Conducting artificial fertilisation, hatching and spawn development of Indian shad, *Tenualosa ilisha*: Pathfinding hilsa domestication

D.N. Chattopadhyay[1], A. Chakraborty[1,2], P.K. Roy[1,3], R.N. Mandal[1*], A. Das[1], A. Hussan, S. Adhikari[1], B.R. Pillai[4] and S.K. Swain[4]

*1. Regional Research Centre, ICAR-Central Institute of Freshwater Aquaculture, Rahara, Kolkata 700118, West Bengal, India; 2. Nature Environment and Wildlife Society, 10 Chowringhee Terrace, Kolkata - 700020, West Bengal, India; 3. Sreeragam Exports Pvt. Ltd. 1830 Chakgaria, Budherhat, Kolkata 700094, West Bengal, India; 4. ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar 2, Odisha, India. *Email: rnmandal2003@yahoo.com*

Hilsa, *Tenualosa ilisha* is a fascinating food fish. Its charming lustre, culinary flavour and medicinal importance make it a favourite of the people of West Bengal, India and Bangladesh in particular. However, it has complex habitat requirements, migrating between marine, estuarine and riverine waters. Usually, adults mature in the sea, and then venture a long journey towards rivers via estuarine environments for breeding.

The domestication of such a fish growing in diverse ecosystems at different life stages has provided quite a challenge to researchers.

Breeding hilsa

Ideally, in breeding hilsa, fertilisation in captivity needs to be performed under controlled conditions. However, male and female broodstock are collected from fishers who catch hilsa with monofilament gill nets while sailing country boats, and artificial fertilisation is performed onboard. Some of the specific constraints which are encountered in hilsa breeding include the following:

- It is difficult to collect enough healthy males and females in breeding condition, with oozing milt and eggs. In particular, it is difficult to obtain them both at the same time.

Family operated boat - earning a living catching hilsa in the tidal flow of the Hooghly River.

- Captured broodstock are highly stressed from the monofilament net and handling, and typically only survive a few minutes after capture.

- Sometimes, gravid females and males are available, but one or both of sexes are not viable enough for breeding.

- Hilsa migrates twice a year during February-March and September-October, around the full and new moon periods, which places a time constraint on the breeding operation.

Breeding prerequisites and necessities

When viable broodstock of both males and females are collected after catch from fishermen, artificial breeding operations are undertaken immediately. First, the maturity status of both females and males is checked, females should have freely oozing yellow eggs and males should ooze white coloured milt on abdominal pressure. If the broodstock are in suitable condition to proceed, then the fish are temporarily placed in a large circular container filled with river water until enough have been collected. However, care must be taken not to cause females to release their eggs into the container or absorption of water leads to closure of the micropyle. Battery operated aeration is provided and a mild water flow is maintained by hand to keep the fish vibrant to some extent.

Once enough broodstock have been collected the stripping operation needs to be started. Breeding of hilsa is by and large performed with stripping method comprising two different techniques such as the dry method and the wet method; the former is better than the latter.

Dry stripping

Removal of water from the buccal cavity, gills and body surface of hilsa is necessary to prevent water mixing with the stripped eggs. To do so, individual fish are kept upside down by hand for a few minutes so that excess water from mouth and gills is removed. Mixing water with eggs causes them to lose fertility. Then the surface of both male and female broodstock is sponged with a clean towel or tissue paper to make the body surface dry. A gentle finger or thumb pressure is applied on both sides of the upper region of the female abdomen so that the eggs are moved and eventually come out through the vent. Stripped eggs are collected and spread in a clean steel or enamel tray. Then the male is stripped to remove milt. Having obtained eggs and milt:

- The eggs and milt are mixed thoroughly with a soft feather to bring sperm in contact with the eggs.

- The small amount of previously conditioned river water (filtered through a bolting silk hapa) is sprinkled gently over the milt-egg mixture to activate the process of fertilisation.

- Immediately after addition of water, the tray needs to be swayed for a moment to mix the water uniformly over the egg-milt mixture.

- The addition of sprinkled water stimulates sperm to start moving very fast and to adhere with the egg membrane.

- Sperm remain active and viable for only 40-50 seconds, during which they must enter via the micropyle and eventually fertilise the egg.

- After 4-5 minutes, a large amount of water is gently added and the mixture must be kept undisturbed.

Post-fertilisation the eggs should be cleaned through repeated washing with conditioned water. The washings involve removal of decanted water, followed by addition of fresh conditioned water 4-5 times. This practice removes clogged milt, mucous, scales, faeces, blood, oil droplets and other waste which might facilitate bacterial or fungal infection. After washing, the fertilised eggs are transferred into circular plastic tub of 20 litres capacity partly filled with conditioned water. Continuous mild aeration with battery operated aerators is required to increase the dissolved oxygen level and make the eggs buoyant.

Water hardening of eggs

Fertilised eggs start swelling and separate from each other just after fertilisation. Swelling of eggs, known as ‘water hardening’, seems to be an indication of successful fertilisation. Usually, eggs require 45 minutes to fully swell. Swollen fertilised eggs measure around 2.0 mm in diameter, are spherical, transparent and contain yellowish yolk with numerous oil globules. The egg membrane is single layered. Comparatively, unfertilised eggs measure around 1.8 mm in diameter and have a whitish yolk.

Female hilsa in breeding condition.

