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Induced breeding of mola carplet (*Amblypharyngodon mola*) for mass seed production

A practical guideline

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Induced breeding of mola carplet (*Amblypharyngodon mola*) for mass seed production: A practical guideline

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Related knowledge products from the project

- Stocking of hatchery produced mola seed (Amblypharyngodon mola): A guideline for farmers
- Induced breeding of small indigenous fish species (SIS): An overview of some trial options
- Guidelines for setting up breeding experiments for small indigenous species (SIS)
- Selection of small indigenous fish for induced breeding trials in the states of Assam and Odisha in India
- Current state of knowledge on induced breeding of nutrient-rich small indigenous fish species

Project webpage

www.worldfishcenter.org/project/taking-nutrition-sensitive-carp-sis-polyculture-technology-scale

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Introduction

- The mola carplet (*Amblypharyngodon mola*) is a small indigenous fish species that naturally inhabits ponds, canals, beels, slow-moving streams, ditches, reservoirs, inundated paddy fields and floodplain wetlands.
- Mola is widely distributed throughout South Asian countries like India, Bangladesh, Nepal, Pakistan, Myanmar and Afghanistan. In India, the species is known locally as moa in Assam and mahurali in Odisha.
- Mola is a surface-dwelling fish that feeds on plankton.
- It exhibits partial spawning behavior with a wide range of spawning periods (February–November), depending on climate conditions, rainfall and broodstock management.
- On the Indian subcontinent, mola is a popular and affordable nutrient-dense food for many people because of its distinctive flavor.
- This small fish is packed with several important micronutrients, making it an excellent candidate species for nutrition-sensitive aquaculture, such as carp-mola polyculture.
- Mola is particularly rich in vitamin A, which protects against eye and skin diseases associated with vitamin A deficiency. Additionally, mola is a good source of essential minerals like iron, zinc and calcium, as well as fatty acids and amino acids that can prevent micronutrient deficiencies in children, adolescents and pregnant women.
- Farming mola locally can increase the availability of these valuable nutrients and make them more accessible to all, especially for vulnerable communities and low- and middle-income consumers.
- Until recently, however, the lack of standardized hatchery breeding techniques for the mass production of mola seed was a significant barrier to scaling up nutrition-sensitive aquaculture to its full potential.
- At the outset, this practical manual offers a unique, step-by-step guideline for induced breeding of mola to produce mass-scale seed in a hatchery. The breeding protocol is simple and easy for existing fish hatchery operators to adopt.
- This eight-step guideline is intended to serve as a protocol that any fish hatchery with an interest in breeding mola can follow, and it makes an important contribution to the development of nutrition-sensitive aquaculture.

Plate 1. A mature mola carplet.

1. Broodstock pond preparation and management

- Dewater the broodstock pond and keep the bottom soil moist until lime application.
- Depending on the pH level of the pond, apply hydrated powdered lime at a rate of 200 g/m^2 and allow it to dry for a week to disinfect the pond and eliminate predators.
- To ensure ideal conditions in a mola broodstock pond, it is crucial to manage natural food sources such as phytoplankton and zooplankton.
- Use organic fertilizers, like cattle dung or compost, applying 1–2 t/ha to the pond bottom.
- Gradually fill the pond with borewell water until it reaches a depth of 2 feet.
- When water filling begins, apply urea at a rate of 10 g/m^2 (40 kg/acre) and single super phosphate (SSP) at a rate of 20 g/m^2 (80 kg/acre) diluted in the pond water.
- To supplement natural food sources, spray fermented mustard oil cake daily at a rate of 1.5 g/m^2 (6 kg/acre) on the pond surface.
- After 15 days, when the phytoplankton density in the pond has reached its optimal level and the color of the water has become green (with a Secchi disk reading of 25–30 cm), it is the ideal time to stock mola broodstock.
- Collect mola broodstock from diverse sources, preferably from large permanent waterbodies. After acclimatizing them to the pond water, stock the mola breeders at a rate of 20–25 fish/m^2. Before stocking, treat them with potassium permanganate ($KmNO_4$) solution kept in a bucket (0.5 g $KmNO_4$ in 100 L of water).
- Give mola breeders powdered feed that has a 35%–40% crude protein content to promote steady gonadal development.
- To stimulate gonadal development of the breeders, add soft water from a river or stored rainwater into the broodstock pond.
- Apply urea weekly at a rate of 2 g/m^2 (8 kg/acre) and SSP at 4 g/m^2 (16 kg/acre), depending on the density of the phytoplankton in the pond.
- Maintain proper biosecurity measures to prevent any outbreaks of disease.

Plate 2. Biosecurity in a mola broodstock pond.

