CMFRI Training manual Ser. No.: 9/2016

Training Manual on Live Feed for Marine Finfish and Shellfish Culture

VISAKHAPATNAM REGIONAL CENTRE
ICAR-CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
OCEAN VIEW LAYOUT, PANDURANGAPURAM
VISAKHAPATNAM - 530003

Stock culture maintenance and mass culture of micro algae

Biswajit Dash, Ritesh Ranjan, Vamsi B and Shiva P

Introduction

Marine micro algae are found in marine systems living both in water as well as in sediment have an important role in ecological pyramid of the marine ecosystem. These are the base of the trophic web and provide energy for all successive trophic level in the marine eco system. These are single celled, chlorophyll bearing organisms uses solar energy and nutrients from water to convert it into organic matter. Out of 8 Lakhs species of different genera of algae available from marine ecosystem very few have been tapped for their use as availability of biomedical compounds and also as feed in different finfish, shellfish and molluscan hatchery. Their role is also very much vital and critical in a successful mariculture hatchery management. An alga serves as a feed for other zooplanktons and also added to the larval rearing tanks to improve quality of water as green water technology. Among microalgae, flagellate and diatom species are cultured in hatcheries in suitably treated seawater enriched with nutrients such as nitrates, phosphates, essential trace elements and vitamins. To support growth of high densities of larvae and juveniles reared in the hatchery high density of micro algal culture is essential. Considering the importance of marine algal culture, different indoor laboratories and outdoor mass culture systems being carried out in various hatcheries in world like Japan, Taiwan, China, Philippines, Indonesia as well as India.

Stock culture development and maintenance

Stock cultures of the preferred species are the basic foundation of culture. They are normally supplied as monospecific cultures without contaminations. Stock cultures are used as inoculum when required. For development of stock culture it is very much important to take up the required culture for a particular hatchery operation. There are thousands of microalgae that differ in size, color, shape, and habitat. Researchers have isolated and identified several species from natural waters that have proven to be nutritious food for growing and from them some are very much important for use in

larval rearing of fish, shrimp or molluscs. Different species of the genus mainly used are in fin fish hatchery are *Pavlova, Dicrateria, Thalassiosira, Isochrysis, Chaetoceros, Dunaliela, Nanochloropsis, Tetraselmis, Chlorella and Nannochloropsis.* Stock culture of a desired species of algae begins with a pure starter culture. It is important to collect starter culture from an established and running hatchery. Usually starter cultures transported in test tubes and inoculated in several new test tubes with a suitable media. Some of these cultures must be kept as stocks for unforeseen situations like old culture contaminated or lost etc. then again revival of the culture will restart and multiplied. The rest are used to inoculate progressively larger glasswares until there is enough culture to start mass production

To have a sustained production of algae main source is supply of good sea water free of any contaminant either chemical or biological. Sterilization of the seawater can be done by many ways like filtration, autoclaving, pasteurization, UV irradiation, chlorination, acidification or ozonization. The critical factors for a successful algal culture stock room which are to be maintained are mainly controlled light, available illumination with required lux, controlled aeration at different stages, stable temperature control, sterile sea water without any contamination with desired salinity, nutrient media for inoculation and skilled workers.