Male hilsa in breeding condition.

Stripping eggs from a female hilsa.

Stripping milt from a male hilsa onto stripped eggs.

Fertilised eggs are further transferred into another container equipped with aeration to increase dissolved oxygen and begin embryonic development. At this stage the fertilised eggs may be transferred into a hatchery or aquaria for further incubation and hatching. Fertilisation rate varies between 95-98%. The fertilised eggs are packed into polythene bags with oxygen and transported to the laboratory of Kalyani Field Station, RRC of Rahara, ICAR-CIFA.

Egg incubation

The transported eggs have both fertilised and unfertilised mass which needs to be segregated prior to incubation. The unfertilised eggs decompose quickly and pollute the water and must be removed to avoid damage to fertilised eggs. If the quality of water deteriorates, the whole batch of eggs will be spoilt, including viable eggs. To remove the unfertilised eggs, spread a small amount of egg mass in a steel tray so that fertilised and unfertilised eggs are clearly visible. The unfertilised eggs can then be removed from the mass with a dropper, without touching the fertilised eggs.

The fertilised eggs are then incubated in a steel tray, or aquarium or hatching pool depending on the number of eggs collected. The most important aspect includes handling of eggs, water management and aeration. Water exchange at regular intervals seems to be the most important operational factor, and aeration must also be provided for proper development of the eggs.

Mixing milt and eggs with a soft feather.

Fertilised eggs being spread in a steel tray.

Rearing of hatchlings

After hatching, the young are collected by dropper to eliminate egg shells and are put into aquaria with the same water and continuous mild aeration. Hatchlings are reared without any natural or supplementary feed for the first four days. On the fourth day, the larvae have reached spawn size of 5 mm/0.5mg. The dead larvae, if any, need to be removed to avoid water quality deterioration. In such a practice, bolting silk may be used while water is being siphoned and later replenished with fresh water.

Packets of fertilised eggs kept in hatching pool for acclimatisation.

Spawn development and management

Usually, by the fourth day larvae are able to swim at the water surface and they can be easily be harvested with a small container. During harvest a small number may be collected to avoid any injury or damage due to stress or handling. Harvest is usually undertaken either morning or evening when the ambience becomes fresh and water temperature becomes comparatively cooler but collecting hatchlings in evening is better. Using a florescent light during hatchling collection improves the contrast between the water and transparent body of hatchlings and makes their movement easier to see and easier to collect them. The spawn are then transferred into another rearing system for fry development.

Conclusion

Domestication of hilsa requires extra effort while nurturing the natal stage which is very sensitive and susceptible to mortality. In particular, the early stage of larvae including hatchlings and spawn development up to the fourth day requires the utmost care and attention. Kalyani Field Station of the Regional Research Centre, Rahara, ICAR-CIFA has developed standardised protocols for incubation and hatching of fertilised eggs, maturation of hatchlings and early spawn development of hilsa, which are the first of their kind for hilsa domestication and culture. This has been made possible through a decade of continuous research, dedication and devotion to both benefit farmers

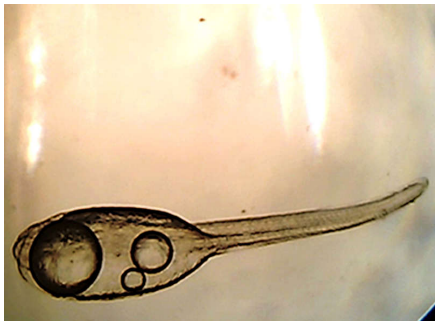
Fertilised eggs incubated in aquaria.

Hatchlings harvested with dropper for transfer to other rearing tanks.

Hatchlings harvested to transfer to another rearing tank.

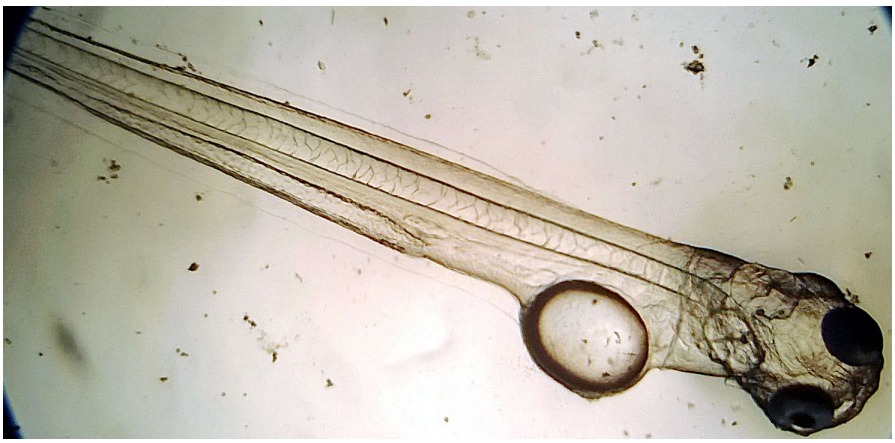
New hatchlings.

Scientists observing the growth of hatchlings.



A close view of 2 day old hatchling.

4 day old hatchlings.



A close view of a 4 day old hatchling.

and contribute to the conservation of wild hilsa populations through development of captive breeding techniques and domestication, to alleviate pressure on wild sourced stocks.

Acknowledgment

The authors are grateful to the Director, ICAR-CIFA, for his encouragement of hilsa research.