Current state of knowledge on induced breeding of nutrient-rich small indigenous fish species

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Authors

Francois Rajts,[1] Sourabh Kumar Dubey,[1] Ben Belton,[2] Arun Padiyar[1] and Shakuntala Haraksingh Thilsted.[3]

Affiliation

[1] WorldFish
[2] International Food Policy Research Institute
[3] Nutrition, Health and Food Security Impact Area Platform, CGIAR

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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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List of abbreviations

CPE	carp pituitary extract
EUS	epizootic ulcerative syndrome
FCR	feed conversion ratio
GSI	gonado somatic index
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit
IMC	Indian major carp
PUFA	polyunsaturated fatty acid
P/E	protein/energy
SIS	small indigenous fish species
SGR	specific growth rate

1. Executive summary

Fish consumption in India, Bangladesh and other Southeast Asian countries is heavily skewed toward cultured and large species, while the availability of small indigenous fish species (SIS) caught from the wild has become increasingly scarce. However, research has shown that several SIS have a higher nutritional value than commonly farmed carp species because they are rich in both vitamins and minerals. Several successful efforts have been made to include SIS in carp-based polyculture systems. The primary challenge in widely establishing nutrition-sensitive carp-SIS polyculture practices is the inability to produce SIS seed in large quantities.

With support from Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ), the Taking Nutrition-Sensitive Carp-SIS Polyculture Technology to Scale project is currently working toward promoting the aquaculture of SIS in the states of Assam and Odisha in India. To achieve effective breeding and mass production of SIS seeds, a comprehensive understanding of the ecology and biology of the targeted SIS is crucial. Proper information on the reproductive biology and other breeding-related parameters of particular SIS would help plan induced breeding properly and achieve success.

To that end, this report aims to assess the current understanding of induced breeding techniques for specific nutrient-dense SIS while also identifying crucial gaps in knowledge to address. The species selected for evaluation were chosen based on their known nutritional value, especially essential vitamins and minerals such as vitamin A, vitamin B12, calcium, iron and selenium, as such species can help mitigate nutritional deficiencies among children and pregnant women. The selection process also considered the ecological niche and food web of each species, with a preference for herbivores or omnivores over predators.

The following species were selected:

- Mola carplet (*Amblypharyngodon mola*) is a dominant phytoplankton feeder. It has high levels of vitamin A, vitamin B12, iron and calcium, and market demand is very high. Although some hatcheries have successfully bred mola in captivity, mass seed production has not yet been popularized.
- Pool barb (*Puntius sophore*) is mainly herbivorous. It is very high in calcium and has almost three times the level of higher polyunsaturated fatty acids (PUFAs) than mola. It fetches high market prices, but, like mola, mass seed production has not yet been achieved.
- Flying barb (*Esomus danrica*) mainly feeds on phytoplankton and zooplankton. It has high levels of iron, iodine, selenium and vitamin B12. Although this species was the first SIS to be artificially reproduced in India, since then seed production for this species has not been developed.
- Banded gourami (*Trichogaster fasciata*) is an omnivore that can thrive in hypoxic water, unlike other fish, and is very effective at eliminating mosquito larvae. It contains high levels of iron, calcium, zinc and vitamin B12, but seed production is still only small-scale, mainly for ornamental purposes.
- Climbing perch (*Anabas testudineus*) is an omnivore. It has high levels of PUFAs, moderate amounts of selenium and a high vitamin A content. Market demand and price are very high, though mass seed production remains at the initial stage.
- Dhela (*Osteobrama cotio*) is a dominant phytoplankton feeder. It has high amounts of calcium, selenium and vitamin A, but seed production is also at the preliminary stage.
- Reba carp (*Cirrhinus reba*) is a minor carp that feeds on plankton, detritus and insect larvae. It has high levels of calcium and vitamins A and D. Despite its small size, market demand is high in Odisha because of its unique taste. There have been reports of induced breeding, but mass seed production has not been developed.

2. Introduction

> Overfishing and anthropogenic activities such as aquatic pollution and siltation, as well as the loss of natural habitat required for spawning and growth, have resulted in declining SIS populations in natural water bodies throughout the Indian subcontinent in recent decades.

The pioneer of induced breeding in India is Dr. Hiralal Chaudhuri, who is widely regarded as the "father of induced breeding in India" (Ghosh 2021). In 1955, Dr. Chaudhuri became the first person to successfully breed SIS flying barb, and 2 years later he did that same with reba carp. These were the first instances of induced breeding in India. Dr. Chaudhuri's ground-breaking work paved the way for further advancements in this field.

However, the development of induced breeding techniques has historically focused on large fish species like carps, catfish and tilapia, while SIS have been considered competitors and, thus, eliminated from aquaculture ponds. Yet several SIS have a higher micronutrient content than commonly farmed carp species and can be eaten whole, which enables greater micronutrient intake (Bogard et al. 2015). This is significant, because the scarcity of SIS caught from the wild, coupled with the high price of SIS, has affected the poorest segments of the population. So there is great potential for SIS polyculture with carp to step in to fill the void.

Previous WorldFish research demonstrated that including SIS like mola in carp polyculture systems did not hinder carp production and improved household income and micronutrient intake (Roos et al. 2007). Additionally, the composite culture of some SIS with carps under semi-intensive conditions can significantly enhance the overall nutritional quality of total fish production (Castine et al. 2017). These findings highlight the potential benefits of reintegrating SIS species into aquaculture practices, particularly for improving nutrition in vulnerable populations.

Standardized hatchery techniques for the mass production of mola and other SIS seed do not yet exist, which is a key technical barrier to scaling up nutrition-sensitive aquaculture to reach many more people. To address this bottleneck, WorldFish is implementing the Taking Nutrition-Sensitive Carp-SIS Polyculture Technology to Scale project in the Indian states of Odisha and Assam. A key goal of the project is to standardize a method of hatchery-based breeding that will develop easily scalable techniques to mass produce SIS seed. The outcome of the project will enhance the nutritional status of underprivileged communities, especially children under 1000 days old and expectant mothers. This effort could also increase the availability of SIS in markets through the large-scale production of SIS seeds, ultimately resulting in reduced prices for consumers.

