

Hatchery-Based Mass Seed Production of Nutrition-Sensitive Pool Barb (*Puntius sophore*) and Swamp Barb (*Puntius chola*)

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Introduction

The tropical Asian fish genus *Puntius* comprises a wide range of small indigenous fish species (SIS) that play an important role in rural nutrition and livelihoods. Among them, the swamp barb (*Puntius chola*) and pool barb (*Puntius sophore*), commonly known as “punti” or “puti” in Bangladesh and the Ganges Delta, are two of the most popular and nutritionally valuable species. Both belong to the Cyprinidae family and are very similar in appearance, making visual identification difficult except at full maturity. A key distinguishing characteristic is the presence of a small pair of maxillary barbels in swamp barb, which are absent in pool barb.

These barbs are omnivorous, column-dwelling feeders that consume green algae, diatoms, crustaceans, rotifers, insect larvae, and organic matter. They exhibit partial and asynchronous spawning, with a prolonged breeding season from April to September influenced by rainfall, water temperature, and water quality. Their distribution spans widely across South and Southeast Asia, including Bangladesh, India, Nepal, Pakistan, Sri Lanka, and Myanmar. They inhabit a variety of freshwater environments such as ponds, canals, beels, ditches, wetlands, and floodplains, and are well adapted to stagnant or slow-flowing waters and low-oxygen conditions—traits that make them compatible with diverse aquaculture systems.

Once abundant in natural water bodies, these species were routinely harvested by rural households and small-scale fishers, contributing significantly to local nutrition and food security. However, in recent decades, wild populations have declined due to habitat degradation, pollution, overfishing, water abstraction, and climate-driven changes in hydrology. As a result, their market prices have increased, reducing access for low-income families that traditionally depended on these fish as an affordable and nutrient-rich food.

Both swamp barb and pool barb are typically consumed whole and are exceptionally nutritious. They provide high-quality protein, fat-soluble vitamins (A, D, E, and K), calcium, iron, zinc, potassium, manganese, essential fatty acids, and amino acids—nutrients that are critical for preventing micronutrient deficiencies, particularly among children, adolescents, and pregnant women (Bogard et al., 2015; Ignowski et al., 2023).

Despite their nutritional value and cultural importance, these SIS have received limited attention in pond aquaculture, especially within carp polyculture systems (Ali et al., 2016; Shepon et al., 2020). Yet integrating them into nutrition-sensitive aquaculture can increase overall fish production while improving household access to small, nutrient-dense fish, thereby supporting both food security and rural livelihoods. A major barrier to expanding their culture has been the absence of standardized, hatchery-based seed production methods.

To address this challenge, WorldFish, under the CGIAR Scaling for Impact (S4I) program, has developed a simple, low-cost, and scalable technique for induced breeding and mass seed production of pool barb and swamp barb. This manual presents a practical, step-by-step guidelines for hatchery operators to produce high-quality SIS seed and to promote the wider adoption of nutrition-sensitive aquaculture in Bangladesh and beyond.



Photo 1. Mature female (left) and mature male (right) punti broodfish. **Photo credit:** Dr. Hazrat Ali, WorldFish.

1. Punti Broodstock Pond Preparation and Management

Proper preparation and management of the broodstock pond are essential for successful punti breeding. The pond should first be completely drained, leaving the bottom soil moist before applying lime. Calcium oxide (CaO) is recommended at 247 kg per ha, with adjustments depending on soil pH. Since the optimal pH for pond soil is 6.5–7.0, acidic soils require higher lime doses, while ponds with pH above 7.0 require less. After liming, the pond should be dried for about one week to disinfect the bottom and eliminate predators.

Once prepared, the pond should be gradually filled with borewell or surface water to a depth of 0.7–1.0 m. Maintaining suitable conditions for broodfish also depends on the availability of natural food, particularly phytoplankton and zooplankton. To enhance natural productivity, inorganic fertilizers such as urea and triple super phosphate (TSP) should be applied at 25–37 kg per ha each. Fermented mustard oil cake (MOC) at 37 kg per ha should also be applied evenly across the water surface to further enrich natural food resources.

