

# SANITARY MEASURES FOR BETTER MANAGEMENT PRACTICES IN LIVE FEED CULTURE

**Biji Xavier, Ritesh Ranjan, R D Suresh, Padmaja Rani and Linga Raju**

The production of marine finfish and shellfish larvae in hatchery requires different types of phytoplankton and zooplankton. The quality of these live feeds directly affects the production cycle of finfish and shellfish larvae since these live feeds are used either directly or indirectly in larval rearing system. Thus proper sanitary measures for better management need to be taken at each level of culture operation of live feeds to maintain the quality and quantity of these live feeds, right from stock culture to mass culture.

Sanitary measures are routine biosecurity programmes that should be taken before, during and after production runs to maximize the health of the stock by minimizing the impact of contamination. These measures are taken to control the movement of different contaminants into and through the live feed stocks. This may begin with facility design, water intake and passes through the entire range of possible sources of contamination throughout the production cycle. Optimum level of sanitization in the system will result in good quality stock, which will reduce the failure risk of the larval production cycle. Hence, it reduces the cost of the production and helps in maximizing the profit from the production cycle.

## Contaminants in phytoplankton

The major contaminants in phytoplankton stock and mass culture are as follows:

- i. Microbes such as bacteria, virus etc
- ii. Ciliates
- iii. Other microalgal species
- iv. Zooplankton in outdoor culture

## Source of contamination

The following are the source of contamination in phytoplankton stock culture:

- i. Glass ware
- ii. Culture media
- iii. Water
- iv. Air
- v. Personnel

### ***Precautions/ sanitary measures***

#### **i. Phytoplankton stock culture**

- ◆ It is necessary to practice aseptic techniques to protect the sterile culture media, glassware and finally the cultures from different contaminants.
- ◆ Personnel hygiene is the first important step to maintain sterile conditions in stock room.
- ◆ The person involved in maintaining stock room should first finish the work related to stock culture and then only carry out any other assigned work.
- ◆ He should always use sanitizer whenever he is handling the stock.
- ◆ The microalgae stock culture room needs to be kept clean and the door should be kept closed at all times to maintain desired temperature.
- ◆ The stock of the algae should be sub cultured under the laminar flow so that contamination in the stock can be avoided.
- ◆ The laminar flow UV light should be kept on at least half an hour before doing inoculation work.
- ◆ The equipments such as weighing balance, microscope and laminar flow need to be cleaned regularly.
- ◆ All glass wares such as culture containers, pipettes etc used in indoor culture are to be cleaned, washed with chromic acid and sterilized in hot air oven to avoid contamination.
- ◆ The culture media except heat labile media need to be autoclaved after preparation to avoid contamination. The heat labile chemicals need to be sterilized by 0.22  $\mu\text{m}$  filtration techniques.

- ◆ Water is a major source of contamination. It needs to be treated with different filtration processes before sterilization.
- ◆ The culture container with water needs to be autoclaved for maintaining stock culture.
- ◆ The air supplied to the culture container must be passed through filters so that contamination can be avoided.
- ◆ Each and every step must be monitored carefully so the contamination can be stopped early on.
- ◆ The working area needs to be cleaned and wiped with alcohol to limit contamination.
- ◆ The air stone, pipes and air control need to be sterilized after each cycle by boiling in water for 15 minutes.
- ◆ Finally only authorized personnel should be allowed entry into algal stock culture room.

### **The ciliates are the major source of contamination in algal culture.**

It can enter either through culture solution or air. The autoclaving of culture solution and use of filter before passing of air to the culture container can help in controlling ciliates in stock culture.



**Sub-culture of microalgae in laminar flow**

## The contamination of one species of microalgae with another species of microalgae:

- ◆ It is common in a stock culture room if it is housing more than one species in the same room.
- ◆ This contamination can be controlled by the personnel involved in stock culture room.
- ◆ They should sub culture one species at a time and at least give half an hour time interval before starting sub culture of another species.
- ◆ In addition, they need to follow personal hygiene before starting sub culture.
- ◆ The pipette or the inoculum loop used for the sub cultures need to be sterilized each time before use.

### ii. Phytoplankton mass culture

- ◆ Design of the water pumping system, water storage facility and culture tanks have effect on the contamination of phytoplankton culture.
- ◆ Proper design of the tanks is required to facilitate easy cleaning and drying so the contamination can be avoided.
- ◆ The tank inner surface needs to be smooth and the corners should be curved in such a way that cleaning should be easy.
- ◆ The bottom of the tank should have some slope so when the water is getting drain it should dry easily.
- ◆ An epoxy or fiber glass or plastic coating inside the tank will help in these things. Among these, epoxy coating is the best solution for making inner wall of tank smooth.
- ◆ The pumping facilities need to be treated every alternate month with chlorinated water (30-40 ppm) to avoid any contamination.
- ◆ The aeration system needs to be sanitized every alternate month by using formalin.
- ◆ After the harvest of live food organisms proper management measures for wastewater treatment is required for proper hatchery production.