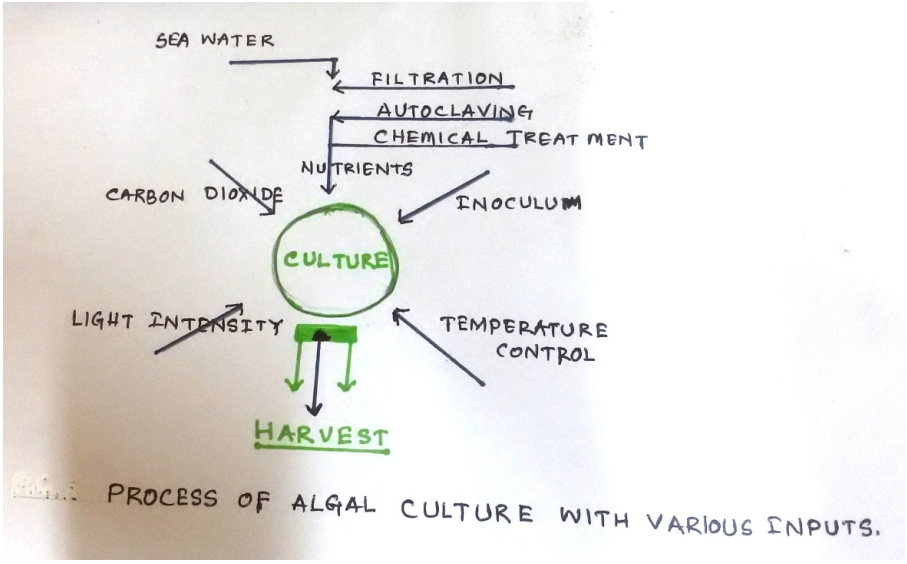


Fig. 1 . Process of Algal culture with various inputs

Always stock culture are maintained in small quantity and in gradual manner these are transferred to different containers observing the quality and density of the algae. In indoor culture it is maintained in a 10 ml test tube, then in 100 ml conical flask, then 250 ml conical flask, then 2 lt haufkin flask and finally to the 20 lt or pet jar or 50lit carboys. Care must be taken to minimize the risk of contaminating the stock and starter cultures with competing microorganisms. Each container should have half level sterilized media for inoculation of the algae and covered with cotton and gauge cloth to avoid contamination. Always harvest and transfer of algae is to be done during the peak of exponential phase so that maximal growth can be obtained.

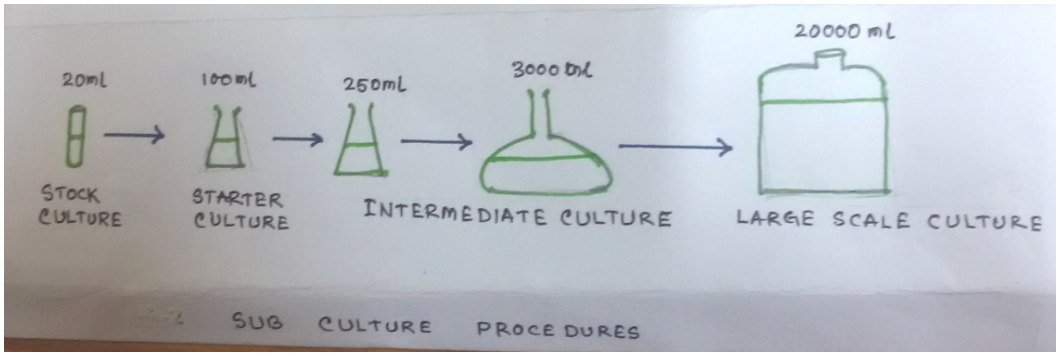


Fig. 2 . Sub culture procedures

Algal multiplication is normally dependant of various steps like sterilize the culture water, add nutrient enrichment, inoculate new culture from the pure algal strain, microscopic observations for growth of the algal counts, and multiply algae in larger containers. Finally the alga has to be supplied with a requisite quantity for outdoor mass culture. For all the above process to continue in a cyclical manner and it is very much essential to maintain different stock culture for a back up and for further use.

Control of light intensity is very much essential as less light is required during the stationary and declining phases. Too much of light will cause the culture for the early declining. For maintaining the stock cultures of all micro-algae during the declining and stationary phases of growth, light intensity of 500 lux is essential while for the

mass culture 2000-3000 lux is required. Maintenance of light and dark hours with timer control is ideal for maintaining the stock.

Since normal room temperature (28-30^{0}C) is not ideal for the maintenance and culture of micro-algae, air conditioned rooms are used for keeping the stock culture. It should have 23-25^{0}C during day time. Some of the A.Cs. can be switched off for few hours during night time to save current and maintaining temperature. All these should be done with automatic controls or timers.

Similarly, aeration is also important for developing and maintaining stock room cultures or mass outdoor culture. Aeration is very much essential to enhance the exponential phase of growth of micro-algae for required temperature. Aeration helps in many ways like the maintenance of oxygen sufficiency in the culture medium and a good agitation in the culture suspension for making the culture moving. Aeration also helps in mixing the nutrient in the culture media uniformly in the medium and preventing the algae from settling at the bottom of the culture tanks. Frequent checking of aeration pipes and decontamination with formalin also enhances the quality of stock. Also it is essential that separate person for maintaining separate culture of algae as it will reduce the chances of mixing by contamination.