After one week, when phytoplankton levels are adequate and the water turns green—with a Secchi disk reading of 25–30 cm—the pond becomes ready for stocking. Punti broodstock should be sourced from diverse and preferably large, permanent waterbodies to ensure good genetic diversity and fish health. After acclimatization, the broodstock should be stocked at 200,000–250,000 fish per ha. Prior to stocking, the fish must be disinfected by immersing them in a potassium permanganate (KMnO₄) solution made by dissolving 0.5 g of KMnO₄ in 100 liters of water.

To support continuous gonadal development, powdered feed containing 35%–40% crude protein should be provided. Gonadal maturation can be further stimulated by adding soft water from rivers or stored rainwater when necessary. Weekly applications of urea and TSP at 12–18 kg per ha each, along with 19 kg per ha of fermented mustard oil cake, help maintain sufficient natural food levels. These quantities should be adjusted according to the observed density of phytoplankton.

During the monsoon season, the pond dike should be kept free of grasses and aquatic weeds up to one foot above the highest water level to reduce hiding places for fish and ease harvesting. Throughout the entire culture period, strict biosecurity practices are essential to prevent disease outbreaks and ensure the health and survival of the broodfish.



Photo 2. Biosecurity in a punti broodstock pond. **Photo credit:** Harun Or Rashid, WorldFish.

2. Taxonomic identification

The pool barb (*Puntius sophore*) and swamp barb (*Puntius chola*) are morphologically similar and often difficult to distinguish in the field. However, accurate identification is important for broodstock selection, breeding, and seed production. The key distinguishing feature is the presence of a single pair of small maxillary barbels in swamp barb (*P. chola*), which are absent in pool barb (*P. sophore*). Other distinguishing characteristics, such as fin coloration, body shape, and spot patterns, become more apparent at maturity and can also aid in identification (Table 1).

Table 1. Basic morphological characteristics of pool barb and swamp barb

Characteristic	Pool Barb (<i>Puntius sophore</i>)	Swamp Barb (<i>Puntius chola</i>)
Coloration	Greenish dorsally with a silvery gloss; entirely silver on the underside.	Greenish dorsally with a silvery gloss; entirely silver on the underside.
Body shape	Deep, moderately elongated, laterally compressed; dorsal margin humped with a peak at the origin of the dorsal fin.	Strongly deep, laterally compressed; dorsal profile arched from snout to dorsal fin origin.
Body color	Olive-green back; silvery-yellowish flanks; silvery abdomen.	Olive-green back; silvery-yellowish flanks; silvery abdomen.
Gill cover	Diffused golden spot on the gill cover.	Diffused golden spot on the gill cover.
Fin coloration	Fins tinged yellow; may appear reddish underneath.	Olive-colored fins; ventral fins tinged with orange.
Pelvic and anal fins	Red, with a mid-lateral red stripe in mature males.	Yellowish to reddish, with a pinkish-red lateral stripe in mature males.
Blotch/spot	Dark round blotch at the base of the dorsal fin and end of the caudal peduncle.	Dark blotch at the base of the dorsal fin and mid-point of the caudal peduncle.
Eyes	Silvery, sometimes tinged with red.	Silvery.
Barbels	Absent.	Single pair of small maxillary barbels.

Accurate identification of mature male and female punti broodfish is essential for successful induced breeding. Mature individuals can be distinguished based on external physical characteristics and behavior, as outlined below in Table 2.

Table 2. Distinguishing mature male and female

Characteristic	Female	Male
Size and color	Larger in size; lighter body color.	Smaller; reddish coloration along the lateral line in maturity.
Abdomen	Soft and clearly distended.	Not distended.



Photo 3. A mature female (top) and male (bottom) punti broodfish. **Photo credit:** Dr. Hazrat Ali, WorldFish.

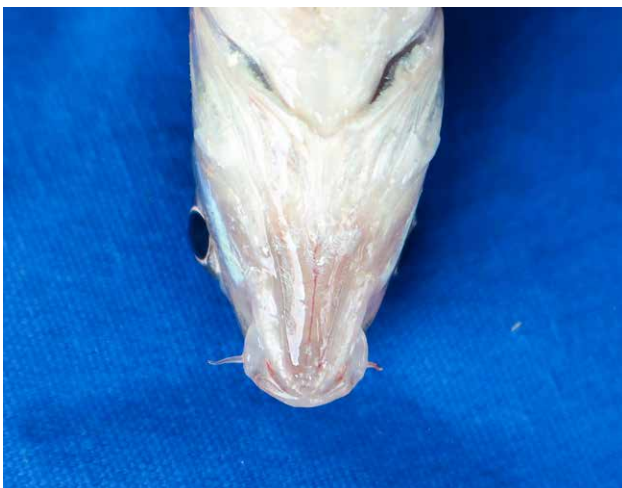


Photo 4. Single pair of maxillary barbels of swamp barb. **Photo credit:** Kalpajit Gogoi, WorldFish.