A deep understanding of the reproductive biology, breeding behavior and habitat of fish is crucial for induced breeding and mass seed production. This knowledge helps hatchery operators choose appropriate techniques, manage environments and identify optimal breeding windows for specific fish. Understanding the biology of SIS gives insight into conditions for breeding behavior, helps anticipate mating behavior and provides knowledge of habitat that can create optimal breeding environments. By replicating conditions that contribute to breeding in the wild, fish breeders can maximize success in hatcheries.

The purpose of this report is to evaluate the current understanding of the reproductive biology and induced breeding techniques of some specific nutrient-dense SIS and to identify critical knowledge gaps in induced breeding of SIS. The species were chosen based on their micronutrient value, especially essential vitamins and minerals such as vitamin A, vitamin B12, calcium, iron and selenium. The selection process took into consideration the ecological niche and food web of each species, with preference given to herbivores or omnivores over predators. These species require less animal protein in their supplementary feed, which is important in rural areas for household ponds of poor communities, as it makes them cheaper to produce and reduces the need of using fishmeal ("fish in-out"). They also have a lower risk of accumulating toxins in their bodily tissues.

The report is organized into five sections. Section 1 provides the brief summary of the report. Section 2 provides an introduction and background that highlights the need for mass seed production of SIS in hatcheries. Section 3 synthesizes the nutrient content, reproductive biology and artificial reproduction of specific SIS. Section 4 details the knowledge gaps in the induced breeding of SIS and makes suggestions for improvement. Section 5 concludes by discussing the advantages of stocking hatchery-produced fry to promote nutrition-sensitive aquaculture.

2.1. Need for mass producing SIS seed in hatcheries

Nutrition-sensitive carp-SIS polyculture can improve the nutrition and income of rural poor communities (Castine et al. 2017). Although the current level of production is not optimal, homestead ponds are crucial for culturing SIS, as they make them readily available for household consumption. According to Castine et al. (2017), the edible parts of SIS produced in a small homestead pond under mola-carp polyculture in Bangladesh can contribute up to 98% of vitamin A, 56% of iron, 30% of calcium and 35% of zinc in dietary requirements, even though they account for just 15% of total fish production. The study suggests that mola could fulfill half of the necessary annual intakes of vitamin A and iron for a family of four, if consumed at home.

It is crucial to identify distinct wild stocks of several SIS for the development of good hatchery stocks through effective breeding technology. This is an important measure for mass seed production and sustainable aquaculture as well as conservation.

To achieve higher mola production, it is important to harvest fast-maturing mola frequently to prevent overpopulation resulting from their breeding activity during the grow-out period (Rajts et al. 1997; Roos et al. 2007). This suggestion is based on the observed population dynamics of most SIS compared to carps,

Selling various *Puntius* species caught from wetlands in Assam.

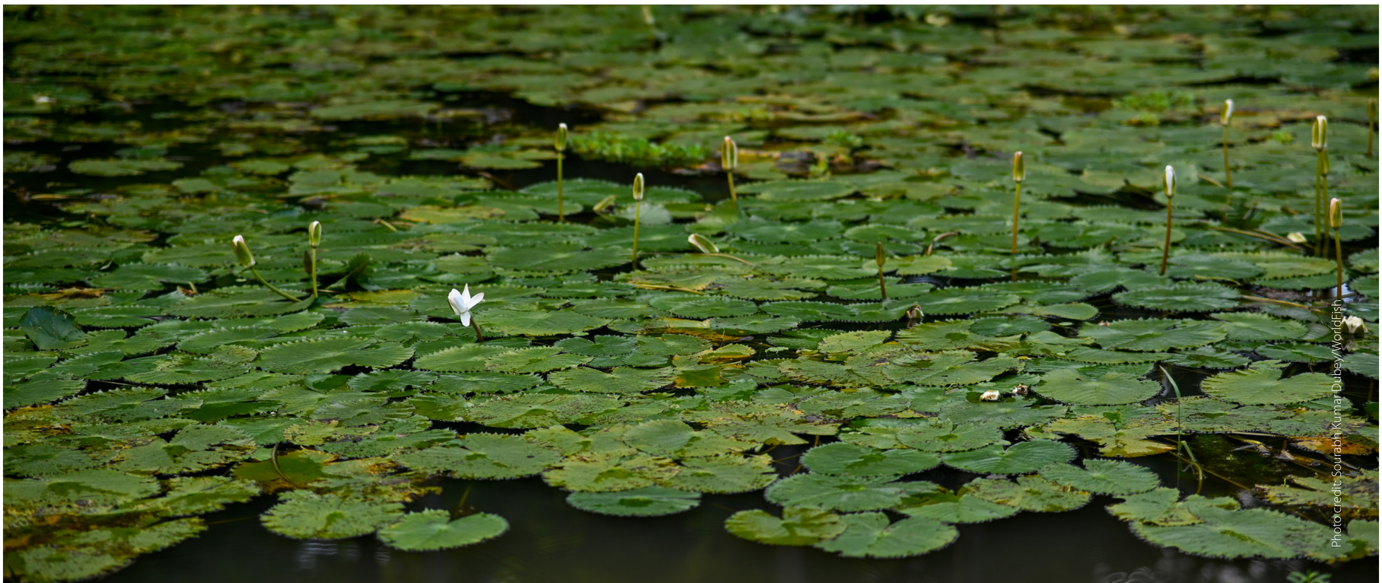
which take longer than most SIS to reach sexual maturity. To avoid excess breeding and food competition with carps, it is necessary to harvest sexually matured SIS either partially or completely from polyculture ponds and restock them with hatchery-reared SIS fry that use feed more efficiently and grow faster.

To maximize the use of estimated dietary niches in large-scale commercial semi-intensive polyculture systems, it is essential to calculate the biomass proportion of different fish species. However, species proportion, seed stocking and harvesting depend on many factors: the length of the culture period, the season, water depth, targeted fish size, the time needed for sexual maturation, the productivity of the water, natural food types and availability, physicochemical characteristics of the water, and the quality and intensity of supplementary feeding.

Relying on natural spawning to establish the right biomass proportion during the culture period by stocking SIS breeders in polyculture ponds is not recommended. As such, mass-producing SIS seed of the same age in hatcheries is critical to ensure a timely supply of good-quality seed for nutrition-sensitive aquaculture. Several measures can extend the breeding period of SIS, such as applying feeding, controlling the temperature, using a greenhouse-type covering for brood preparation ponds, using artificial stimulation techniques through water quality management, and applying hormones.

