in the pond, respiration from excessively dense phytoplankton can cause morning DO levels to drop significantly. This generally happens during the night after cloudy days, when the DO reserve is already low in the pond in the evening (section 7.1.4).

Low morning DO levels can also cause phytoplankton to die off. On top of this, fast-decomposing dead algal biomass produces carbon dioxide, ammonia, and other toxic gases (section 7.1.4.), which decrease zooplankton density because of the loss of phytoplankton and continuous consumption by the fry. Without appropriate zooplankton density in the pond, the starving fry will become infected by protozoan and other parasites. To prevent mortalities, it is suggested to harvest the fry and restock them in another pond, if the development stage of the fry will allow it.

10.3.3. Harvesting

Harvesting should be done when the fry become big enough to endure handling and transportation. The minimum size of fry allowing safe harvesting should be identified during trials for each SIS. Harvesting fry is a delicate process, and the number of times they are handled should be minimized.

Before harvesting, netting should be used to train the fry, followed by splashing water from into the net for 10 minutes before releasing the fry back into the pond. Feeding should be stopped 1 day prior to harvesting. The best method is to drive the fry from the pond into a collecting hapa that is kept in a harvesting pit.

If the water level in the pond is reduced, the DO will drop during night. When fresh water is slowly put back into the pond through the hapa outlet, the fish will swim out of the pond against the flow of the water and into the collecting hapa. This should be done early in the morning. Fresh, oxygen-rich water can be also driven into one side of the net to attract fry if a seine net of fine mesh is used inside the pond.

11. Feed composition trials using locally available ingredients

In natural conditions, fish stocks and food availability are balanced. If natural food is lacking, this will result in partial die-off. Fish will migrate in search of a better environment to feed. If they cannot find more food, their growth rate will be slow and they could even get sick and die. In aquaculture, stocking densities are much higher than in the wild. Organic and inorganic fertilization is necessary to enhance natural food production to provide food for the high biomass of fish stocked. However, enhanced natural food alone is generally sufficient only at the beginning of the culture period.

In semi-intensive culture, applying supplementary feed will increase fish production even further. Supplementary feed is not necessarily a complete and balanced feed though. It is usually an inexpensive agricultural by-product with an inadequate nutrient balance that will not meet the entire nutritional requirements of the concerned fish species. Well-maintained natural food contributes to the supply of essential amino acids, fatty acids and minerals, which supplementary feeds lack.

Although the natural food can compensate to some extent for the missing nutrients in supplementary feed, it is highly beneficial if the nutritional composition of supplementary feed is balanced with the requirements of the cultured fish. Industrial "finishing" feed contains less protein than grower feed, which increases profits for the industry. Toward the end of the culture cycle, however, the protein content in the feed must be higher because the increased biomass of the fish has exhausted the amount of natural food in the pond. The exception is when a specific type of fish with a high fat content, such as climbing perch, is intentionally produced to satisfy market demand.

Feed composition trials with three replications are suggested in a series of small hapas that are made from different mesh sizes, using materials adapted to the size of stocked fish (Annex 1). The mesh can be made of 250–300 micron hapa material, a nylon mosquito net or a 5 mm mesh net, as per the size of the fish. The suggested size of the hapas is 1.2 m x 1.2 m x 1.2 m, with a water volume of about 1.5 m^3 (Plate 14). The hapas should be exchanged frequently with cleaned ones to prevent isolation of inner water by algal growth on the net (called biofouling) and by wastes which restricting the flow of water through them. Bird protection is also required.

For feeding trials, feed formulas should be prepared from the hatchling to fry (about 1 g) stages, and for both growers and breeders. The feed ingredients used should be available in local markets. Using farm-made feed is suggested. Cooked feed improves digestibility and water stability, which can be used for larger size stages of fry when able to feed on the bottom. This improves the feed conversion ratio (FCR) and specific growth rate (SGR) and reduces the level of pollution in pondwater from undigested feed or nutrients leaching out.

Feed can be prepared at the farm by cooking cassava flour quickly until it reaches a gelatin-like consistency, and then mixing in other ingredients. Gelatinized starch provides energy and acts as a binder, thus preventing nutrients from leaching out of the feed (Plate 15). After the mixture cools, ingredients with a low heat tolerance, such as vitamins, or medicines can be mixed into the cooked dough.

Plate 14. Gelatinized cassava flour before mixing with other ingredients.

Plate 15. Trial hapas with bird protection at a research and development farm of EWOS Ltd. in Vietnam.

12. Transportation guidelines for fish seed, breeders and market fish

Successful transportation of live fish mainly depends on the species, age, density and health of the fish, how they are handled, how long they are transported, and the quality of the water used to transport them. Even if the fish survive transportation, the survival rate in the receiving pond can be poor if their health is affected by inappropriate handling or other unfavorable conditions.

Fish and equipment should be prepared before transportation. A checklist for transportation using a truck is shown in Annex 4. Currently, handling and transportation puts a greater stress on many SIS more than on large domesticated species, which have been cultured for hundreds or even thousands of years.

12.1. Conditioning SIS for harvesting and transportation

Training fish for crowding and handling conditions is necessary to reduce the level of stress for SIS during transportation. The frequency and length of conditioning depends on species and age of the fish. Conditioning is not used for hatchlings, as at this stage the young fish have only just filled the air bladder, and still have an attached yolk that provides energy.