3. Punti Broodstock Harvesting and Conditioning

Proper harvesting and conditioning of punti broodfish are crucial for maintaining their health and ensuring successful breeding. A dedicated broodstock pond should be used exclusively for punti to avoid injuries that may occur when harvesting alongside larger species such as carp. Harvesting is best conducted early in the morning, before water temperatures rise, to minimize stress on the fish.

During harvest, broodfish must be handled gently and transferred immediately in cool, well-oxygenated water to the conditioning tanks. To reduce jumping during transportation, leafy plant branches may be placed inside the containers. Upon arrival, the fish should be carefully moved into a fine-mesh hapa that has been prepared in the conditioning tank beforehand.

The broodstock should then be kept under a continuous water shower for 24 hours to help them acclimate and prepare physiologically for spawning. This conditioning period also allows the fish to empty their digestive tracts, promoting a cleaner environment and reducing the risk of disease during breeding. Finally, the hapa should be securely covered with a fine mosquito net to prevent the broodfish from escaping.



Photo 5. Mature male and female punti broodfish in a fine mesh hapa inside a conditioning tank.

Photo credit: Harun Or Rashid, WorldFish.

4. Punti Induced Breeding Arrangement

Successful induced breeding of punti requires a controlled environment that ensures optimal conditions for spawning, fertilization, and hatching. This involves the proper setup of dedicated breeding tanks, incubation units, hapas, and a reliable aeration system to maintain suitable water quality and oxygen levels throughout the breeding process.

4.1 Breeding and Incubation Tank

The breeding and incubation tank is the core component of the induced breeding system, serving

multiple purposes including broodstock conditioning, hormone-induced spawning, egg incubation, and early hatchling care. In small-scale hatcheries, breeding is commonly carried out in a fiber-reinforced plastic (FRP) rectangular tank (see Photo 6). A standard FRP tank measures 2.5 m in length, 1.25 m in width, and 0.6 m in height, with the floor built at a 1% slope toward an outlet pipe to allow efficient water flow and drainage. An iron frame is installed along the top edges of the tank to support two nested hapas. Above the tank, a 3/4-inch shower pipe fitted with 1 mm holes spaced 1 cm apart delivers a continuous, gentle water spray to keep broodstock well-oxygenated and comfortable, creating ideal conditions for spawning.

For commercial-scale hatcheries, a concrete tank is more suitable (see Photo 7). The recommended internal dimensions are 3.0 m × 1.5 m × 0.6 m, providing approximately 2.25 m³ of water at a depth of 50 cm. Like the FRP tank, the concrete tank should have a 1% floor slope leading to a 1.5-inch vertical drainage outlet that can be easily detached for cleaning and routine maintenance. This ensures proper hygiene and smooth operation of the breeding system.