Box 1. Why data on breeding and other biological parameters of SIS varies

Several authors have investigated the first maturity size, gonado-somatic index (GSI), fecundity and other biological parameters of SIS, but their findings have been inconsistent. This variation in results can be attributed to different environmental factors, such as climate, water quality and food availability. Additionally, the overexploitation of stocks could have contributed to differences in the first maturity size and maximum size. In small waterbodies, where fishing is intensive and the entire fish population is harvested repeatedly, only a few small specimens survive, resulting in negative selection for growth, size at first maturity, and maximum size. Wohlfarth (1986) has noted that overfishing drastically reduces population size, selectively removing the larger fish, which results in the selection of smaller fish. Furthermore, Kenchington (2002) has pointed out that fishing can alter genetic diversity within a population, even when numbers are high, and that removing large fish often favors slow-growing, early-maturing fish. Therefore, as a precaution, it is recommended that SIS founder stocks for hatcheries be collected from large permanent water bodies.



A homestead pond in Odisha, a favorable abode for air-breathing SIS.

3. Existing state of knowledge on induced breeding of SIS

3.1. Mola (*Amblypharyngodon mola*)

Mola has been studied extensively because of its exceptional nutritional value, as it is rich in calcium, iron, and vitamins A and B12 (Zafri and Ahmad 1981). Mola has small gill rakers connected with a membrane that enables it to filter phytoplankton, which is its primary food source. Chlorophyceae is its most preferred food (Gupta and Banerjee 2013). In Assam, where mola is responsible for up to 4% of the catch from capture fisheries, phytoplankton represents 95% of its diet (Budhin 2017). Both gravid and mature mola are highly valued in markets across India and Bangladesh.

Plate 1. Harvested mola from carp-mola polyculture ponds.

3.1.1. Reproductive biology

Mola is a prolific breeder. First maturity occurs as early as 3 months of age (Rajts et al. 1997), and it is a fractional spawner, as noted by Gupta and Banerjee (2013). Several factors influence the reproductive biology of mola, including climate, water quality, food availability, size and habitat. To achieve successful spawning, an appropriate substrate must be provided for broodstock to lay their slightly adhesive eggs. A summary of the current understanding of the reproductive biology of mola is provided in Table 1.

3.1.2. Induced breeding

Various methods can stimulate mola to breed, such as introducing freshwater (especially rainwater), increasing water levels, and the presence of submerged but freshly inundated land with the appropriate substrate for eggs. Rice plants, remaining rice straw or artificial grass all work well as substrate. Dubbed "Dubics ponds," this type of breeding pond was previously used for breeding common carp in Europe during the 19th century. Rajts et al. (1997) successfully used Dubics ponds for induced mola breeding in Bangladesh.

In northwest Bangladesh, farmers have developed interconnected ponds with rice fields to breed mola. Excess freshwater is added to the pond to flood the rice field, and the mola migrate to the rice field for breeding. During spawning, the rice straw serves as a substrate for eggs and generates infusoria and other zooplankton, which serve as food sources for young fry (Thilsted et al. 2014; Saha 2019).

Kohinoor et al. (2005) studied the reproductive biology of mola for a year and found that the GSI values for females ranged from 1.78±0.88 to 17.06±2.66. The highest female GSI values were in July. Monthly observations of the diameter of the ova indicated that the fish spawned at least twice per year, once in May–July and again in September–October (Kohinoor et al. 2005).

Several authors have demonstrated the possibility of repeatedly inducing mola to breed. For example, Ghosh et al. (2018) conducted a successful breeding trial in West Bengal. They found that environmental manipulation, such as flushing ponds with water from different sources, can induce mola to breed five times in one year. Every time the pond was flushed, the mola spawned, as well as after heavy rain.

Mondal et al. (2020) maintained mola breeders in hapas in ponds throughout the year to study their breeding habits. Ripe ova were found for 9 months, from April to December, oocytes developed in different stages batch-wise, and natural breeding occurred several times throughout the culture period. This is consistent with the findings of Ghosh et al. (2018), which showed that environmental stimuli could stimulate mola to breed in captivity several times a year.

Saha (2019) reported using carp pituitary extract (CPE) to successfully induce breeding. They found that 25 mg/kg was the best dose of CPE for inducing mola to spawn in the hatchery, with a latency time of 6–8 hours and hatching occurring 17–18 hours after fertilization, at about 27°C. Saha estimated the average weight of 4-day-old hatchlings was 0.7 mg. The hatchlings were grown in a nursery pond and reached 5.14 ± 0.42 cm in total length and 1.54 ± 0.33 g in weight in 34 days. Saha et al. (2014) also reported techniques for the successful transportation of live mola breeders, which are very sensitive to handling.

Particulars	Value	Reference
Age of maturity (months)	3	Rajts et al. (1997)
Weight at maturity, female (g)	0.25–11.82	Rahman et al. (2018)
Weight at maturity, male (g)	0.39–6.86	Rahman et al. (2018)
Length at first maturity, female (cm)	3.57–9.94	Rahman et al. (2018)
Length at first maturity, male (cm)	3.69–8.88	Rahman et al. (2018)
Breeding habit	Batch spawner	Froese and Pauly (2022)
Parental care	None	Froese and Pauly (2022)
Breeding ground (preferable)	Freshly flooded dry land with plant substrate	Rajts et al. (1997)
Natural breeding per year	2	Kohinoor et al. (2005)
Natural breeding per year in the Kaptai reservoir, Bangladesh	3	Azadi and Mamun (2004)
Semi-natural breeding per year (induced by environmental manipulation)	5	Ghosh et al. (2018)
GSI for females (January and June)	1.78 and 17.06	Kohinoor et al. (2005)
Eggs per female 5–5.5 cm and 8.1–8.5 cm ♀	1023 and 6806	Hoque and Rahman (2008)
Relative fecundity 5–5.5 cm and 8.1–8.5 cm ♀	200 and 850	Hoque and Rahman (2008)*
Relative fecundity in the Kaptai reservoir, Bangladesh	995/g	Azadi and Mamun (2004)
Diameter of vitellogenic oocyte µm	27–30	Hoque and Rahman (2008)
Pituitary gland (PG) dose for induced breeding	2 mg/kg ♀ and 1 mg/kg ♂	Khondaker±
PG dose for induced breeding	25 mg/kg ♀	Saha (2019)
Latency period for ovulation (hours at 27°C)	6–7	Khondaker±
Time of egg development to hatching (hours at 27°C)	12	Saha (2019)
Larval development time to first feeding (hours at 27°C)	72	Khondaker±
	48	Saha (2019)
Number of 4-day-old hatchlings per kg	1.456 million	Saha (2019)
Size in nursery pond (34 days old)	5.15 cm; 1.54 g	Saha (2019)

** Calculated from data in Hoque and Rahman 2008. The relative number increases with size.*
± Personal communication, hatchery owner, Bangladesh.