2. Harvesting and conditioning breeders

- Maintain an exclusive broodstock pond. Keeping mola broodstock in a separate pond minimizes the risk of injury from larger fish such as carps during harvesting.
- When harvesting mola breeders with carps, use a segregator net with a larger mesh size to separate the mola breeders.
- Always harvest in the morning hours before the pond water heats up.
- Harvest the mola breeders carefully and then transport the broodstock quickly, preferably in cold water, to the conditioning tanks.
- To prevent the breeders from jumping during transportation, place plant branches with leaves inside the container.
- After transporting the mola breeders to the conditioning tanks, move them into a pre-installed fine mesh hapa.
- Condition the breeders under constant showering for a whole day to acclimatize them to the water and stimulate readiness for spawning.
- Cover the hapa with a fine mesh mosquito net.

Plate 3. Stocking breeders in a fine mesh hapa inside a conditioning tank.

Plate 4. Conditioning breeders under constant showering prior to hormone administration.

3. Identifying mature males and females

Females

- Females are larger in size and lighter in color.
- They have a soft and conspicuously distended abdomen.
- Mature females have smooth pelvic fins and a deeply forked caudal fin.

Males

- Males are comparatively brighter, thinner and smaller than females.
- They do not have a distended abdomen.
- They exhibit active swimming behavior.
- Mature males have a yellowish caudal fin.

Plate 5. A mature female (top) and male mola (bottom).

4. Induced breeding arrangement

Hapa arrangement

- Install one hapa in the conditioning tank to receive the captured breeders for conditioning and to release waste, as discussed in step 2.
- In the breeding tank, arrange two hapas to receive injected breeders.
- First, install an outer hapa with a 250 micron nylon mesh. Then, with the aid of a galvanized iron frame, fix an inner hapa with a 10 mm mesh inside the outer hapa.
- Maintain the water level in the breeding tank at 1 foot below the upper edge of the hapa.
- Supply a constant, gentle shower of oxygen-rich water from an overhead tank equipped with an aeration tower.

Plate 6. Outer and inner hapas installed in the breeding tank.

Plate 7. An inner hapa installed with aid of a galvanized iron frame.

Aeration tower

- An aeration tower is a metallic structure that is installed on top of an overhead tank to increase the oxygen level in borewell water before it is supplied to the conditioning and breeding tanks.
- It is made up of four rectangular galvanized iron sheets with many holes (10 mm in diameter) to allow the water to pass through and break into droplets.
- It removes carbon dioxide and other harmful gases from the borewell water as it is pumped through the tower on its way to the overhead tank.
- The process of passing borewell water through the aeration tower can reduce the carbon dioxide content in the water by up to 70 percent.
- In addition to reducing carbon dioxide levels, an aeration tower also enriches the dissolved oxygen content of the supplied water up to the saturation level, which increases the survival of larvae.

Plate 8. An aeration tower installed within the overhead water reservoir tank in a hatchery.

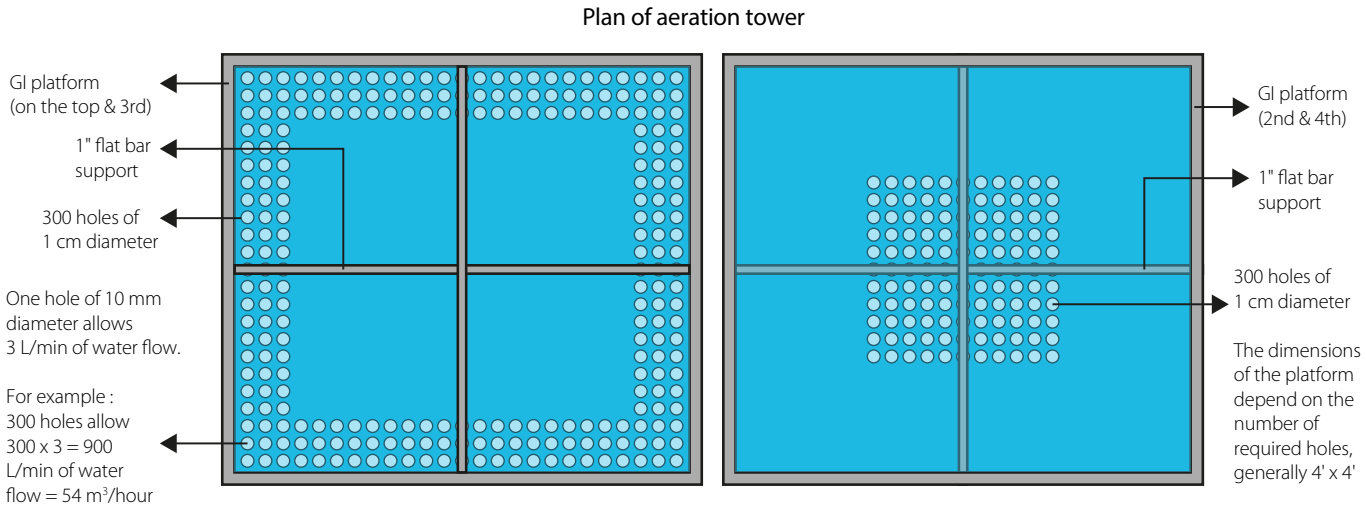

Figure 1. Metal sheets with holes.