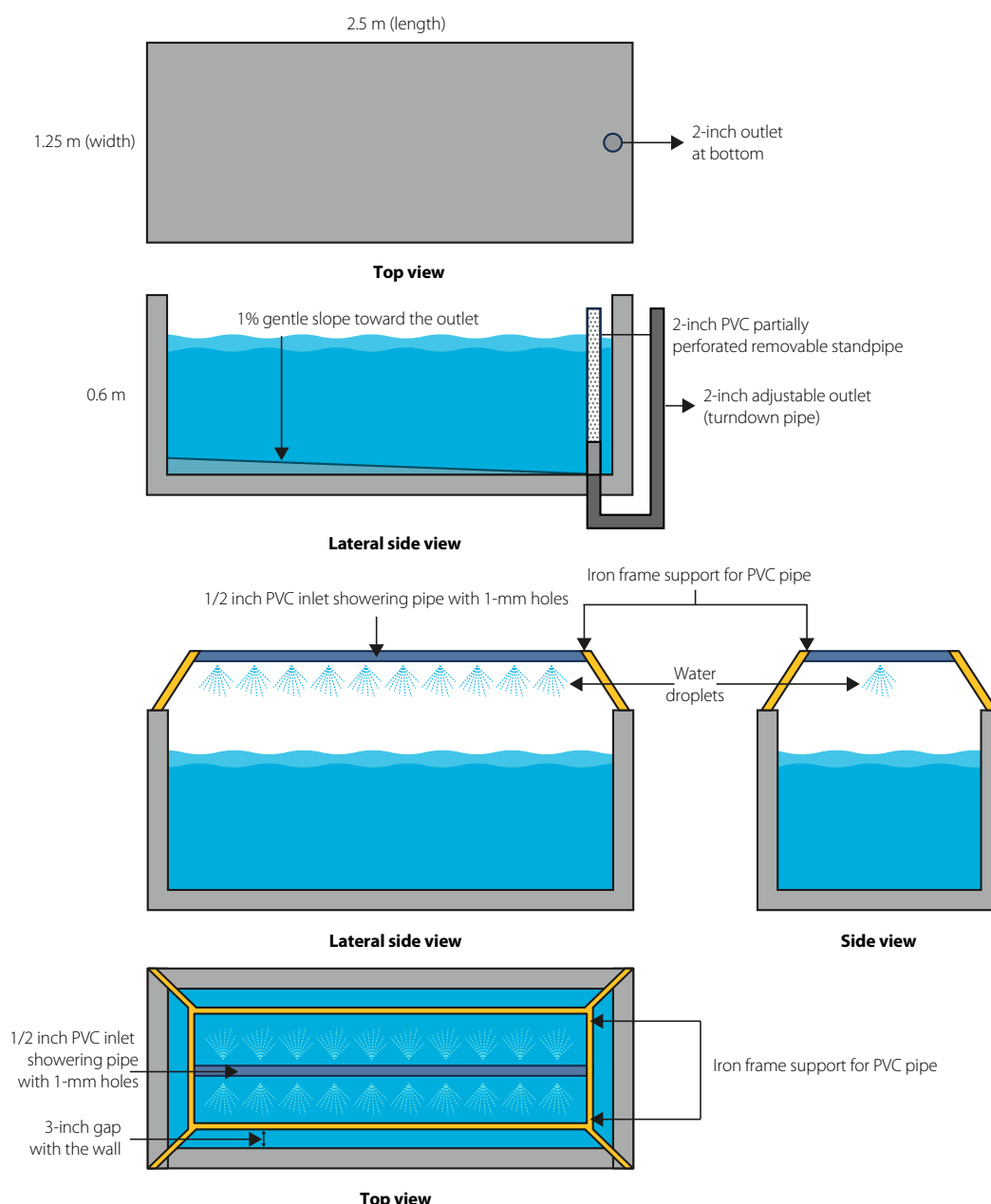


Photo 6. FRP model of a breeding and incubation tank (modified from Rajts et al., 2024).