Source: Modified from Rajts and Shelley 2020.

Table 1. The reproductive biology of mola.*

3.2. Pool barb (*Puntius sophore*)

Pool barb is primarily herbivorous, as reported by Mitra et al. (2006). Although composite culture with carps has proven successful (Wahab et al. 2003), availability of pool barb is mainly restricted to the dry season, despite high market demand. However, pool barb has a higher PUFA content than mola, with almost three times the amount, as reported by Mustafa et al. (2015). Pool barb is also an excellent source of calcium (Bogard et al. 2015).

Plate 2. Mature female pool barb.

3.2.1. Reproductive biology

Kohinoor et al. (2005) studied the reproductive biology of pool barb for 12 months and found that the GSI of females varied between 1.16±0.64 and 24.81±1.50, with the highest values obtained during July. They also found that pool barb spawned at least twice a year, once in May–July and again in September–October. Similar results of estimated two breeding periods per year were found for mola and chela (*Chela cachius*).

Hossain et al. (2012) reported that the size at first sexual maturity of pool barb in the Pabna River was 5 cm. Mean total fecundity was 5300±2700, ranging from 1580 to 16,590, while relative fecundity ranged from 466 to 4036 (mean 1100±580). Ahamed et al. (2015) found that the size of first maturity for females was 4–4.2 cm standard length in the Old Brahmaputra River, Bangladesh.

Hasan et al. (2018) recorded the maximum GSI value for pool barb as 15.43±2.20 in April at Gazipur and 15.60±1.74 in June at Jessore. Maximum fecundity in Gazipur was in April (5053±878.27), and June in Jessore (5433±968.26). The study also found that pool barb's spawning season runs from March to July, with peaks in the Gazipur and Jessore regions occurring in April and May, respectively. It is important to note that the breeding season of SIS could vary from one location to another because of environmental and other factors (Box 1).

3.2.2. Induced breeding

Ghosh et al. (2018) succeeded at breeding pool barb in semi-natural conditions. The researchers found that flushing with natural water played a crucial role in the spawning of the fish. The fish spawned three times a year in a pond with a natural water flush and twice with an artificial water flush but only once in ponds with no water flush. The pond with natural water flush had the highest GSI value (12.22) compared to the pond with domestic sewage (10.10).

Miss Kerala (*Puntius denisonii/Dawkinsia denisonii*), also known as Denison barb or red line torpedo barb, is a closely related species to pool barb and has been successfully bred in captivity (Mercy et al. 2013). The species is native to fast-flowing rivers and streams of the Western Ghats in India. Fertilized eggs of Miss Kerala are adhesive and demersal, with a diameter ranging from 1184 to 1312 µm. Hatching occurred 36 hours after fertilization at a water temperature of 27.5°C, with a mean larval length of 3.5±0.2 mm and a high yolk content. The yolk sac remained for 3–4 days, and organogenesis was completed 15–20 days after hatching.

Several authors have successfully reproduced the endangered species of the genus Puntius known as olive barb (*Systomus sarana*). Udit et al. (2014) induced spawning in the species by applying Ovatide at a rate of 1.2 ml/kg for females and 0.8 ml/kg for males. The latency period was 8–9 hours at a water temperature of 26.5°C–28.5°C. The diameter of fertilized eggs was 1.3 mm. The fertilization rate was 90.5%, and the hatching rate was 75.39%, with hatching occurring in 15–16 hours.

3.3. Flying barb (*Esomus danrica*)

The flying barb feeds primarily on zooplankton and detritus. This species is known for its high micronutrient content, particularly its rich sources of iron, iodine, selenium and vitamin B12 (Bogard et al. 2015). Despite reaching a maximum recorded length of 13 cm, the flying barb attains first sexual maturity at 4 cm in the Ganges River of Bangladesh (Hossain et al. 2016). There are reports of successful polyculture of flying barb with Indian major carps (IMCs) in ponds, resulting in more than a 5.5% increase in fish productivity and complementary growth of IMCs (Debnath and Sahoo 2020). Although there is high market demand for flying barb, its availability is generally restricted to the dry season.

Plate 3. Juvenile flying barb.

3.3.1. Reproductive biology

The absolute fecundity of female flying barb varies between 392 and 2412 (Froese and Pauly 2021), while the diameter of the mature ova is 0.31–0.45 mm and for ripe ova 0.41–0.6 mm (Mondal et al. 2019). The highest GSI has been recorded in June and July, followed by September, with breeding observed in both June and September. Proportions of mature ova vary throughout the year, except for January. This suggests that manipulating the environment can stimulate the species to reproduce before and after the normal breeding season.

3.3.2. Induced breeding

The first recorded instance of induced breeding of flying barb occurred in 1955, using pituitary extracts (Chaudhuri and Alikunhi 1957). However, no successful induced breeding of this species has been reported since. Kumara et al (2013) attempted to induce breeding in a closely related species, Sri Lankan flying barb *Esomus thermoicos*, which is an endemic species in Sri Lanka. The

Red pigmentation of pool barb caught from the Deepor Beel in Assam, a Ramsar site.

authors discovered that sand hindered breeding, but they successfully bred six pairs in an aquarium with breeding intervals as short as 14 days. These findings suggest that an artificial grass substrate would likely be beneficial to mass produce seed of the closely related flying barb, and that loose fine sand or muddy pond bottoms should be avoided. Additionally, it is probable that flying barb is also a fractional spawner, as it typically deposits its semi-adhesive eggs on floating or marginal vegetation.

3.4. Banded gourami (*Trichogaster fasciata*)

The banded gourami, popularly known as kholisa is a highly resilient SIS that can thrive in harsh environments, such as hypoxic water, where most fish cannot survive. This species possesses an accessory air-breathing organ that allows it to live in shallow, weed-infested waters with extremely high fluctuations of dissolved oxygen and toxic gases (Felts et al. 1996). As an omnivore, the banded gourami feeds on a wide range of food items, such as algae, diatoms, zooplankton, insects and mosquito larvae, as well as decaying organic matter (Khongngain et al. 2017). Additionally, it contains high levels of iron, calcium, zinc and vitamin B12 (Bogard et al. 2015). There is demand for banded gourami in the markets, but its availability is mainly restricted to the dry season and during monsoon rains, when fishers capture schools of kholisa migrating for breeding. This species is also popular in the ornamental fish trade.