Fry should be kept in a hapa for about 6 hours before transportation to allow them to empty their digestive tube prior to packing. Water should be continuously falling vertically into the hapa to ensure sufficient DO and eliminate metabolic wastes. Care should be taken to ensure water does not flow horizontally through the net of the hapa, as the net can damage the skin of the mouth of the fish when crowded fish try to escape against the inflowing water (Plate 3).

For adult SIS, the first step of conditioning is netting the pond using a net with an appropriate mesh size to avoid injuries. The crowded fish should receive fresh, oxygen-rich water inside the net for a significant period of time and then be released before they get tired. This procedure should be repeated several times over a period of 10 days before harvesting.

Monitoring the growth and checking the health of the fish, in the same way as would be done during the culture period, also helps to habituate the fish to netting. Similar to fry, adult fish should be kept in hapas in concrete tanks before transportation. The length of time depends on the species and size of the fish, but can last up to 24 hours.

12.2. SIS transportation

At every stage of harvest and transfer, from the conditioning device to the transportation tank, SIS should always be carried in water, never in dry conditions. Fish that can breathe air can be transported in open containers without using oxygen, but small fish should be transported in plastic bags filled with water and oxygen (Plate 16). For large fish or large quantities of small fish, fiberglass tanks should be used.

The following are the steps needed to prepare the number and amount of fish to be carried in each device:

- First, select fish randomly from the conditioning hapa to establish the average weight.
- Determine the allowable total weight of fish packed in one unit of the carrying device, taking into consideration the species, the volume of the carrying water, expected duration of transportation, temperature, and the possibility of exchanging water. Examples of SIS transportation are rare and poorly documented. For an approximate guide, Table 3 lists transportation guidelines for carp juveniles.
- Calculate the number of fish for one unit of the carrying device by dividing the allowable weight by the average weight of the fish. Record the number.
- Keep a plastic bucket on the weighing scale and fill it with water at a minimum of twice the allowable weight of fish. Record the weight.
- Pour the fish slowly into the bucket up to the allowable weight.
- Empty the bucket into the carrying device together with the water.

Hatchling		Dhani	Fry	Fingerlings										
Average weight (g)	0.002	0.1	1–2	4	6	8	10	12	14	16	18	20	22	24
Rohu per liter (g)	19	22	25	33	36	37	38	40	42	43	45	48	50	53
Silver carp per liter (g)	15	16	18	23	25	26	27	28	29	30	32	33	35	37
Water (Liters)	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Oxygen (Liters)	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Source: Modified from Hamilton et al. 2020.

Table 3. Recommended weight of carps for transportation in plastic bags at temperatures 27°C–30°C for up to 12 hours.

Source: Hamilton et al. 2020.

Plate 16. Packing carp spawn in Jessore using plastic bags for transporting hatchlings (left) and using a rotating disk to shorten packing time (right). (Designed by Francois Rajts.).

- Keep the amount of hatchlings to about 150 g per 10 L of water for 6 hours. Take care when transporting hatchlings long distances, as hatchlings can starve if they have already absorbed their yolk before transportation. In this case, it is suggested to feed the hatchlings beforehand to get them sufficient energy during transportation. Reduce the density to half so that faeces do not pollute the carrying water. In addition, hatchlings of diurnal species should be released into the receiving pond during the daytime so that they can find food in the pond before the night.

12.3. Broodfish and market fish transportation

Just as with cultured large fish, not all SIS are affected to the same extent during transportation and handling. For example, existing data for silver carp can be used as an approximate guide for mola, while data for the less sensitive rohu can be used for pool barb. However, establishing a transportation guide for each SIS under this program is suggested.

For breeders, slight sedation could help reduce stress during transportation, and the density in the transportation tank should be half that of the market fish. However, this is not recommended for market fish because of restrictions on using chemicals in fish destined for human consumption. For sedation trials of breeders, clove oil (Eugenol) is suggested. Other known fish anaesthetics are either too expensive or dangerous for the operators and are prohibited nowadays anyway (for example Quinaldine).

To transport breeders and market fish, large special tanks with about a 1 m^3 capacity are convenient. A standard oxygen distribution system is required, using compressed oxygen in cylinders, an oxygen pressure regulator and a perforated oxygen distributor pipe.

12.4. Water quality requirements for transportation

The oxygen level in the water is the most important factor for the well-being of the fish and to avoid mortalities during or after transportation. However, fish use oxygen from the water and release metabolic wastes, such as carbon dioxide and ammonia, both of which are toxic. Even if the DO content of water remains high, the fish will not be able to absorb it if the amount of carbon dioxide is too high.

The oxygen requirements of fish are positively correlated with temperature, so gradually cooling the transportation water by few degrees helps reduce oxygen consumption. Approximately 20 kg of ice will drop the temperature of 1 m^3 of water by 1°C. Ice should be kept in a plastic bag in the transportation tank to avoid direct contact with fish and to lower the temperature slowly. Upon arrival at the destination, the temperature and other water quality parameters should be gradually adjusted to the new environment.

Ammonia can also accumulate in the water during transportation. Ammonia and carbon dioxide are both difficult to remove from the water, so water exchange might be necessary for long transportation times.

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Annex 1. Sample protocol for feed experimentation

Introduction

This covers the effect of different, locally made feeds on survival, growth performance and feed conversion rate of mola carplet (*Amblypharyngodon mola*, Hamilton, 1822) from early fry to 1 g in size.