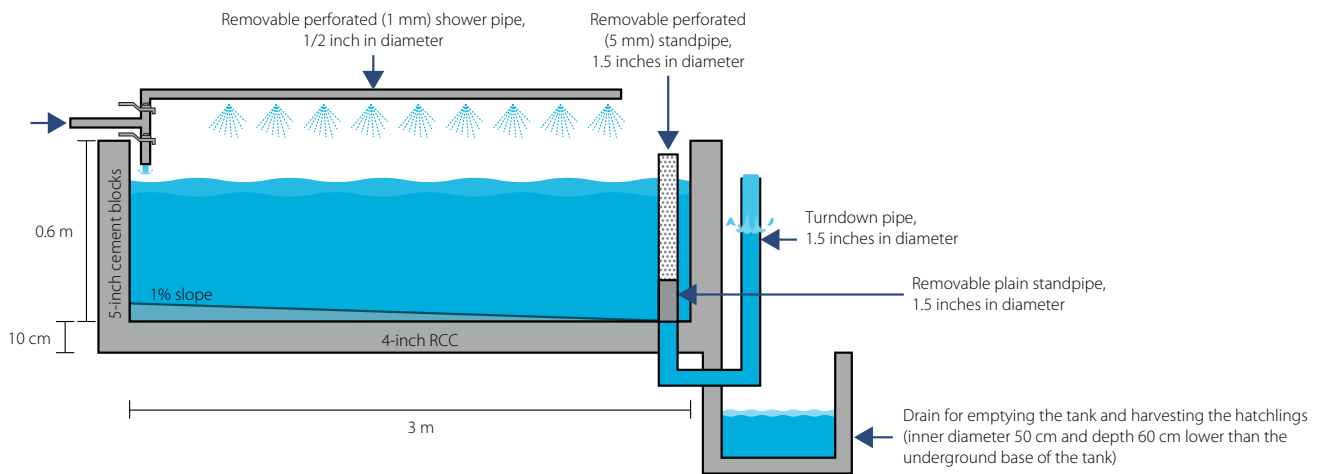


Photo 7. Concrete breeding and incubation tank (modified from Rajts et al., 2024).

4.2 Hapa Arrangement

Hapas play a critical role in separating and managing broodstock during different stages of the breeding cycle. A conditioning hapa should first be installed in the conditioning tank to hold broodfish before breeding. This allows efficient waste removal and ensures proper acclimatization prior to hormone induction. In the breeding tank, two hapas are used to contain the hormone-treated broodstock securely. The outer hapa is constructed from 250-micron monofilament mesh, and a smaller inner hapa with a 10 mm mesh is fixed inside it using the iron support frame. This nested structure ensures safe containment of the breeders while promoting good water flow and effective egg collection.

A gentle water shower from the overhead tank should be maintained continuously over the hapas to improve oxygen availability and simulate natural breeding triggers. When using surface water from ponds, rivers, or canals, the water must be filtered through a 100-micron monofilament mesh to remove sediment, plankton, and debris before entering the system.



Photo 8. Installation of inner and outer hapas in an FRP tank. **Photo credit:** Dr. Hazrat Ali, WorldFish.



Photo 9. Installation of 100-micron monofilament mesh to filter water prior to use for hatchery operation. **Photo credit:** Harun Or Rashid, WorldFish.

4.3 Aeration Tower

The aeration tower enriches the oxygen content of borewell or surface water before it enters the breeding and conditioning tanks. This system efficiently increases dissolved oxygen (DO) levels while reducing energy costs by eliminating the need for electric aerators. The tower is typically made of metal and mounted on top of an overhead water tank.

It consists of four perforated galvanized iron sheets fitted with 10 mm holes, which disperse the incoming water into fine droplets as it flows downward. This droplet formation greatly increases the surface area for gas exchange, allowing carbon dioxide and other harmful gases to escape. Through this process, CO₂ levels can be reduced by up to 70%, while DO levels rise close to saturation. This significantly improves overall water quality and creates optimal conditions for pundi broodstock health and successful breeding.



Photo 10. An aeration tower in an overhead water reservoir tank at a hatchery. **Photo credit:** Harun Or Rashid, WorldFish.

5. Hormone Dose and Administration

For induced breeding of punti, any commercially available synthetic gonadotropin-releasing hormone analogue (S-GnRH α) combined with a dopamine antagonist may be used, as these products are widely available under different trade names. The recommended dosage is 0.75 ml per kg of body weight for males and 1.50 ml per kg of body weight for females. Hormone potency and broodfish readiness can vary across batches and brands; therefore, it is advisable to conduct a small trial before full-scale induction. A male-to-female ratio of 1:1 should be maintained throughout hormone-induced breeding.

To prepare the inducing solution for 1 kg of female punti, dilute 1.50 ml of hormone with 4.50 ml of sterile water, yielding 6.00 ml of diluted solution per kg of body weight. Dilution is essential because the hormone is highly viscous and only a very small dose is needed for punti. Sterile water or a 0.65% sterile saline (NaCl) solution may be used for dilution.

Hormone injections should be administered into the dorsal musculature—specifically above the lateral line and below the dorsal fin—using a 1-ml insulin syringe with 50 fine graduations to ensure accurate dosing. The ideal time for injection is late afternoon to early evening, between 17:00 and 19:00, when broodfish respond most effectively. After injection, the broodfish should be gently transferred to a breeding hapa made of 10-mm mesh and securely covered with a nylon mosquito net to prevent escape or injury. A continuous, gentle water shower must be maintained over the hapa to mimic natural rainfall and stimulate spawning behavior.