Plate 4. Banded gourami.

3.4.1. Reproductive biology

Banded gourami can easily breed in an aquarium when the water hardness is suddenly reduced, similar to what occurs during heavy monsoon rains in its natural habitat (Rajts, personal observation, 1987). According to Mitra et al. (2007), its absolute fecundity is between 1095 (6.1 cm and 5.3 g) and 19,291 (8.6 cm and 13.9 g), while its relative fecundity is 206–1392 and its length at first maturity is 6 cm. It breeds from March to September, when the diameter of its ripe ova is 0.66–0.78 mm.

3.4.2. Induced breeding

Gouramis exhibit a unique reproductive behavior, wherein males play a crucial role in building bubble nests attached to floating substrates and provide paternal care. The eggs, which contain droplets of oil, float on the water's surface. During breeding, the male prepares a bubble nest among dense vegetation, and the fertilized eggs are automatically lifted into these nests. The male then protects and cares for the eggs and larvae until they reach the free-swimming stage. In a recent study, Swain et al. (2021) examined different substrates for breeding nests to enhance the breeding performance of honey gourami (*Trichogaster chuna*). They found that using Bermuda grass as the nest-building substrate resulted in higher seed production.

Islam et al. (2017) suggested that using Ovaprim at a rate of 0.5 ml/kg of the weight of females was more effective than other spawning stimulants in the rates of inducing ovulation, fertilization and hatching in banded gourami. Abujam et al. (2015) reported that the optimal dose of Ovaprim for these rates in banded gourami was 0.3–0.5 ml/kg, compared to higher doses. In another study, Hossen et al. (2014) investigated the effects of PUFAs on the spawning performance of banded gourami. The researchers found that supplementing the diet with 1% squid extracted phospholipids enriched with PUFAs resulted in enhanced spawning performance.

3.5. Climbing perch (*Anabas testudineus*)

The genus *Anabas* is represented by two species in India: climbing perch and Gangetic koi (*Anabas cobojius*). Locally known as koi, climbing perch is an omnivorous fish with high levels of PUFAs and vitamin A, as well as moderate levels of selenium, making it a highly sought-after species with a lucrative market (Bogard et al. 2015).

Yet, the introduction of imported strains from tropical river basins poses a threat to the genetic purity of endemic strains of climbing perch. Interbreeding climbing perch with fast-growing imported strains from tropical areas of Vietnam, whether in hatcheries or the wild, puts the species at risk of losing its genetic purity and disease resistance during colder winters in subtropical habitats. For example, EUS frequently affects the introduced silver barb (*Barbonymus gonionotus*) from tropical areas during the cold season in northern Bangladesh, leading farmers to harvest it before winter. In addition, the flavor, price and likely nutritive value of exotic strains and their hybrids of climbing perch are inferior to those of the local strains. To safeguard the genetic pool of endemic strains, stakeholders must implement strict controls on imports, releases/escapes to the wild and hybridizations. It is also crucial to raise awareness about the risks of importation and uncontrolled hatchery operations among all stakeholders.

Plate 5. Climbing perch.

3.5.1. Reproductive biology

Like walking catfish and freshwater prawn, climbing perch migrates from culture ponds to search for breeding grounds. It is a seasonal breeder, with breeding activity occurring mainly during the monsoon season. Spawning occurs in shallow water, and the fish's eggs typically float on the surface of the water. Sexual maturity occurs when the fish reach a total length of 10–12 cm.

3.5.2. Induced breeding

For induced breeding, a single-dose injection of either carp pituitary or synthetic hormone can achieve fecundity anywhere from 5000 to 35,000. The latency time for induced breeding is 6 hours at 28°C. *Anabas* is a scattered breeder, so the parents abandon the fertilized eggs, which float on the surface. The incubation period for eggs to hatch is 18–24 hours, depending on the temperature. Under natural conditions, the effect of the wind can damage many floating eggs on the shoreline.

Sarkar et al. (2005) successfully used Wova-FH synthetic hormone at a rate of 0.3 ml/kg of weight to induce breeding in climbing perch. According to Perera et al. (2013), climbing perch does not breed naturally under captive conditions, so successful ovulation was only obtained when treated with Ovaprim at 0.5 mL kg^{-1} of weight. Chaturvedi et al. (2015) successfully reproduced climbing perch using Ovatide at a rate of 0.6 ml/kg for females and 0.4 ml/kg for males, though the water temperature was not recorded. Breeding occurred 18–28 hours following a single injection, and the diameter of the fertilized eggs, which were bright clear and transparent, ranged between 0.6 and 0.7 mm. The hatched larvae measured 2.0–2.3 mm on day one, 2.6–2.8 mm on day two and 3.0-3.6 mm on day three, after they had absorbed their yolk sac. The air-breathing organ developed on the 11th day, with the larvae measuring 10.6–11.8 mm. Rearing was done in cement tanks inside the building, at a water depth of 6–8 inches, with the fish fed plankton, egg custard and chopped mollusks. Bhattacharyya and Homechaudhuri (2009) stated that Ovaprim was useful in breeding climbing perch, with high rates of fertilization and hatching, though many of the brooders died.

Plate 6. The Hau Giang strain of climbing perch in the Mekong Delta is frequently imported into India.

3.6. Dhela (*Osteobrama cotio*)

Dhela is a freshwater minnow that is widespread in Pakistan, India and Bangladesh. It can reach a maximum length of 15 cm (Menon 1999) and primarily feeds on phytoplankton (Rafin et al. 2019). In rural areas near the riverbanks of Brahmaputra, Barak and the lower stretches of the Teesta River, fresh dhela is a staple food that is typically consumed fresh after being caught (Kumar and Goswami 2013).

Plate 7. Dhela.

3.6.1. Reproductive biology

Observations by Parameswaran et al. (1971) showed that dhela breeds in ponds in Assam beginning in late April or early May until the end of July. Hussain et al. (2003) documented the reproductive biology of dhela and reported that the breeding season starts with the onset of the monsoon rains in May, peaks in June–July and ends before September. They also found that the 50 females they studied had three different stages of egg development simultaneously, suggesting they could breed several times a year. Table 2 summarizes the details of their findings. Although there is limited research into the fecundity of dhela in different regions, Parameswaran et al. (1971) found that the fecundity of dhela collected from Assam waters varied from 3006 to 10,970.