Equipments required for the stock culture maintenance of microalgae

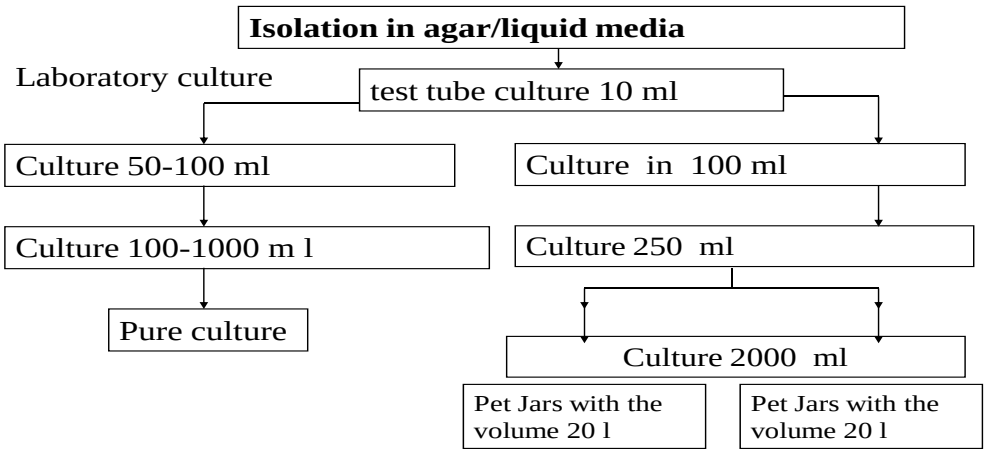
The minimal equipments required for a successful microalgal hatchery operation are numerous culture glasswares of different sizes, light, pure algal stock, seawater (or brackish water), seawater pump, blower or other aeration equipment, temperature controls or thermostats, chemicals, balance, autoclave, oven, salinometer, hydrometer, or refractometer, pH meter, sedgwick rafter or hemacytometer, microscope with digital counter with suitable magnification and most important is involvement of skilled or knowledgeable staff.

The glass wares or plastic wares for stock culture and mass culture in outdoor should be cleaned thoroughly prior to sterilization either by sun drying or by autoclaving. The glass wares used for the indoor culture need to be cleaned with chromic acid. For outdoor culture of micro algae, the carbuoys used are cleaned by

common salt and rinsing with tap water 4-5 times and keep for sun drying. The glass wares have to be kept in an oven at temperature 100 ^{0}C for an hour. The culture tubes, conical flasks and Haufkin culture flasks have to be plugged with cotton before keeping in the oven.

Procedure of inoculation

Add 10ml of the culture medium to the test tube (20ml) and inoculate the medium with single drop of the pure stock culture. Cover the mouth of the test tube with aluminium foil tightly. Keep the culture in the temperature controlled room with sufficient light intensity. Shake the culture gently everyday and observe for the culture. The culture will be observed from 5th to7 th day. The test tube culture will be served as the starter culture and precede the culture into 100 ml, 250 ml and 3000 ml conical flasks. A single test tube inoculum can be used for the inoculation of three or four culture (100 ml conical flask). The culture can be continued to 250 ml conical flask using the culture inoculum from 100 ml conical flask. Once the test tube inoculum is developed the culture will be observed on every third or fourth day depending on the quantity of inoculam was added to the culture medium. In every step the culture should be transferred aseptically without any contamination. The unicellular algae grow exponentially by subsequent multiplication of cells in suitable conditions of light, temperature and aeration.



Mass outdoor culture in 100 l transparent tanks and further in 1 ton taks

Table 1. Amount (ml/L) of medium added for the indoor culture of some specific algae

Algae	Convey medium (ml/L)				F/2 medium (ml/L)			
	A	B	C	D	A	B	C	D
Chaetoceros	-	-	-	-	1	1	1	1.5
Isochrysis	1	0.5	0.1	-	-	-	-	-
Nanochloropsis	1	0.5	0.1	-	-	-	-	
Thalasiosera	1	0.5	0.1	-	1	1	1	1.5
Skeletonema	1	0.5	0.1	-	1	1	1	1.5
Chlorella	1	0.5	0.1	-	-	-	-	-

Growth cycle of algal culture

After the inoculation of the cells with required culture medium the cells passes through various growth phases in the culture with suitable conditions of light, temperature and aeration. Cell count increases in a characteristic sigmoid fashion.

1. Lag phase or induction phase*:* After the addition of stoke culture inoculum for the subculture of micro algae, there will be a no cell division phase for few hours which is known as lag or induction phase.

2. Exponential phase: after the lag phase, the cells are acclimatized and start dividing, grow fast by utilizing nutrients, aeration and light. This growth phase is called exponential phase and reaches maximum cell concentration during this period.

3. Declining phase: After reaching the growth phase, the cells will be showing less growth or slow growth. This stunted growth stage is known as declining phase.