5. Hormone dose and administration

- Use any commercially available synthetic gonadotropin-releasing hormone analogue (S-GnRHa with domperidone) with various trade names as an inducing agent.
- The hormone dose for males is 0.25 ml/kg of their bodyweight and 0.5 ml/kg for females.
- Before beginning large-scale operations, it is recommended to test breeding, because the strength of the different brands of hormones can vary, as well as the readiness of breeders.
- Maintain a male to female sex ratio of 2:1.
- It is necessary to dilute the hormone in sterile water or a 0.65% sterile sodium chloride (NaCl) solution because of the high viscosity of the hormone and the very small dose that is required for mola.
- Inject 1 kg of breeders with an inducing solution that is diluted in 15 times the amount of water (0.5 ml hormone + 7.5 ml water).
- Administer the inducing solution into the peritoneal cavity of the mola broodfish with a 1 ml insulin diabetic syringe of 40 graduations.
- Inject the breeders during the evening hours (17:00–21:00).
- Immediately after administering the inducing solution, shift the brooders to the 10 mm mesh inner hapa and cover it with a nylon mosquito net to prevent the fish from jumping out.
- Maintain a constant water shower to simulate a rainy environment.

Plate 9. Synthetic hormone and a diabetic syringe used for induced breeding.

Plate 10. Injecting an inducing agent into the peritoneal cavity of a female breeder.

Plate 11. Placing breeders in a 10 mm mesh inner hapa immediately after administering the inducing solution.

Plate 12. Covering the breeding tank and maintaining a constant shower after administering the hormone.

Calculating the hormone dose for induced breeding of mola: An easy guide

Hormone dose required

The required dose of a synthetic gonadotropin releasing hormone (e.g. WOVA-FH) for males is 0.25 ml/kg of their bodyweight and 0.5 ml/kg for females.

How to prepare and calculate the hormone dose

For 1 kg of female mola breeders, first dilute 0.5 ml of hormone in 15 times the amount of water. That means 0.5 ml hormone + 7.5 ml water = 8 ml of inducing solution for 1 kg of mola.

For example:

- 1000 g of mola = 8 ml
- 1 g of mola = 8 ÷ 1000 = 0.008 ml
- 5 g of mola = 5 x 0.008 = 0.04 ml
- 7 g of mola = 7 x 0.008 = 0.056 ml
- 10 g of mola = 10 x 0.008 = 0.08 ml

Administering the hormone solution

Inject the prepared inducing solution into the peritoneal cavity of mola broodfish. The preferred method is an insulin diabetic syringe of 1 ml capacity with 40 graduations. One graduation of an insulin syringe will contain 0.025 ml (1 ÷ 40 = 0.025 ml).

For example:

- 5 g of mola: 0.04 ÷ 0.025 = 1.6 graduations of syringe
- 7 g of mola: 0.056 ÷ 0.025 = 2.24 graduations of syringe
- 10 g of mola: 0.08 ÷ 0.025 = 3.2 graduations of syringe

6. Spawning and eggs incubation

- Remove all injected mola breeders by lifting the inner hapa the next morning (09:00–11:00). Stock the breeders in separate ponds for spent fish.
- Spawning occurs after an average latency period of 6 to 8 hours (at 29.5°C) following hormone administration.
- Hatching occurs 12–14 hours after fertilization at a water temperature of 28.5°C–30°C.
- Check the laid eggs attached to the bottom part of the 250 micron outer hapa.
- Fertilized eggs are light brown to yellowish in color, clear in appearance, demersal and semi-adhesive.

Plate 13. Checking the eggs the next morning.

Plate 14. Eggs attached to the bottom part of the outer hapa.

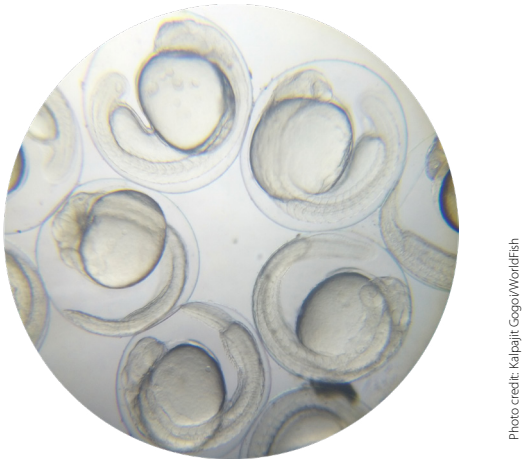

Plate 15. Microscopic view of fertilized ova in the final stage of development.