3.6.2. Induced breeding

Although there appear to be no records of induced breeding trials for dhela, Das et al. (2017) conducted successful breeding trials in captivity of the closely related species *Osteobrama belangeri*, locally known as pengba. They used an inducing agent, Gonopro-FH (Salmon GnRH), with doses ranging from 0.7 to 0.8 ml/kg of weight for females and 0.4 to 0.5 ml/kg for males. The latency period was 9.1±0.09 hours at a mean water temperature of 25.3°C±0.18°C and a pH of 6.3–6.5. The species demonstrated high fecundity, with a 180-g female releasing approximately 65,136 eggs, resulting in a corresponding relative fecundity of 362.13 eggs per gram of bodyweight. The fertilized eggs are non-adhesive, spherical, translucent and demersal, measuring about 2.3±0.06 mm in diameter. Incubation lasted for 19.6±0.4 hours at a relatively low temperature of 19°C–26°C, likely contributing to a hatching rate of 61.9%.

Particulars	Value
Natural breeding per year	Possibly several
GSI female (June–July)	15.31
GSI female (September)	3.79
Relative fecundity 2.2 cm, 1.12 g	512
Relative fecundity 5.7 cm, 7.1 g	6849
Main fecundity (mean body length 3.880±1.216 cm and mean bodyweight 3.667±1.82 g and gonad weight 0.605±0.302 g)	3273+±1708

Source: Hussain et al. 2003.

Table 2. Reproductive biology of dhela.

3.7. Reba carp (*Cirrhinus reba*)

Reba carp is a minor carp species that is indigenous to the Indian subcontinent (Talwar and Jhingran 1991) and holds significant commercial importance. Known locally as *bhagna bata*, *lasim bhangan* (in Assam) and *pohola* (in Odisha), reba carp is highly sought after by consumers for its taste and quality and is a good source of protein, minerals and fatty acids (Rahman 2005; Afroz and Begum 2014) as well as vitamin A, vitamin D, calcium and potassium (ICAR 2019). In nature, reba carp is a bottom-feeding omnivore, preferring plankton as its primary food source (Mathialagan et al. 2017). Mahfuj et al. (2021) observed insulated geographical reba carp stocks in Bangladesh and found distinct morphological differences. The researchers suggest that further genetic investigations and an assessment of the ecological impact of reba carp stocks would aid conservation efforts.

Plate 8. Mature male reba carp.

3.7.1. Reproductive biology

Reba carp typically reaches first maturity at a total length of 18.62 cm. It reproduces during the monsoon season and breeds in flooded floodplains, much like other IMCs. This species breeds annually, at water temperatures ranging from 26°C to 30°C. Its single spawning period occurs during the southwest monsoons, extending from May to July in Assam and Bangladesh and from June to August in West Bengal, with a peak in June. Additionally, it is worth noting that artificially lengthening daylight longer than a natural day can accelerate the later phase of maturation in this species.

3.7.2. Induced breeding

Although reba carp can reach full maturity in pond conditions, they do not typically spawn in ponds. Lashari et al. (2007) studied the GSI of this species and found that values ranged from 0.45 to 5.05 in males and 1.05 to 12.5 in females, with one peak in July. The highest GSI values were from June to August, ranging from 4.60 to 5.05 in males and 9.80 to 12.5 in females.

Fecundity data is useful for planning commercial hatchery operations, as fecundity greatly varies with the size of available breeders. For reba carp, Khan (1986) also examined its fecundity in the Baigul reservoir in Uttar Pradesh, reporting that

Fisherwomen selling various SIS caught from wetlands in Assam.

it varied from 22,356 to 437,400 in fish 120–320 mm in length. Fecundity in females is typically between 200,000 and 250,000 eggs per kilogram of bodyweight and increases with increasing total length, ovary length, ovary weight and bodyweight (Hussain and Mazid 2001).

Although some authors (Akhteruzzaman et al. 1998; Sarkar et al. 2004; Rahman et al. 2009) have conducted induced breeding and nursing of fry for reba carp, commercial production of this species has not yet been established (Mohanta et al. 2008). However, previous attempts at induced breeding using CPE have been successful (Dutta 2001; Gupta et al. 2016), with the best results obtained using primary and secondary injections of 2 and 5 mg/kg in females and 2 mg/kg in males. Induced breeding trials of some minor carp species were made in Tripura. Some of the results are presented in Table 3 (Datta 2012).

In another study, Sarkar et al. (2004) used Ovaprim at a rate of 0.5 ml/kg of weight for females and 0.4 ml/kg for males, which resulted in spawning within 4–5 hours of injection and completion within 6–7 hours. The fertilization rate was high, ranging from 90% to 95%, and average fecundity was 420,000. Additionally, the average diameter of the eggs was 2.24 mm, and average weight was 0.0042 mg.

Recently, Bhagawati et al. (2023) succeeded at mass producing reba carp. Induced breeding was carried out at an air temperature of 24°C–25°C, producing approximately 600,000 hatchlings. Ovafish, a synthetic inducing agent, was administered at a rate of 0.5 ml/kg of weight for females and 0.25 ml/kg for males. Both sexes were then released into the spawning pool after injection. After 7–8 hours, the females released their eggs, at an average fecundity of about 55,000 per 200 g of female brooders. The hatchlings were 1.75–2.1 mm in length and had a prominent dark band, as seen under a microscope.

Species	Primary dose (PG mg/kg)		Interval between two doses (hours)	Decisive dose (PG mg/kg)	Ovulation (hours)	Hatching (hours)
Kuria labeo (*Labeo gonius*)	Female	2	6	5	7–8	16–18
	Male	-	-	2		
Bata (*Labeo bata*)	Female	1	6	5	7–8	16–18
	Male	-	-	1		
Reba carp	Female	1	6	5	7–8	14–16
	Male	-	-	1		
Olive barb	Female	-	6	5	6–7	14–16
	Male	-	-	2		

Source: Extracted from Datta 2012.

Table 3. Development of artificial propagation techniques for minor carps.