Although many SIS prefer to feed on plankton, detritus and periphyton, most will eat supplementary feed given to carps. Developing adapted feed for some important SIS would help establish larger aquaculture SIS production. To improve mola and other SIS production, WorldFish, BISA and GIZ have partnered together as part of the Taking Nutrition-Sensitive Carp-SIS Polyculture Technology to Scale project. The purpose is to identify locally available ingredients and introduce a simple technique for processing and improving the digestibility of feed for SIS. Under this trial, different feed formulations will be tested to identify the best formulations for rearing early fry to fingerlings. Every formula will be made exclusively from locally available ingredients.

Objectives

The overall objective of this experiment is to increase fish production and, consequently, improve the protein and micronutrient supply of the population, with particular attention paid to children and pregnant women. The specific objectives are to compare the effect of different feed formulations on the growth performance of juvenile mola. This would allow for the following:

- confirming the viability of using local ingredients for supplementary feed production, for producing mola seed
- promoting mola culture by introducing techniques of mola seed production
- improved growth and survival rate of mola by applying quality seed for stocking in monoculture or polyculture ponds
- reduced FCR by using nearly adapted feed
- shortened growth period
- increased profit for farms
- lower market prices
- better nutrition for children and pregnant woman
- improved water quality of ponds through better digestion of feed.

Details of experiment

Materials

Place: The experiment will be take place under the applied research program of the project in selected fish farm(s).

Duration: The experiment will last for 2 months.

Pond: Ponds with an area of 400 m^2 and a minimum water depth of 1.5 m will be used.

Hapas: The experiment will be done in nine hapas measuring 1.2 m x 1.2 m x 1.2 m.

Water: The water in the pond can come from the rising underground water table during the rainy season and/or from a nearby small river or borehole. The water quality must be similar to that in ponds of the region.

Fish: Early mola fry about 10 days old pre-nursed in a nursery pond will be used.

Stocking density: Two hundred early fry will be stocked in each hapa measuring 1.44 m^2. The water depth in the hapas will be 1 m, corresponding to the density of 138.8 fish per cubic meter.

Natural food: Care will be taken to replace the hapas in a timely manner with clean ones that have increased mesh sizes to allow plankton to enter. All hapas will be replaced at the same time.

Supplementary feed: Three types of formulated farm-made supplementary feed will be used based on locally available ingredients. The nutritional value of the formulated feed will be confirmed by proximate analysis in a laboratory. The feed will consist of hot gelatinized cassava flour mixed with all ingredients, then sundried, broken down into small particles and sieved to adapt to the size of fish's mouths (section 12.3.). An example of different diet formulations is shown in Table 4. However, the final feed ingredients applied and their cost will be decided based on availability.

Method

Three replications will be applied using three hapas for each treatment, nine hapas in total. The experiment design is presented in Extension 1.

Stocking

Ten-day-old early mola fry will be brought from a nursery pond. The fry will be harvested from the nursery pond of the farm and stocked for 1 day in a common hapa for acclimatization and recovery from the stress of harvesting. The fry will be randomly selected and distributed throughout the nine trial hapas at 200 fry per hapa. The layout of the hapas placed along both sides of a walkway in the middle of the pond is shown in Figure 3.

Feed code	A	B	C
Blood meal (%)		5	5
Local fishmeal made from dried marine fish waste collected in markets (%)	54		
Yeast (%)	2	2	2
Cassava flour (%)	5	5	5
Rice bran (%)	39	27	27
Soybean cake (%)		40	
Sesame cake (%)		20	20
MOC (%)			40
Linseed oil (%)		1	1
DM (%)	91.7	90.3	91.9
Ash (%)	18.0	6.9	8.0
GE MJ/kg	17.7	17.4	17.3
Crude protein (%)	32.8	32.8	32.8
Lipid (%)	9.2	8.3	8.25
Fiber (%)	9.4	9.2	9.2
Cost (USD/kg)			

Table 4. Specifications of the trial diets for mola nursery culture (calculated from a raw material matrix).

