Green water technology as an essential support to larval rearing of hilsa shad

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A mature hilsa, Tenualosa ilisha.

'Green water technology' is a technique that promotes phytoplankton to grow profusely, making cultured water turn a lush green colour, hence its name. The term 'green water technology', sometimes referred to 'green-water culture' or simply 'green water' includes several methods by which desirable microalgae are produced for the purpose of rearing larval fish and crustaceans. Naturally occurring phytoplankton, which serve as feed for fish and crustacean larvae, are grown and proliferate under a controlled system. Single celled microalgae are produced in green water culture because they have useful features such as natural dispersion, remain buoyant in the water column for a long period, and do not cause fouling in the cultured water. *Chlorella* is one of the best of a few microalgae commonly used in the production of large amounts of green water. Green water is efficient and can be used in diverse culture methodologies including extensive, intensive, and mesocosm systems used in larval fish rearing across the globe. It is productive and helps to maintain desired oxygen levels.

Microalgae as a means of suitable food to feeding larvae

Being autotrophs, microalgae are the first link in the food chain of the aquatic trophic system and are harnessed in aquaculture as a direct food source for fish and crustacean larvae. The role and benefits of microalgae are to:

- Provide a direct nutrient source to the larvae.
- Contribute to the maintenance of other live foods and their nutritional balance.
- Enhance environmental conditions of the culture water, enhancing larval feeding by increasing turbidity, scattering and attenuating light, and enhancing visual contrast.
- Lower the stress level of larvae.
- Ameliorate water quality due by stripping of excess nitrogenous substances and increasing dissolved oxygen level.
- Provide digestive stimulants.
- Assist in diversifying the microflora of the larval gut.
- Provide beneficial antibacterial properties.

Certain microalgae add essential fatty acids to the rearing water. These fatty acids, which may eventually transfer to fish larvae through prey, promote larval growth and survival. Microalgae contain a vast number of highly bioactive compounds including amino acids, vitamins, pigments (including antioxidants), and minerals. These provide an immense benefit to the health of fish larvae through the food chain. The overall larval rearing system benefits from

Application of green water in hilsa fry rearing pool.

microalgae either as a direct food source or an indirect food source by stimulating the production of rotifers, *Artemia* and copepods, and other zooplankton used as food for the larvae of a variety of carnivorous fish species.

Larval rearing of hilsa shad vis-à-vis role of microalgae

Hilsa – a food fish of sheer importance

Hilsa shad, *Tenualosa ilisha*, a euryhaline fish species, undertakes long migrations crossing diverse water bodies from river to sea to fulfil its life cycle. Adult hilsa travel from the deep sea and travel up estuaries to reach freshwater rivers, where they breed. Juveniles then undertake a reverse journey, following the path of the adults from the rivers to reach deep sea habitat. Hilsa have been recorded in India, Bangladesh, Myanmar, Pakistan, Sumatra, Indonesia, Iran, Iraq, Kuwait, Sri Lanka, and Vietnam, occurring in waters such as the Bay of Bengal, Indian Ocean, Persian Gulf, and Arabian Sea. Hilsa is highly nutritious, being rich in protein, fat, vitamins and minerals, including essential amino acids, and n-3 polyunsaturated fatty acids (n-3PUFA), particularly eicosapentaenoic acid and docosahexaenoic acid.

Rearing hilsa larvae

To culture hilsa in captivity, one important factor among others is to rear larvae up to fry stage in freshwater. The main obstacle is the lack of knowledge on the biology of the larvae, including different stages of larval development, for example:

- The age when the larval mouth opens.
- The size of the mouth aperture.
- The age when larvae first accept food.
- The kind of foods larvae will accept.
- The size requirements for foods to enter the mouth.
- The suitability and favoured or preferred foods of larvae.
- Are the larvae active or passive feeders.
- Favourable water conditions to facilitate feeding and growth.
- The condition of alimentary tract and its capacity to accept and digest different types of food.

These and many other questions must be studied when a new species is considered for aquaculture. The most critical task is to choose preferred natural foods in the early stage of larval growth on which the larval survival is greatly dependent and assured.

Chlorella vulgaris – microalgae – suitability as larval feed

Chlorella vulgaris is a single-celled protein source, with a diameter of approximately 3-10 µm. It is the best choice for single cell protein production because it has simple and inexpensive growth requirements chiefly nitrogen, and phosphorus, and grows rapidly under sufficient light. It is known to be an effective, economical, and preferred source of protein since it has a short life cycle, good tolerance to environmental variations, and its cell size is appropriate for the demands of many fish larvae. *C. vulgaris* has an adequate nutritional value and high digestibility as a food for rearing fish larvae. It contains the following nutrients (%): Protein, 40-50%; lipids, 10-15%; and carbohydrate, 12-16%, apart from n-3 highly unsaturated fatty acid (HUFA), poly unsaturated fatty acid (PUFA) including a significant concentration of eicosapentaenoic acid (EPA, 20:5n-3) and of docosahexaenoic acid (DHA, 22:6n-3). Hilsa larvae will accept *Chlorella* at first feeding, grow well and survive satisfactorily, when larvae have still a rudimentary digestive system and lack a developed stomach. Much of protein digestion takes place in the hind gut epithelial cells. Such a digestive system usually prefers receiving easily digestible and highly nutritious food; in such cases, *Chlorella* is a most preferred source.