- ◆ Drainage pipes carrying wastewater need to be of suitable diameter for water draining and for avoiding backflow.
- ◆ In outdoor culture system, carboys must be cleaned using common salt, rinsed with tap water and keep upside down for sun drying.
- ◆ All tanks should be cleaned, disinfected and dried before use.
- ◆ The water used for the mass culture of phytoplankton needs to be filtered before stocking in the tank.
- ◆ The chemicals required for the mass culture need to be prepared in filtered fresh water.



**Cleaning of outdoor algal tan**

### **Contaminants in zooplankton**

The major contaminants in zooplankton culture are as follows:

- i. Microbes such as bacteria, virus etc
- ii. Ciliates
- iii. Other zooplankton

## Source of contamination

The following are the source of contamination in zooplankton culture:

- i. Culture container
- ii. Water
- iii. Phytoplankton
- iv. Personnel

## *Precautions/ sanitary measures*

- ◆ All tanks should be cleaned, disinfected and dried before use.
- ◆ The water used for mass culture of zooplankton needs to be filtered before stocking in the tank.
- ◆ The use of the same siphon pipe for the all tanks should be avoided. Each tank should have a separate siphon pipe.
- ◆ Each tank should have a separate set of items such as filters, mugs, and buckets etc required for day to day activity.
- ◆ Generally, in commercial facilities, contamination of one zooplankton species culture with another zooplankton species is the most likely cause of the crash of culture. Therefore, it is important to keep these cultures strictly apart.
- ◆ The presence of other zooplankton species may pose a problem, so care should be taken while adding fresh seawater and feed to the culture tank.
- ◆ The water used for the phytoplankton culture as well as copepod culture should be filtered with 10  $\mu\text{m}$  filter bag.
- ◆ Generally ciliate proliferation is more in over-fed culture tanks. Sometimes these ciliates act as a feed for some zooplankton during the periods of low phytoplankton concentrations. However ciliates also compete for the same feed with the zooplankton, thus care should be taken to avoid contamination with ciliates.

- ◆ It is advisable to empty the culture tank using 40-60  $\mu\text{m}$  mesh size gauze if ciliate contamination is more, which retains the zooplankton, but allows the ciliates to be washed out. Then, culture can be started afresh.
- ◆ Generally, bacteria constitute a part of the diet of zooplankton. Sometimes, cultures may succumb to uncontrolled proliferation of bacteria. Some bacteria, such as *Vibrio* sp., are known to infect zooplankton in eutrophic coastal waters, resulting in low survival rates with further contamination of the fish larval rearing system which leads to mass mortality of the fish larvae also.
- ◆ Sea water is one of the main routes that causes contamination in phytoplankton culture. Sanitation protocols need to be followed to maintain water quality which has been provided in the next chapter on water quality for live feed in aquaculture

## Conclusion

In order to bridge the gap between demand and supply of marine aquatic resources it is necessary to bring significant changes in live feed production technology. Appropriate sanitary measures are required to combat contamination and maintain hygiene. Technological developments for improving the sanitary conditions of marine hatcheries are critical. Further, awareness for sanitary cleanliness and personal hygiene among the workers is highly essential for improving the quality and quantity in the supply of live feed.

## References

- Cai Yimin, Pang Huili, Tan Zhongfang, Wang Yanping, Zhang Jianguo and Chuncheng Xu. 2014. Application of Lactic Acid Bacteria for Animal Production. *Lactic Acid Bacteria*, 443-491.
- Head E.J.H. 1988. Copepod feeding behavior and the measurement of grazing rates *in vivo* and *in vitro*. *Hydrobiologia*, Volume 167, Issue 1, pp 31-41.
- Kimura T., Yoshimizu M., Tajima K., Ezura Y. and Sakai M. 1976. Disinfection of hatchery water supply by ultraviolet (U.V.) irradiation. I. Susceptibility of some fish-pathogenic bacterium and microorganisms Inhabiting pond waters. *Bull. Jap Soc. Sci. Fish.*, 42. 207-211.

- Loka Jayasree, Sonali, S. M., Saha Purbali and Philipose, K. K. 2016. Bacterial flora of water and rotifers in outdoor mass culture tanks fed with different microalgal diets. *J. Life Sci.*, 10: 123-127.
- Munn Colin B. 2005. *Oceans and Health: Pathogens in the Marine Environment*. Oceans and Health: Pathogens in the Marine Environment. pp.1-28.
- Wang Lawrence K., Yuan Pao-Chiang and Hung Yung-Tse 2005. Halogenation and Disinfection. *Physicochemical Treatment Processes*. Volume 3 of the series *Handbook of Environmental Engineering* pp 271-314.
- White G.C. 1999. *Handbook of chlorination and alternative disinfectants*, 4th edn. Wiley, New York.