Plate 16. Microscopic view of a newly hatched mola spawn or hatchling.

7. Harvesting spawn or hatchlings

- Hatchlings or spawn are typically harvested 60–72 hours after hatching, just before the yolk sac is fully absorbed.
- To collect the spawn or hatchlings, gently lift the outer hapa from one end while constantly splashing water from the outside.
- This splashing motion helps detach the hatchlings from the wall of the outer hapa.
- Once detached, carefully lift the hatchlings with a measuring cup and place them in a leak-proof polythene bag filled with clean and cool oxygen-rich water.

Plate 17. Collecting and corralling mola hatchlings in an outer hapa.

Plate 18. Placing hatchlings in plastic bags protected by containers.

Plate 19. Careful handling and harvesting of the hatchlings from the breeding tank.

8. Packaging and transportation

- Fill a polythene bag with one-third water.
- After placing the hatchlings in the bag, fill the space above the water's surface with compressed pure oxygen from an oxygen cylinder. Once this is done, seal the bag tightly using a jute rope or rubber band to make sure that no air enters or escapes from the bag.
- To ensure that the hatchlings are protected during transportation over long distances and to maximize space, place the airtight bag in a sturdy cardboard box or a bag to prevent damage and punctures.
- With appropriate oxygen packaging, hatchlings can be safely transported for up to 24 hours in slightly cooled water (by ≤3 °C) without any complications.
- Make sure the density of the hatchlings does not exceed 25 g in 10 L of water. The density also depends on the duration of transportation.
- Upon arrival, it is important to acclimatize the hatchlings to their new pond environment gradually. Do this slowly by adding water from the receiving pond to adjust the temperature and other water quality parameters before releasing the hatchlings.

Plate 20. Filling mola seed bags with oxygen.

Plate 21. Sealed airtight mola seed bags.

Plate 22. Placing mola seed bags inside cardboard boxes for long-distance transportation.

Plate 23. Mola seed packages ready for transportation.

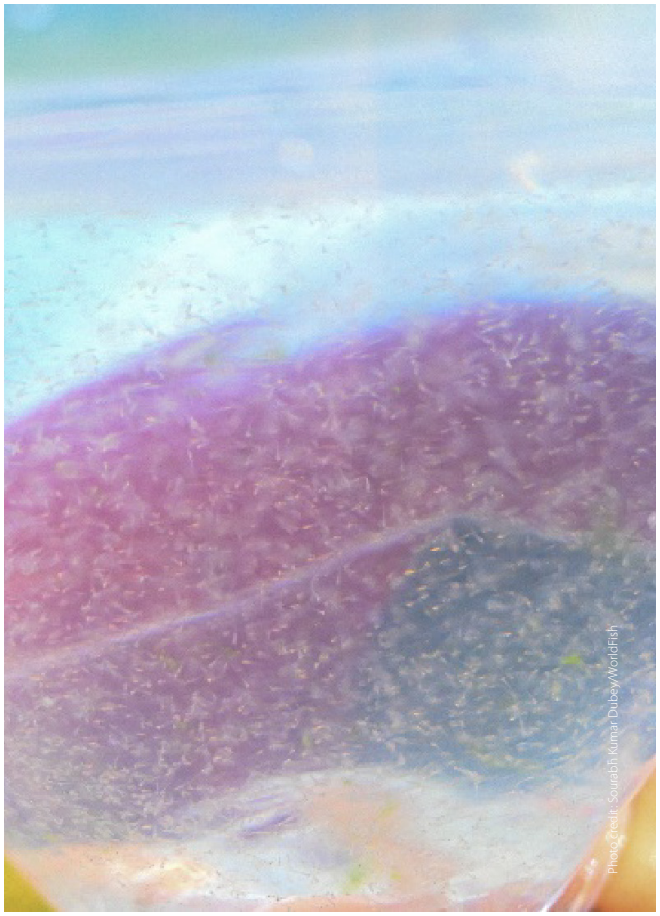

Plate 24. Oxygen-packaged mola hatchlings.

Plate 25. Oxygen-packaged mola fry.

Plate 26. A hatchery operator with mola seed.

Plate 27. Selling mola seed from a hatchery in Odisha.

About WorldFish

WorldFish is an international, not-for-profit research organization that works to reduce hunger and poverty by improving aquatic food systems, including fisheries and aquaculture. It collaborates with numerous international, regional and national partners to deliver transformational impacts to millions of people who depend on fish for food, nutrition and income in the developing world.

The WorldFish headquarters is in Penang, Malaysia, with regional offices across Africa, Asia and the Pacific. The organization is a member of CGIAR, the world's largest research partnership for a food secure future dedicated to reducing poverty, enhancing food and nutrition security and improving natural resources.

For more information, please visit www.worldfishcenter.org