Photo 11. Synthetic hormone and 1-ml syringe used for punti induced breeding. **Photo credit:** Dr. Hazrat Ali, WorldFish.



Photo 12. Administering the inducing solution into the dorsal musculature, just above the lateral line and below the dorsal fin of punti breeder. **Photo credit:** Harun Or Rashid, WorldFish.



Photo 13. Administering the inducing solution into the punti breeder. **Photo credit:** Dr. Hazrat Ali, WorldFish.

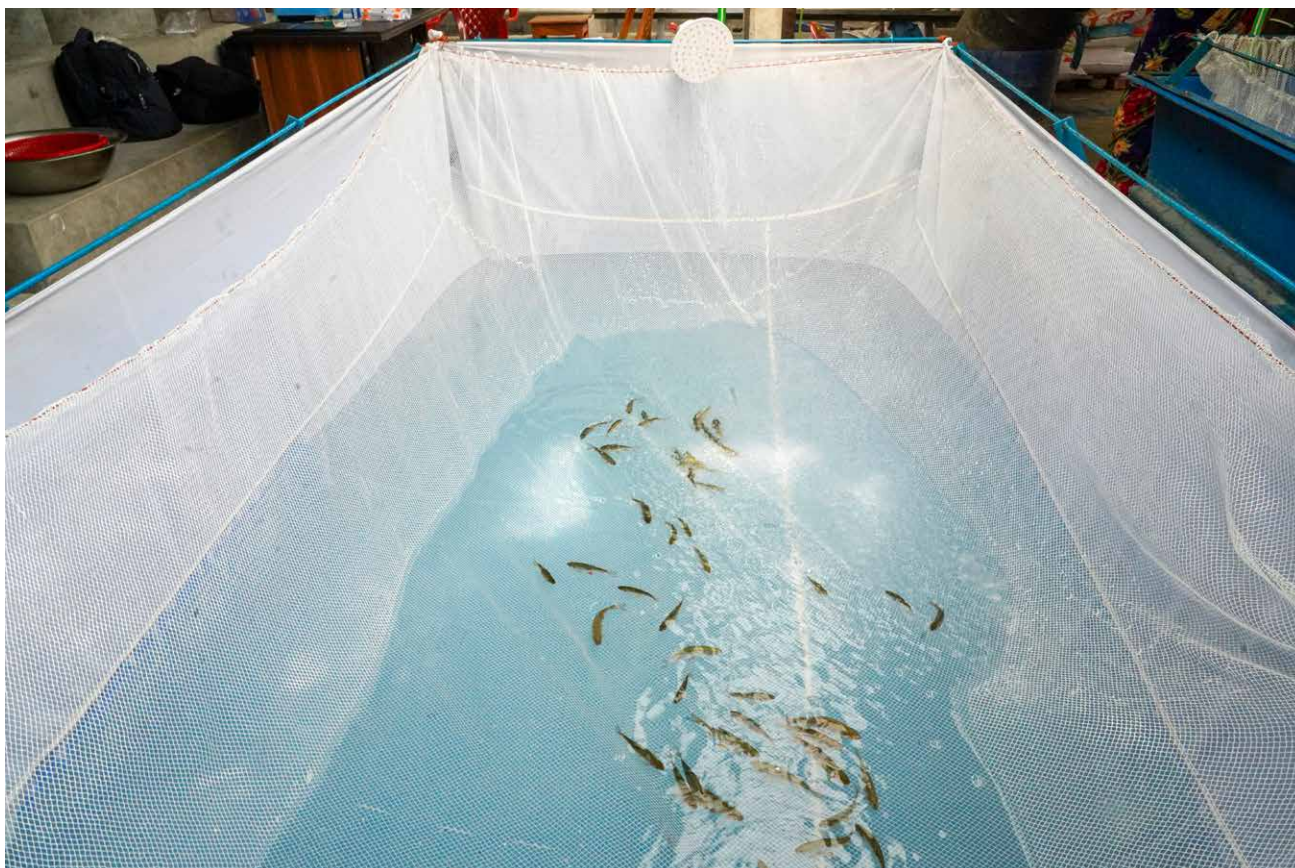


Photo 14. Placing breeders in a 10 mm mesh inner hapa immediately after administering the inducing solution. **Photo credit:** Dr. Hazrat Ali, WorldFish.