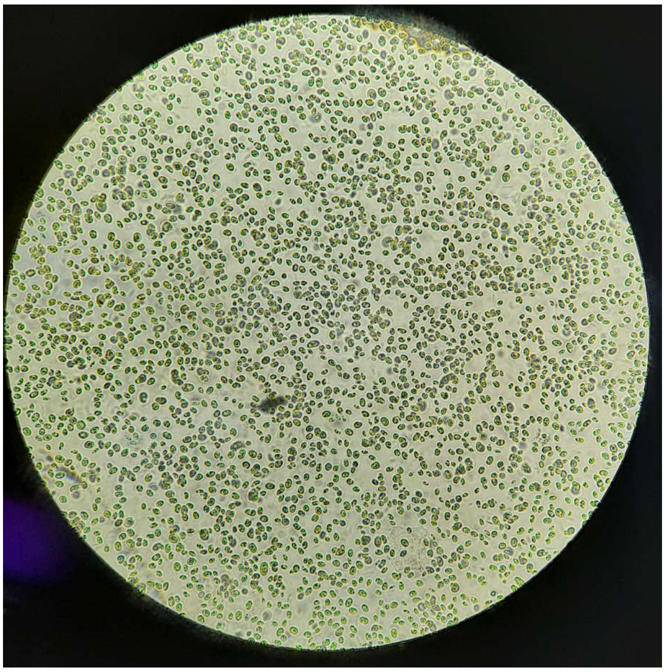
Green water as the basis of co-feeding larvae

Co-feeding is the application of two types of foods – a method of feeding found to be effective compared to single feeding in the early stages of larval growth. It has shown improved growth and survival of hilsa larvae as per experiments conducted in RRC, ICAR-CIFA, Rahara. *C. vulgaris* was produced as green water and applied to larval feeding, in combination with other zooplankton foods.

The schedule of co-feeding for different ages of hilsa larvae was undertaken during 46 days of larval rearing (Chattopadhyay et al., 2019). In the experiment, feeding started on the 4th day of rearing and continued until the 50th day. The 46 day feeding period was demarcated into five stages:

- 1st stage: Day 4-50. Daily application of green water (*C. vulgaris*).
- 2nd stage: Day 6-10: Feeding with *Brachionus calyciflorus* of lower population density.
- 3rd stage: Day 11-25: Feeding with *Brachionus calyciflorus* at a higher population density.
- 4th stage: Day 8-50: Feeding with mixed phytoplankton (diatoms, *Pandorina*, *Scenedesmus*, *Closterium*)
- 5th stage, 26-50: Feeding with mixed zooplankton (*Cyclops*, *Diaptomus*, *Diaphanosom*).

Selected plankton were produced separately in sterilised circular and rectangular FRP tanks in an outdoor system, while a few others were produced in ponds.

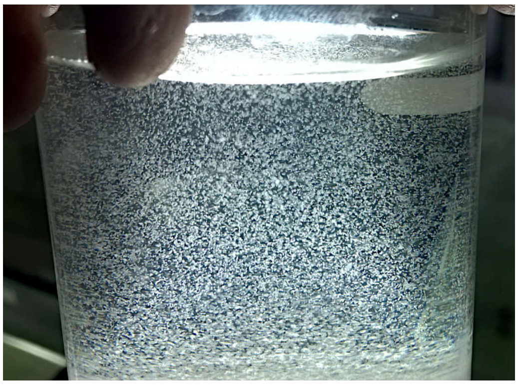


A microscopic view of Chlorella vulgaris, single cell protein.

Scientists observing the density of Chlorella culture.

Above: Rotifer culture in cemented raceway with green water. Below right: Dense rotifer population displayed in a glass.