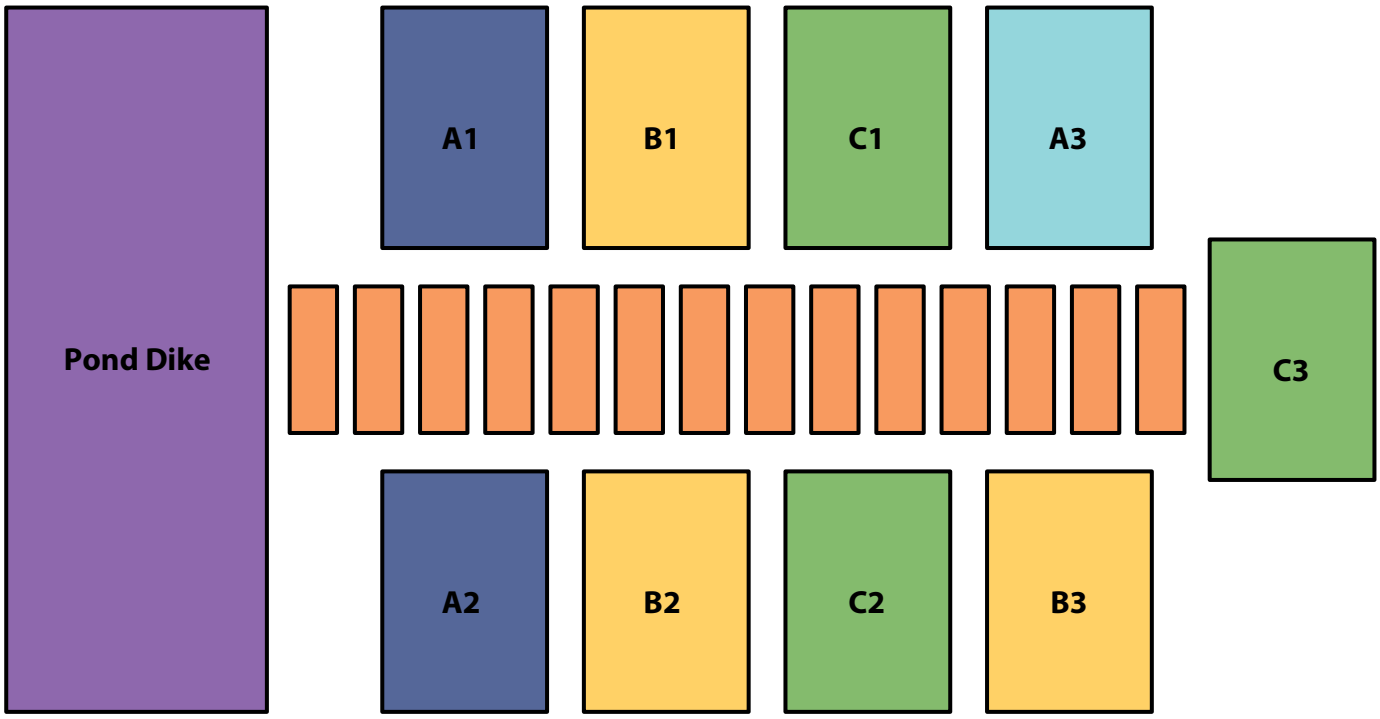

Figure 3. Layout of trial hapas.

Feeding

The daily quantity of feed will be determined by the biomass of the mola population in each cage. This will be controlled fortnightly through random sampling, and feeding rates will be adjusted accordingly. Three different diet formulas (Table 5) will be applied, and the fry will be fed four times a day. Daily feed rates are shown in Table 5.

Water quality management

Fish appetite, energy requirements, digestion and general health are optimum when the water quality is high. The water level and physicochemical characteristics of the pond will be monitored and, if necessary, water exchange, liming or other measures will be taken to maintain good water quality. Water quality data will include measuring temperature, pH and transparency every 3 days, while other parameters such as DO, ammonia, nitrite and alkalinity will be checked weekly. The difference in water quality inside and outside the hapas will determine the need to replace the hapas.

Monitoring

For stocking at the start of the trial, the fish will be weighed in bulk and individually counted to determine the total number, total biomass and the average weight stocked in each hapa. Weighing will be done in a plastic bucket filled previously with about five times more water than the estimated weight of fish. Weighing will be done using an electronic scale with a 2 kg capacity and 0.1 g sensitivity. During the trial, 20 fish will be randomly sampled every 7 days in the same way. Data will be recorded on stocking, daily feeding,

Fish size (g)	Amount of daily feed % of fish biomass	Number of feedings per day
0.1–0.5	20	4
0.51–0.75	15	4
0.75–1	10	4

Table 5. Application rates for trial diets.

daily mortality, random sampling, water quality and harvesting. Forms for data recording are shown in Extensions 2, 3 and 4. Random sampling data and mortalities will be used to correct the estimated biomass in each pond, and feeding rates will be adjusted to estimate the biomass in each hapa weekly. Any disease issues will be reported and any treatments required will be logged.

Health management

To prevent diseases, special attention should be paid to the applied feed quality by keeping the cages clean and free from algal fouling and ensuring adequate water quality in the pond. If treatment is required, however, it will be applied equally to all cages (treatment A, B and C) so that any negative effect on growth is shared.

Parameters	Activity
Average weight of fry	Weigh a minimum of 20 fry every 7 days before feeding in the morning.
Mortality	Note the mortality of fish until 18:00 every day.
Feeding record	Note the daily feed codes, number of feedings and total quantity of feed applied.
Environment	T (^{0}C): Measured weekly at 07:00–08:00 and 14:00–15:00
	pH: Measured weekly at 07:00–08:00 and 14:00–15:00
	Alkalinity: Measured weekly at 14:00–15:00
	Transparency: Measured every third day around noon
	NH_3: Measured weekly at 14:00–15:00
	NO_2: Measure weekly at 14:00–15:00
	DO: Measured weekly at 07:00–08:00

Table 6. Monitoring activity.

Analysis

Statistical analysis will be applied for evaluation of results. To evaluate the effectiveness of different feed formulations on fish production, the survival rate, net weight gain, SGR and FCR will all be evaluated. The cost estimate of the trial is shown in Extension 5.

Cage No.	Feed code	Number of cages	Density of fish (fish/m^2)	Volume (m^3)	Density of fish/m^3
A1	A	1	138	1.50	133
A2		1	138	1.50	133
A3		1	138	1.50	133
B1	B	1	138	1.50	133
B2		1	138	1.50	133
B3		1	138	1.50	133
C1	C	1	138	1.50	133
C2		1	138	1.50	133
C3		1	138	1.50	133

Extension 1. Experimental design.

Code of cage _____			Day _____ Month _____ 2021										
Day	Fish information		Feed						Dead fish		Treatment		Remarks
	Number of fish	Total fish (kg)	Code of feed	First feeding (g)	Second feeding (g)	Third feeding	Fourth feeding	Total feed (g)	Number	Total quantity (g)	Name of product	Quantity used (g)	
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
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19													
20													
21													
22													
23													
24													
25													
26													
27													
28													
29													
30													
31													
Total													

Extension 2. Daily record.