4. Knowledge gaps, and suggestions for improvement

4.1. Age of breeders

Most trials to culture SIS have been carried out by stocking breeders of diverse and unknown ages, typically sourced from the wild. Fast-maturing SIS are bred in culture ponds at the age of 3–4 months, but a portion of the offspring is lost because of cannibalism or competition for food. Also, some of the older breeders could die, making it challenging to accurately identify the lifespan, survival rate during culture, growth rate, feed conversion ratio (FCR) and specific growth rate (SGR). To address this issue, producing a cohort of SIS seed of the same age in a hatchery for use in trials is recommended.

4.2. Breeding tools in hatchery

Although acceptable for larger fish, the stripping method is generally not recommended for SIS. As a result, it is necessary to develop suitable structures for breeding and collecting adhesive eggs, using a non-decomposing and easy-to-handle substrate. Additionally, an incubation system is needed to mass produce SIS hatchlings.

4.3. Duration of hormone administration

Injecting small fish with hormones is a delicate process that requires careful attention. In the case of mola, for example, it takes four to six workers to inject 600 females and 1200 males in just 2 hours. Any longer and it could compromise the success of fertilization.

There are several reasons for this. First, males can become fatigued from chasing the first matured females, resulting in poor fertilization of later matured females. Second, the quantity of milt available to fertilize the eggs of later matured females could be insufficient, as it is often used up with the first maturing females. Third, if it takes longer than 2 hours to administer the hormone, the liberation of protease enzymes produced by hatching matured larvae during the incubation of fertilized eggs could result in immature, later-laid eggs to hatch prematurely. Last, fish could consume the eggs after breeding, making it necessary to separate breeders from the eggs as soon as spawning is complete. As such, it is essential to synchronize breeding and administer hormones within a short period, which requires a high number of workers.

4.4. Alternatives to avoid hormone administration

Since SIS are small, a large number of breeders is needed for each batch of induced breeding in order to achieve mass seed production. However, the traditional method of injecting the hormone can be problematic, as it can cause injuries and take too much time. Scientists have discussed the use of hormones in animals cultured for human consumption regarding possible residuals and effects on consumers, and the practice is already prohibited in some countries. Furthermore, it can counteract the simultaneous spawning of all individuals in the batch. To avoid this, an alternative approach would be to use environmental stimuli to induce breeding.

One possible method is to use the bundh system, which is traditionally used to induce breeding of IMCs in West Bengal. This system involves using a type of perennial water storage for rainwater runoff that is connected to a breeding pond. Breeders are kept in the pond, and sudden changes in water quality are created to induce flow and turbulence, which triggers breeding (Kumar 1992). Another alternative approach is to use methods commonly used in the aquarium trade. These include manipulating the physicochemical parameters of the water, such as using soft rainwater, adjusting the photoperiod, creating water currents and providing substratum for eggs. Additionally, oral hormonal treatments can be tested to stimulate breeding.

4.5. Synchronization of gonadal development

To mass produce SIS seed, a large number of breeders must be reproduced in a single batch. However, selecting a high number of ripe breeders from a broodstock pond is challenging and time-consuming, and can lead to stress and mortality. As such, it is essential to develop a suitable adaptive method to synchronize gonadal development in each SIS before inducing breeding. This will help reduce the stress and mortality associated with selecting breeders, making the process more efficient and cost-effective.

4.6. Development of organic manure application

Using animal dung as organic manure in sufficient quantities can be challenging because of competing uses and difficulties in sourcing. Furthermore, prior to use, it is necessary to treat the manure through composting or fermentation. Yet such treatment can result in the loss of essential nutrients for phytoplankton growth, such as carbon and nitrogen (Tiquia et al. 2002).

Jute retting, a process commonly thought to be toxic to fish, is actually not harmful and is even used as a vegetable food in Asia. Like any organic manure, excessive use of jute retting can kill fish. When used properly, however, it can be used as a substitute for organic manure to promote plankton development. India has a significant opportunity to use jute retting as manure in ponds, especially in association with pond aeration. According to Rajpoot et al. (2019), jute is cultivated on 710,000 ha in India, with West Bengal, Assam and Bihar accounting for approximately 98% of the country's jute area and production. Urgent research is needed to explore the potential of this resource for fisheries development.

4.7. Development of adapted supplementary feed for SIS broodstocks

In broodstock ponds, fish are kept at high densities, unlike in their natural habitats, where they have access to a variety of food sources. To compensate for this, supplementary feed is needed. However, the nutritional needs of different SIS species are not well understood. Using only rice bran, mustard oil cake or soybean cake as supplementary feed can negatively affect the quality and quantity of SIS seed produced because of the absence of essential amino acids, fatty acids and micronutrients, and the presence of antinutritive elements.

There are several examples of how providing adapted supplementary feed can improve the quality of fish seed. For instance, El-Sayed et al. (2008) found that Nile tilapia broodstock significantly reduced fecundity at decreasing protein/energy (P/E) ratios in their feed. The best spawning performance was achieved at 40% of a dietary protein and energy level of 16.7 MJ GE/kg, with a P/E ratio of 23.6. These results highlight the need for trials to identify the best feeds for breeders of different SIS to ensure high-quality seed production.

4.8. First feed for SIS larvae

Identifying the best first food in the hatchery for each SIS is urgently needed. Considering different options, such as rotifers, artemia nauplii (both natural and enriched) and micro-encapsulated chicken eggs, can help identify which ones are best. The latter is prepared on the farm using locally available ingredients and can be enriched with vitamins, essential fatty acids, essential amino acids and enzymes.

4.9. Feeding in nursery ponds

After stocking hatchery-produced larvae in nursery ponds, the survival of fry primarily depends on their ability to locate appropriate zooplankton based on species, size and density. It is possible to stimulate the development of zooplankton when preparing the pond for stocking, but success is not always guaranteed. Later emerging cladocerans and copepods compete and prey upon protozoans and rotifers, resulting in declining populations. Additionally, copepods often prey on both rotifers and stocked larvae.

To promote fast growth and to avoid predators, it is crucial to adapt food and make supplementary feed available. Hatcheries typically feed boiled poultry egg yolks and protein-rich soybean meal in nursery ponds. However, young fish, particularly larvae, have difficulty digesting complex proteins because their digestive systems are not yet fully functional. Here, research is needed on culturing adapted natural food, formulating easily digestible slow-sinking supplementary feed and controlling predators in order to enhance survival rates in nursery ponds.