Photo 15. Covering the breeding tank after administering the hormone to prevent escape or injury of punti breeder. **Photo credit:** Dr. Hazrat Ali, WorldFish.

Box 1. Hormone Dose Calculation

Standard hormone dose

The required dose of a synthetic gonadotropin-releasing hormone (S-GnRHa) is 0.75 ml/kg of body weight for males and 1.5 ml/kg for females.

How to prepare and calculate the dose

For 1 kg of female punti breeders, dilute 1.50 ml of hormone with 4.50 ml of sterile water. This results in 6.00 ml of inducing solution for 1 kg of punti.

For example:

- 1000 g of punti = 6.0 ml
- 1 g of punti = $6.0 \div 1000 = 0.006$ ml
- 5 g of punti = $5 \times 0.006 = 0.030$ ml
- 10 g of punti = $10 \times 0.006 = 0.060$ ml
- 15 g of punti = $15 \times 0.006 = 0.090$ ml
- 20 g of punti = $20 \times 0.006 = 0.120$ ml

Administering the solution

Inject the prepared inducing solution into the dorsal musculature, positioned just above the lateral line and below the dorsal fin of the punti broodfish. The preferred method is to use a 1-ml diabetic insulin syringe with 50 fine graduations to ensure accurate dosing. Each graduation of the insulin syringe represents 0.02 ml ($1 \div 50 = 0.02$ ml).

For example:

- 5 g of punti: $0.030 \div 0.02 = 1.50$ graduations of a syringe
- 10 g of punti: $0.060 \div 0.02 = 3.00$ graduations of a syringe
- 15 g of punti: $0.090 \div 0.02 = 4.50$ graduations of a syringe
- 20 g of punti: $0.120 \div 0.02 = 6.00$ graduations of a syringe

6. Spawning and Egg Incubation

Spawning and egg incubation are critical steps in the successful breeding of punti. Proper timing, gentle handling, and maintaining suitable environmental conditions during this stage ensure healthy embryo development and improve hatchling survival. After hormone injection, all treated punti breeders should be removed the following morning between 09:00 and 11:00 by lifting the inner hapa and transferring them to a separate recovery pond. Spawning typically occurs 6-8 hours after hormone administration, particularly when the water temperature is around 29.5°C. Hatching usually takes place 12-14 hours after fertilization at temperatures between 28.5°C and 30°C. During this period, the outer hapa should be checked for eggs, which settle on the bottom of the 250-micron mesh. Fertilized eggs are typically light brown to yellowish, transparent, demersal (sink to the bottom), and semi-adhesive in texture.



Photo 16. Eggs attached to the bottom part of the outer hapa. **Photo credit:** Ahmed Jaman, WorldFish.

7. Harvesting Spawn or Hatchlings

Timely harvesting of hatchlings is essential for ensuring their survival and preparing them for successful nursery rearing. Hatchlings are generally ready for collection 60–72 hours after hatching, just before the yolk sac is fully absorbed. Harvesting is done by gently lifting one end of the outer hapa while splashing water along the outside to help the hatchlings detach from the mesh. Once loosened, they should be carefully collected using a clean measuring cup. The hatchlings are then transferred into a leak-proof polythene bag filled with cool, oxygen-rich water to maintain their health during transport.



Photo 17. Collecting and corralling punti hatchlings in an outer hapa. **Photo credit:** Harun Or Rashid, WorldFish.



Photo 18. Harvested punti hatchlings from the breeding tank. **Photo credit:** Harun Or Rashid, WorldFish.

8. Packaging and Transportation

Proper packaging and transportation are vital for maintaining the health and survival of hatchlings during transit. Each polythene bag should be filled one-third with clean water before the hatchlings are added. The remaining space is filled with compressed pure oxygen, and the bag is sealed tightly with a jute rope or strong rubber band to make it completely airtight. Bags should be placed horizontally to maximize the water–oxygen interface, ensuring adequate oxygenation throughout transport. For longer journeys, the sealed bags should be placed inside sturdy cardboard boxes or protective carrying bags to prevent punctures and maintain temperature stability. With proper oxygenation and water temperatures kept below 30°C, hatchlings can be transported safely for up to 24 hours. Stocking density should not exceed 25 g—roughly 50,000 to 75,000 hatchlings—per 10 liters of water, and adjustments should be made depending on travel duration and weather. At the destination, hatchlings must be acclimated gradually by mixing pond water into the transport bag before release.