Code of cage _____		Day _____ Month _____ 2021		
SN	**Weight (g)**	**Total length (mm)**	**Height (mm)**	**Remarks**
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
Average				
Range				

Extension 3. Random fish sampling record.

Date	Time	pH	DO (mg/L)	T °C	Secchi discm	Ammonia N mg/L	Water exchange	Pond treatment	Remarks

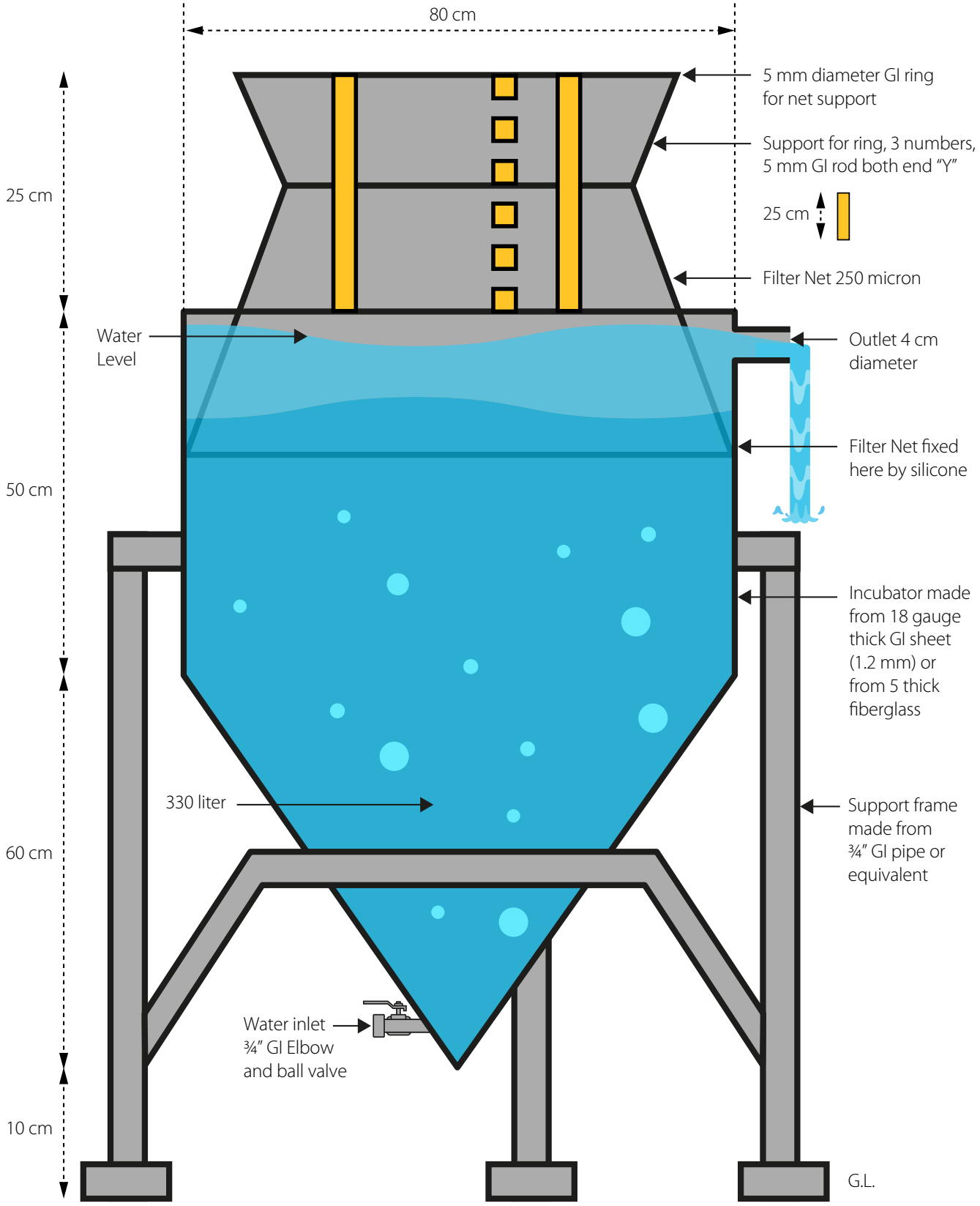
Extension 4. Water quality management.

Cost of investment	Number	Unit cost (USD)	Total cost (USD)
Structure and equipment			
Walkway and cage support	1	85	85
Signboard	1	20	20
Hapa 1.2 m x 1.2 m x 1.2 m, mesh 0.5 mm, 2 mm and 5 mm (20 each)	60	7.6	456
Individual feed containers for each cage (1 L capacity)	9	1	9
Electronic scale, capacity 2 kg, sensitivity 0.1 g	1	76	76
Fish measuring box	1	9	9
Buckets for fed preparation and sampling	5	2	10
Hand counter	1	2	2
Scoop net	1	10	10
Total structure/equipment cost			677
OPERATIONAL COST			
Labor			
Research assistant (2 months)	2	300	600
Guard	2	250	500
Subtotal			1100
Other			
Fuel/electricity for pumps, generator	1	100	100
Procurement/transportation of trial fish	1	40	40
Fish feed	1	50	50
Chemicals/medicines for pond, fish treatments, lime	1	100	100
Cost for outside laboratory analysis (feed, fish)	1	190	190
Unforeseen costs	1	93	93
Subtotal			573
GRAND TOTAL			2350

Extension 5. Estimated cost

Annex 2. Mobile cylindro-conical incubator

Sketch of a mobile cylindro-conical incubator with a 330 L capacity.