4. Stationary phase: The declining phase will be continuing for few days without any cell division and this period is known as stationary phase. Sometimes, the culture may start dividing the cells with suitable conditions.

5. Death phase*:* Prolonged stationary phase will lead to the death phase, where algal cells will lose their viability and the cells dies. This phase is called death phase.

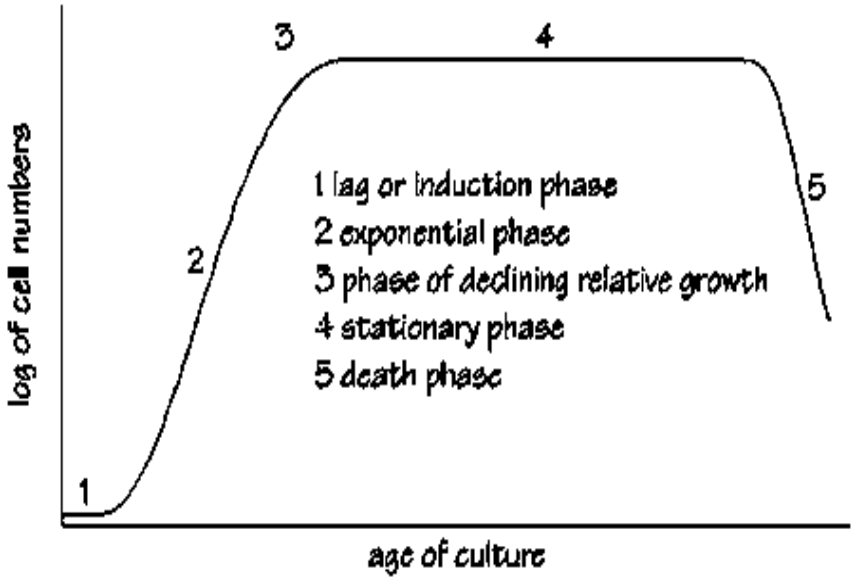


Fig. 3 . Growth cycle of algal culture

Growth slows as the algal population becomes more crowded. Nutrients are depleted, metabolites build, and light penetration decreases because of self-shading. The cultures have reached their stationary phase for the current conditions and will not increase in density. Algae harvested near this maximum density are a high quality food and provide the most efficient use of hatchery labor and space.

Fig. 4 . Stock culture and multiplication laboratory

Algal cell counting

Counting is normally done in Sedgwick Rafter Counting Chamber or Haemocytometers. Haemocytometers are thick glass slides with two chambers on the upper surface, each measuring 1.0 x 1.0 mm. A special cover slip is placed over these two chambers giving a depth of 0.1 mm making the total volume of each chamber 0.1mm^3. With the cover slip in position, one or two drops of the algal sample are introduced by means of a Pasteur pipette to fill both chambers. Cell density is estimated as follows. Haemocytometer has got 9 chambers (4 sides having 16 divisions and 5 chambers of multiple divisions) we have to restrict the counting for at least 4 chambers. Take the average number of cells in one ml.