Co-feeding as a potential nourishment of larval rearing



Co-feeding serves to improve the nutritional condition of the larvae. In this feeding technique, larvae get necessary nutrients from different categories of plankton. In natural habitats, where a variety of live food resources are available, the larvae can have balanced nutrients to promote their growth and survival. In captive rearing of larvae, the co-feeding strategy has been adopted to simulate their natural habitat with regard to the availability of a variety of natural foods. The particular importance of co-feeding is that different live foods are the main source of nourishment of cultured fish larvae. Live foods contain an appropriate energy content and required nutrients. Live foods can also be easily enriched with additional nutrients through dietary manipulation during culture. Despite the recent progress in the development of inert diets for fish larvae, the aquaculturists still rely on live foods during the early life stages of larval growth, particularly at the first-feeding stage of most species of interest for aquaculture. Due to the poorly developed digestive systems of first feeding stages, most of the larvae are unable to digest formulated diets, while live foods are generally preferred and give better growth and survival. The early stages of fish and prawn larvae are inherently attracted towards live foods, because their instinctive behaviour drives them towards organisms that are easily detected and captured while swimming, especially those that move or have any type of motility in the water column. Larvae are believed to be visual feeders adapted to capturing moving prey; the movement of live food is likely to stimulate larval feeding responses. Live foods that have a thin exoskeleton seem more palatable to the delicate soft bodied larvae once taken into the mouth, compared with hard ones and dry formulated diet. Live foods with a body size in the range of 70-350 μm are suitable for co-feeding larvae. The high population growth rate of live foods is also advantageous for larvae, which feed upon them by filtration or capture from suspension.

Chlorella culture in a series of outdoor FRP tanks.

Production of green water – a farmer-friendly cost-effective technique

Mass production of *Chlorella* – easy to do

The tanks or containers in which *Chlorella* culture takes place need to be cleaned with bleaching powder so that containers are free from unwanted microorganisms, algae or any other plankton (Chattopadhyay et al., 2019). Cleaned containers are put under bright sunlight for 12 hours so that the action of bleaching powder disappears. The containers are then filled with bore water, assuming that water is not contaminated with other algal species. Culture water is to be prepared with the addition of inorganic fertilisers such as ammonium sulphate $(NH_4)_2SO_4$, urea CH_4N_2O, and single super phosphate $Ca(H_2PO4)_2$ with the ratio of 10:01:01, respectively. In amount, ammonium sulphate, urea, and single super phosphate are to be added in each tank @ 0.1 g/L, 0.01 g/L and 0.01 g/L, respectively. For example, for 1,000 L production of green water, one should add 100g, 10g, and 10g of ammonium sulphate, urea, and single super phosphate, respectively. All these fertilisers are available commercially in fertiliser shop. After addition of fertilisers the culture water need to be stirred thoroughly and kept as such for 12 hours. *Chlorella* is then inoculated @0.025 ml/L (17 x 10^6 cells of *Chlorella* per ml of stock as counted under the microscope, but there is no strict norm of stock inoculum to have such measured cells into fertilised water. All the cultured tanks should be put in bright sunlight and aerated continuously. The tank water usually turns green four days after inoculation due to increase in cell density. It may also vary and take a few days more, if the inoculum does not have a sufficient *Chlorella* mass. After 6-8 days of culture, green water can be harvested.

Chlorella inoculum maintained in a conical flask.

Strategic culture practice of *Chlorella* – a preferable method

Batch culture is preferable as a strategy for *Chlorella* culture. In open outdoor systems there is no control over contamination of culture water. In such case, continuous culture may not be suitable because many other phytoplankton and zooplankton, particularly *Brachionus* and *Asplanchna*, may grow in peak *Chlorella* production and deplete to near zero within 2-3 days. In an indoor lab under controlled conditions, mass culture of *Chlorella* can be maintained with fluorescent light for more than 15 days with a high population density, but this is costly. Now, it is the option of farmers to adopt either method, based on the available infrastructure and their cost bearing capacity.

Growth curve of *Chlorella* – an established scientific principle

Growth and development of *Chlorella* (as with most of the microalgae) in batch culture follows a typical growth curve. It starts with four sequential phases as (i) an inoculation phase where population growth initiates, (ii) an exponential peak growth phase (iii) a stationary phase where population growth ceases, and (iv) a declining phase as mortality rate

Chlorella culture in an indoor laboratory setting.

exceeds growth rate. In the case of mass culture, batch culture may not be suitable from a commercial point of view. After a certain period of continuous culture, tanks need to be cleaned thoroughly as before and a new set of fertilisers added, otherwise cell deformity may occur, and the quality of *Chlorella* cells may deteriorate.

Conclusion

There are several techniques as well as growth media available for production of green water on a mass scale. However, RRC, ICAR-CIFA, Rahara has developed its own technique as easy as to follow that enable farmers to produce a colossal amount of *Chlorella vulgaris* as a green water in outdoor system, which is reliable, economical and sure to achieve. CIFA's is a much tested technique used in hilsa shad larval rearing as evidenced to achieve above 88% larval survival through green water application and co-feeding with other planktons and formulated diet as well.

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Reference

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