Sketch of incubator (330 liter capacity)
Designed by Rajts

Annex 3. Comparison of a funnel-type circular incubator with special attention to SIS breeding

Chinese circular incubator	Funnel-type incubator
To keep the eggs in motion, the water flow must be 10 cm per second, independently of the quantity of incubated eggs. As a result, water consumption will be relatively high.	The water flow is better adjusted to the actual quantity of eggs or larvae. Eggs will continuously fall down against the inflowing water at the bottom of the funnel.
Because the water mixes in the circular tank, pathogens are not systematically removed. During methylene blue treatment, the water remains blue for a long time and the color intensity gradually declines.	Mixing used water with inflowing water declines. Most of the used water is eliminated at the top of incubator, together with harmful metabolic products and pathogens. The water does not return to the bottom of the incubator, so it could potentially infect another eggs or larvae, provided that the weighted rubber ball is in place to disperse the inflowing water. The process can be observed in a transparent incubator during treatment of eggs with methylene blue. The blue water overflows at the top without mixing with the lower layers.
The incubation capacity is about 500 eggs per liter, because the eggs tend to accumulate and deposit toward the center of the incubator.	The incubation capacity is up to 3000 eggs per liter.
Customers want to buy several species at the same time, but the number of fish species reproduced in one batch is limited by the number of circular incubators.	With 20–50 incubators in one hatchery, it is possible and generally practiced to incubate different species separately at the same time.
The eggs of many breeders must be kept together in one incubator because of its large capacity. However, some females will ovulate a few hours late. During hatching, fish larvae produce protease enzymes to dissolve their eggshell. The enzyme results in premature hatching of late fertilized eggs. When synthetic hormones are used, late ovulation can be frequent. Pituitary extract results in more synchronized ovulation.	A high number of incubators makes it possible to incubate late ovulated eggs separately.
This type of incubator is too large for SIS breeding trials, with some exceptions if used for spawning trials.	Incubators with smaller capacities are better for induced breeding trials of SIS in smaller quantities and several species simultaneously.

Annex 4. Checklist for fish transportation in trucks

Prior starting check the followings
1. The fish are in good health, check also for parasites
2. The fish in pond has been trained by several netting during the last week
3. The fish has been starved for 12 h for fry and 24 h for fingerling and 48 hours for adult fish
4. The receiving farm or traders has been informed and confirmed
5. The truck has been cleaned/disinfected and the documents,engine, break, lighting are in good condition
6. For more than 8 hours trip/day two drivers are necessary
7. Oxygen reductors are operational, one spare is suggested
8. Oxygen cylynders (at least four of 10 m3 capacity) are full
9. Oxygen cylinders are fixed vertically. Condensed water with rast in the cylinder can damage the pressure regulator if entering there from horizontally placed cylinder
10. Oxygen pipes are in good condition, underwater distribution pipe not damaged
11. Wrench for Oxygen cylynder is available
12. Outils, are kept in box in dry condition
13. Balance for fish weighing is available and working
14. Fish carrying basins are in good condition
15. Long handled dip net is available
17. Thermometer should be available
18. If possible, the transportation tanks should be filled the previous evening and kept open to cool down the water
19. The coldest water source should be used for transportation; either from tube-well (26 -28 C), or from pond if it colder
20. Water from tube-well should be well aerated for at least 30 minutes, prior fish loaded (CO_2 content should be <10 mg/l)
21. Prior starting loading the fish, oxygen supply should be started
22. Tanks should be filled full. Moving water in partially filled tank is damaging fish and interfering with driving. The cover of the tanks are in good condition; strong rope available in case the closing system is damaged

During transportation
1. Oxygen suply be continuously checked
2. During transportation the condition of fish should be checked every hour
3. Avoid stop the truck for more than 10 minutes and if stopped, keep it under shelter
4. Water need to be exchanged, if quality deterriorated. Possible sites for exchanging water should be identified in advance
5. While taking water from pond, the end of suction pipe should be at about 1.0 m depth, to avoid taking hot surface water, but not deeper to avoid pumping of hypoxic water from deep layer of stratified water. Entrance of mud from bottom should be avoided
6. The end of suction pipe should be prowided with protective screen
7. At delivery site water should be pumped from the pond into the tanks to equalize water quality