Average counts per chamber x 10^{-4} = Total number of cells/ ml

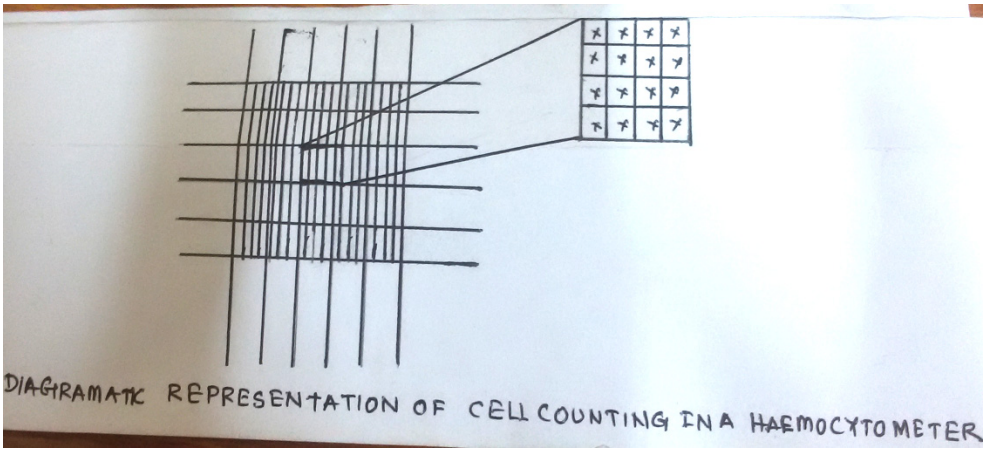


Fig. 5 . Diagramatic representation of cell counting in a haemocytometer

Mass culture of microalgae

Large scale culture of microalgae is normally taken up to obtain maximum possible yield per day, especially nannoplankton flagellates and selected species of diatoms are necessary for feeding the rearing larval forms in a hatchery. Mass culture can be done in the outdoor 100 L tanks taking the inoculum from the 20 L jar from indoor and further transferd to 1 tonne tanks. These containers will have the maximum concentration of the cells in the growing phase on the 5-6th day and can be harvested. After estimating the cell concentration, using a haemocyotometer, the culture is supplied to the outdoor algal hatchery for mass culture should have maximum concentration and purity to have good yield.

Mixed culture medium	
Potassium nitrate	1.2 g
Sodium Ortho-phosphate	0.66 g
EDTA (Na)	0.66 g
Sodium silicate	0.66 g

Dissolve the first 3 chemicals in 25 ml of dist. water and sodium silicate can be dissolved separately in 25 ml of dist. water. Add this to 100 L of fresh unfiltered seawater (freswater can filter through organdy net. 0.33 mm mesh size to remove zooplankton). Pour the water in 3-4 white lined basins or fibre-glass tanks and keep the same in open sunlight. Within 24 hrs, a slight yellow discoloration can be noted. On examination, we can notice the growth of planktonic diatoms and other nannoplankters. If the temperature is very high and sunlight is very bright. only the blooming of *Chaetoceros spp*. could be observed.

Besides the above mentioned laboratory prepared chemicals, which act as nutrients, commercial fertilizers can be used for the mass culture of micro-algae. In open tanks for economy purposes, the following fertilizing medium can be used:

Commercial fertilizer medium/ 1t	g
Urea	46
20-0-0	10
16-20-0	1

Commercial fertilizer medium/ 1t	g
Ammonium sulphate	100
Urea	10
Tripple super phosphate	10

The maximum density of algal cells in outdoor culture depends mainly on factors like atmospheric light and temperature, salinity of sea water without any contaminants suitable to the cultured species, and a good density of indoor inoculum. In outdoor culture with 100 L tanks around 10-15 million cells per ml can be achieved within 2 days of

inoculation and around 3-5 million cells per ml can be achieved in 1 ton outdoor tanks with suitable environmental conditions.

Fig. 6. Outdoor mass culture of micro algae in 100 L carboys

Fig. 7. Outdoor mass culture of micro algae in 1t FRP tanks

Harvest of the culture

The culture should be harvested during the exponential phase of the micro-algae after determining the cell concentration. If the culture has entered the declining or stationary phase, the metabolites will be very high and the cells may not be in healthy condition. The rearing larval organisms may not show the expected growth if fed with this feed. The trouble which comes frequently like air pressure, salinity, pH, culture contamination, nutrient media for culturing the algae needs immediate attention. Frequent

observation to avoid contamination is highly required as the algal feed is very much important in terms of nutrients for the other organisms like rotifers and copepods to feed and also pure culture will not contaminate while adopting the green water technology for the larval rearing system for marine fishes or shell fishes.

References

Anderson, A. R. 2005. Algal culture techniques. Academic press. pp. 596.

Bourne, N., Hodgson, C.A. & Whyte, J.N.C. 1989. A Manual for Scallop Culture in British Columbia. Canadian Tech. Rep. *Fish and Aquatic Sciences*, No. **1694**: 215 pp.

Guillard, R.L**.** 1975. Culture of phytoplankton for feeding marine invertebrates, p. 29 - 60. In: Culture of Marine Invertebrates P.B. Smith (ed). Plenum Press, New York.

Stein, J.R. 1973. Handbook of Phycological Methods.: Culture Methods and Growth Measurements. Cambridge University Press, Cambridge, England **8**: 448 pp.

Trotta, P**.** 1981. A simple and inexpensive system for continuous monoxenic mass culture of marine microalgae. Aquaculture, **22:** 383 - 387

Ukeles, R**.** 1973. Continuous culture - a method for the production of unicellular algal foods. In: J.R. Stein (ed), Handbook of Phycological Methods, Culture Methods and Growth Measurements. Cambridge University Press, Cambridge: 233 – 254.

Training Programme on

"Live Feed for Marine Finfish and Shell fish Culture"

17th - 22nd March 2016

Visakhapatnam Regional centre of CMFRI

Ocean View Layout

Pandurangapuram,Visakhapatnam