Photo 19. Placing hatchlings in plastic bags with oxygen for transportation. **Photo credit:** Harun Or Rashid, WorldFish.

9. Nursery rearing of punti hatchlings

9.1 Nursery Pond Preparation

Proper nursery pond preparation is crucial for successful punti fry production. The pond should be completely drained, leaving the bottom soil moist before applying lime. Calcium oxide (CaO) should be applied at 247 kg per ha, with adjustments based on soil pH. The ideal pH range is 6.5–7.0, requiring higher lime rates in acidic soils and reduced amounts when pH exceeds 7.0. After liming, the pond should be dried for approximately one week to disinfect the bottom and eliminate predators. Once dry, the pond is gradually filled to a depth of about 0.60 m using borewell or filtered surface water. If using pond, river, or canal water, it must be screened through a 100-micron monofilament mesh to prevent entry of predatory insects or unwanted fry. To boost natural productivity, urea and TSP should be applied at 25–37 kg per ha each, followed by 37 kg per ha of fermented mustard oil cake (MOC), sprayed uniformly across the pond. Before stocking, the pond should be netted several times with a mosquito net to remove backswimmers and other predatory insects and larvae.



Photo 20. Preparing the nursery pond after drying. **Photo credit:** Harun Or Rashid, WorldFish.



Photo 21. Applying lime in the nursery pond. **Photo credit:** Harun Or Rashid, WorldFish.

9.2 Stocking Hatchlings

Punti hatchlings should be stocked on the third or fourth day after fertilization. Stocking is best carried out in the morning, gradually acclimating the hatchlings by balancing the temperature of the transport and pond water to reduce stress. The recommended stocking density is 200 hatchlings per square meter, equivalent to approximately 2 million per ha. Strict biosecurity measures should be implemented, including installing bird fencing around the nursery pond to protect hatchlings from predators. It is also recommended to add 5–6 cm of fresh water daily until the pond reaches a maximum depth of 1.0 m, providing optimal conditions for fry growth.



Photo 22. Acclimatizing punti hatchlings prior to stocking in the nursery pond. **Photo credit:** Harun Or Rashid, WorldFish.

9.3 Supplementary Feeding and Fertilization

During the early nursery phase, hatchlings should initially be fed microencapsulated duck or chicken eggs at a rate of 3–4 eggs per 100,000 hatchlings per day, divided into four feedings. After five days, egg feeding should be discontinued and replaced with fine fish meal or a formulated powdered nursery feed containing 40% protein. To maintain optimal natural productivity, weekly applications of urea and TSP at 12–18 kg per ha each, along with 19 kg per ha of fermented mustard oil cake, are recommended. Fertilizer application should be adjusted based on phytoplankton density, monitored using a Secchi disk reading of 25–30 cm. Over-fertilization must be avoided, as excessive nutrient buildup can quickly degrade water quality and negatively affect fry survival.



Photo 23. Applying supplementary feed in nursery pond. **Photo credit:** Harun Or Rashid, WorldFish.

9.4 Harvesting

Punti fry should be harvested after three weeks of nursery rearing and stocked into grow-out ponds at 5–10 fry per square meter, equivalent to approximately 50,000–75,000 fry per ha. Timely harvesting within this three-week period is essential to prevent parasite infestations, disease outbreaks, and unnecessary mortality. By this time, the fry will have consumed most of the available zooplankton, which cannot regenerate quickly enough to support further growth.

9.5 Fry Transportation

For transportation, feeding should be stopped one day prior to harvest. Fry must be collected carefully using a fine-mesh seine net to minimize injury and scale loss. Immediately after harvest, the fry should be transferred to a conditioning tank fitted with a fine-mesh hapa and maintained under a continuous water shower for at least three hours to allow them to clear their gut contents. After conditioning, 1,000–1,500 fry can be safely packed into a 10-liter polyethylene bag filled with oxygen, following the same procedure described in Section 8.



Photo 24. Oxygen-packed punti fry ready for transport from nursery to grow-out pond. **Photo credit:** Harun Or Rashid, WorldFish.

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