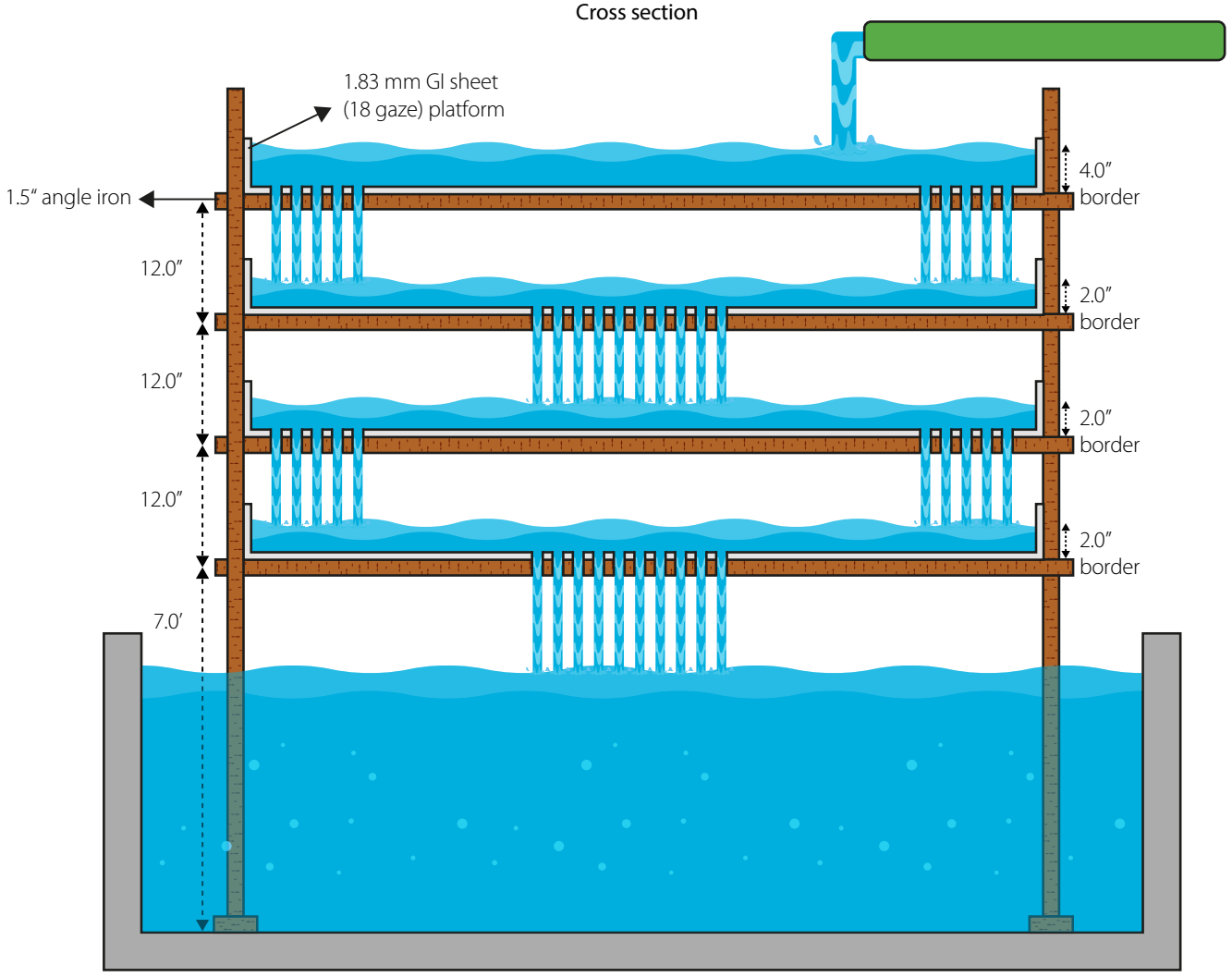
Annex 5. Site criteria for SIS hatchery selection

Name of hatchery owner:			
Address/phone No:			
e-mail :			
Date of visit:			
Visited by:			
Criteria		Reply to questions	Score*
1	The hatchery and ponds are operational		
2	Present key activities and outputs from the hatchery		
3	Have experience in SIS breeding, output, what species, how many years?		
4	Room for use as laboratory and for likely installation of scientific equipment		
5	Rest room for staff		
6	Experienced and reliable hatchery/landowner/partner and staff. Considering the continuous nature of hatchery works, devoted staff would be an advantage. One batch of hatchery operation generally lasts not less than one week and supervision is necessary during 24 hours per day. For this reason, the hatchery should have sufficient staff to apply rotation of skilled staff for 24 hours a day		
7	The owner agrees to provide his staff for required trial operations, hatchery and fishing equipment, one full set of breeding tanks/devices for experimental breeding of SIS, as well as at least four small ponds of about 200 m^2 area for exclusively use by the SIS breeding trials		
8	Land/farm is free of any legal complication		
9	The site is situated close to the house of landowner for security reason		
10	Farm is equipped with sanitary installation		
11	The highest experienced flood level is not exceeding the top of pond dikes; the floor level of the hatchery and pump house, stores etc.		
12	Full time electricity supply. In addition: existing standby generator, as well as standby diesel pump for hatchery water supply, in case of electricity failure		
13	Existing water supply to hatchery from borehole. The quality of water from borehole is suitable for hatchery use (esp. constant temperature, pH 7.0-8.5; Iron content <0.5 ppm; TDS <2000 mg/l). High TDS is preferable, in view of the process of stimulating gonadal development and breeding by manipulating the environment, as sudden dilution of water by using rainwater. Ammonia, Methane and H2S are toxic and should not be present.		
14	Additional possibility of water supply from runoff of rainwater: small stream, ditches, old bundh; Possibility to install gravity water supply from stream and to release used water via effluent treatment to the same stream is saving water of aquifer and operation cost		