4.10. Controlling harmful insects

In nursery ponds, excessive development of backswimmers (*Notonecta* sp.) and other insects often leads to them eating fry during harvest, and these insects can get mixed up with the fry in the harvesting net, sometimes in equal quantities. To prevent this, it is necessary to remove the insects before harvesting the SIS fry.

4.11. Accurate measurement of produced hatchlings

Because SIS are small, it is difficult to measure their hatchlings for sale using the same volumetric method as large carps. As such, a precise measurement technique is needed to determine the number of hatchery-produced SIS hatchlings. This will enable accurate stocking of nursery ponds and proper identification of the survival rate from fertilized eggs to hatchlings and fry.

4.12. Animal welfare for breeders

In several cases, successful induced breeding has been associated with high mortality of breeders. This can be attributed to various factors, such as injuries from hormone injections, transportation stress, the use of breeders collected from the wild, and handling without adequate sedation.

To reduce the mortality of breeders, it is important to develop breeder-friendly methods. This can involve domestication efforts to acclimate breeders to captivity, as well as adopting adapted sedation techniques to minimize stress during handling and injecting. It is also important to identify the optimal location in each species for injecting hormones to avoid injury and improve breeding outcomes. Mitigating the risks associated with induced breeding requires a holistic approach that prioritizes animal welfare and scientific rigor.

A mature female reba carp in Assam.

5. Advantages of stocking hatchery-produced fry

5.1. Stocking carp-SIS polyculture ponds

Stocking hatchery-produced SIS seed offers several potential advantages over the previous practice of stocking broodfish harvested from natural water bodies. Stocking wild breeders in seasonal ponds, where water is only available for a short period, can be challenging because ponds can dry up before their progeny attain market size. Moreover, ponds stocked with wild breeders contain SIS populations of various age groups, resulting in the production of fish of variable sizes that are less preferred in the market. Stocking wild fish also risks introducing pathogens into farmers' ponds. Seed produced by the spontaneous natural spawning of mola in a pond can also face problems such as competition for food, cannibalism and predation.

In contrast, hatchery-produced SIS seed are of uniform size and age and can be stocked at the optimal time and density to maximize survival and growth. Hatchery management practices such as quarantining brooders, maintaining biosecurity and disinfecting fish make infections less likely. Moreover, hatchery-produced seed can be conditioned and packed in clean well-oxygenated water before delivery to farmers, reducing mortalities during transportation and stocking. Stocking hatchery-produced fry can also ensure a uniform size of SIS at harvest, making them more marketable and fetching better prices. Hatchery-based seed production also offers the possibility of stimulating spawning much earlier than in the wild, giving farmers a head-start over nature.

Table 4 presents a fundamental comparison between stocking wild SIS breeders and hatchery-reared SIS seed in carp polyculture systems.

5.2. Stocking seasonal waterbodies

As shown in Table 5, there is a great advantage to stocking hatchlings instead of breeders in seasonal waters, such as ditches, reservoirs and seasonal wetlands.

Paddy fields in Odisha are a haven for SIS.

Wild breeders	Hatchery-produced seed
Breeding SIS in polyculture ponds is seasonal, depending on climatic conditions, and is generally too late in comparison with the stocking period of carp fingerlings.	The programmed stocking of SIS seed can be done in a timely manner.
SIS breeding is not synchronized, resulting in multiple breeding events and significant differences in age and size among the population. This leads to intense competition for resources, including food, and can even result in cannibalism. The negative impacts of these factors can ultimately reduce the market value of SIS.	Stocking same-aged seed results in harvested fish of uniform size that fetch better prices.
Because they are bred several consecutive times, part of the population is older and matures earlier. The feed efficiency of the sexually matured older part of the stock and that of the remaining breeders is inferior to that of the younger part of the population. This results in a higher FCR and competition with carps in polyculture.	The seed from hatcheries can be harvested at the same sexually matured stage and the ponds can be restocked again with faster-growing fry or fingerlings.
Several authors have reported that mola die after spawning, though this has not been proven.	Using hatchery-produced stocks of the same age can help accurately identify the life cycle of mola and other SIS.
It is not possible to accurately monitor the survival rate, conduct periodic stock assessments to make feeding adjustments, calculate the FCR, SGR and precisely identify the life of spawn.	All concerned scientific tasks can be monitored accurately.
Because of the lack of a substrate for the eggs to adhere to, the eggs often fall into the mud on the bottom and die.	Using an artificial or natural substratum or removing the sticky layer of eggs and then incubating them in modern devices can control the mortality of eggs/larvae.
The availability of suitable-sized larval food in polyculture ponds is often inadequate, leading to poor survival rates of hatchlings until they reach the fry stage.	The survival rate of hatchlings to fry is higher in specially managed nursery ponds.
The unpredictable outcomes of breeding make it challenging to determine the appropriate proportion of SIS biomass in a polyculture system. Insufficient SIS survival rates make it difficult to effectively use available dietary niches within the habitat, while excessive survival rates can impede the growth of other species and create competition within the fish stock, increasing susceptibility to disease outbreaks.	Stocking hatchery-produced SIS fry can establish the precise proportion of SIS biomass in polyculture ponds.
It is impossible to accurately assess the proportion of the correct species, competition for food and space in trial ponds.	Instead of breeding SIS in the pond, stocking a known number of SIS fry can make it easier to understand species proportion trials in composite culture.

Table 4. Comparison between stocking wild SIS breeders and hatchery-reared SIS seed in carp polyculture systems.

Wild breeders	Hatchery-produced seed
Stocking breeders is more costly than hatchlings because adult mola are sensitive to transportation, and mortality is frequent.	Transportation costs less and is the same as for carps.
Stocking breeders requires deeper water than hatchlings. This results in delayed stocking in seasonal waters, which shortens the production period.	Hatchlings can be stocked earlier, as soon as the waterbody has 1 foot of water from the monsoon rains. This results in a longer production period.
The longer period prior to stocking and successive breeding gives more time for aquatic insects to develop. This affects the survival of eggs, larvae and hatchlings as the insects prey upon them.	As predator insects cannot develop within a limited time, the survival rates of eggs, larvae and hatchling is higher.
Not all the breeders spawn at the same time. Breeding occurs on different days depending on the climatic conditions and readiness of gonads. This results in cannibalism and competition for food and space by older fry.	Stocking hatchlings results in a homogenous population of SIS.

Table 5. Advantages of stocking hatchery-produced hatchlings in seasonal waterbodies.

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