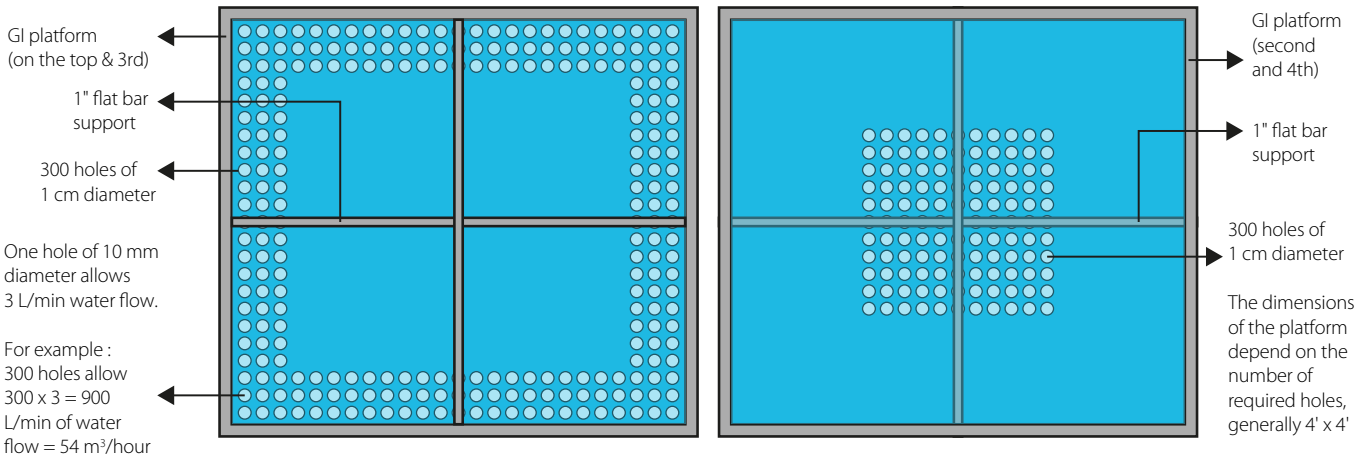
15	Existing aeration tower for aerate the water prior reaching the elevated storage tank (overhead tank) and also prior reaching the pond for Iron oxidation/sedimentation (if excess Iron present in water from borehole)		
16	Capacity of aeration tower should ensure >90 % DO concentration of water in overhead tank and removal of CO2 to a maximum of 15 mg/l. Actual measurements?		
17	Autonomy of overhead tank allows 4 hours supply at full operation without refilling; if not how long?		
18	The hatchery has about 100 m^2 free space for likely installation of additional tanks, incubators. It is to prevent interference with usual hatchery operation by the owner, as well as installation of additional devices may be necessary.		
19	Available land for likely requirement of construction of small ponds would be an advantage. It would require about 500 m^2 area for brood stock, nurseries of SIS and for feeding trials		
20	Soil quality is suitable for pond construction		
21	Existing ponds are retaining water through the year, or additional water supply is ensured		
22	Expected total water spread area for trials is >1,000 m^2 is available/ possible		
23	Gravity-flow water supply and drainage for ponds is preferred		
24	Drying of ponds is possible (bottom of ponds are above the underground water table)		
25	The trials are made for development of SIS culture in S/E Asia. Therefore, the owner should allow conducting training courses on trial results in his hatchery and pond sites		
26	Farm geographic situation and road connection allows demonstration activity and easy marketing of farm product		
27	Biosecurity: Although hazardous chemicals are not used or in minutest quantity, effluents from the hatchery should not be released directly into natural water bodies. Possibility should be there for releasing the used water into ponds, where the residence time of water should be not less than one week? In case of recycling the water for hatchery use, provision should be there to use at least two effluent treatment ponds in series, prior the water is re-used. In the second pond, no fish should be present and growth of dense aquatic vegetation should be allowed to ensure clean water? Existence of fencing around the farm would be an advantage? Hand and foot sanitising at the entrance of the farm available?		
28	Biosecurity: possibility should be there for effluent treatment of used water		
29	Existence of fencing around the farm		
	Total		
*Score: 0 = Poor; 5 = Best			

Annex 6. Sketch of aeration tower

(Capacity 54 m3/h)
Cross section



Plan of aeration tower



Annex 7. Saturation point of dissolved oxygen in freshwater at sea level atmospheric pressure at different temperatures

Temperature °C	Oxygen Solubility mg/l	Temperature °C	Oxygen Solubility mg/l
10	11.29	21	8.92
11	11.03	22	8.74
12	10.78	23	8.58
13	10.54	24	8.42
14	10.31	25	8.26
15	10.08	26	8.11
16	9.87	27	7.97
17	9.67	28	7.83
18	9.47	29	7.69
19	9.28	30	7.56
20	9.09	35	6.93

Source: Boyd (1998).

About WorldFish

WorldFish is a nonprofit research and innovation institution that creates, advances and translates scientific research on aquatic food systems into scalable solutions with transformational impact on human well-being and the environment. Our research data, evidence and insights shape better practices, policies and investment decisions for sustainable development in low- and middle-income countries.

We have a global presence across 20 countries in Asia, Africa and the Pacific with 460 staff of 30 nationalities deployed where the greatest sustainable development challenges can be addressed through holistic aquatic food systems solutions.

Our research and innovation work spans climate change, food security and nutrition, sustainable fisheries and aquaculture, the blue economy and ocean governance, One Health, genetics and AgriTech, and it integrates evidence and perspectives on gender, youth and social inclusion. Our approach empowers people for change over the long term: research excellence and engagement with national and international partners are at the heart of our efforts to set new agendas, build capacities and support better decision-making on the critical issues of our times.

WorldFish is part of One CGIAR, the world's largest agricultural innovation network.

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