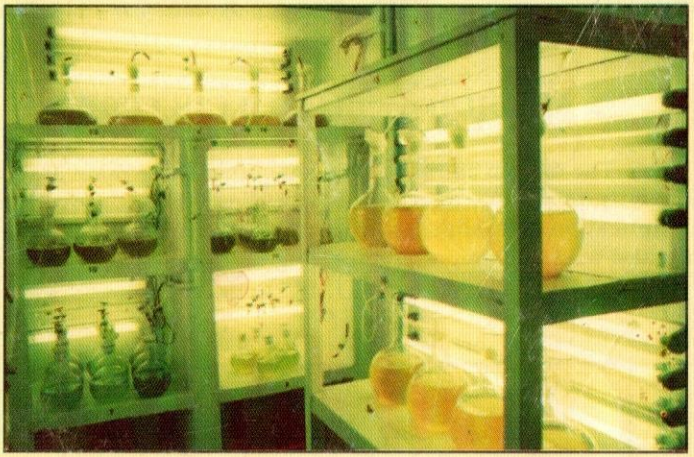
CIBA EXTENSION SERIES NO. 18

CULTURED DIATOMS AS FEED FOR HATCHERY RAISED PENAEID SHRIMP LARVAE



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CULTURED DIATOMS AS FEED FOR HATCHERY RAISED PENAEID SHRIMP LARVAE

Successful rearing of shrimplarvae in hatcheries depends mainly on feeding the larvae with appropriate feed. The floating microscopic plants or phytoplankters, play an important role in the dietary regime of larvae reared in hatcheries. They directly or indirectly form an essential link in larval food chain. Hence, culture of phytoplankton as live food organisms, receives due importance in all hatcheries.

Chaetoceros sp., *Skeletonema* sp., *Thalassiosira* sp. (Fig. 1), *Isochrysis* sp. and *Chlorella* sp. are some of the phytoplankton food species which are mass-cultured in hatcheries to feed the shellfish larvae individually or collectively. Among these species, diatoms such as *Chaetoceros* spp. and *Skeletonema* spp. are preferred as algal diet to penaeid shrimp larvae. Hence, culture of diatoms assumes immense importance in penaeid shrimp hatcheries.

2. METHOD OF DIATOM CULTURE

The diatom culture process involves three main aspects, namely, preparation of a suitable medium for its growth and multiplication, obtaining a starter or inoculum by isolating it from the wild collection and scaling the operation to optimum level.

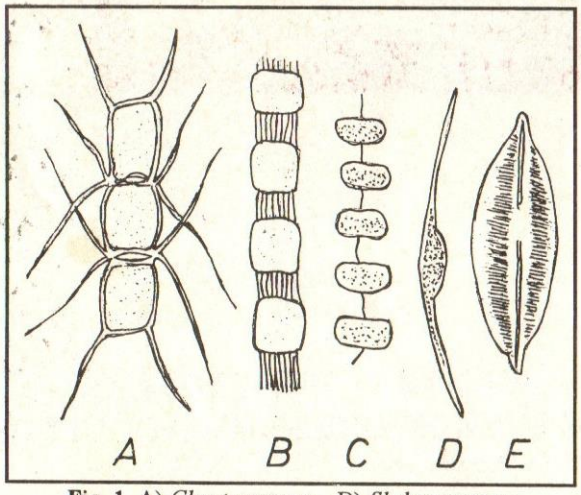


Fig. 1. A) *Chaetoceros* sp. B) *Skeletonema* sp. C) *Thalassiosira* sp. D) *Nitzchia* sp. E) *Navicula* sp.

3. MEDIUM PREPARATION

The natural or artificial sea water will form a very good base-medium for diatom culture, which should be initially sterilized to remove all living organisms and then it should be enriched with the required nutrients.

STERILIZATION

Sea water should be initially filtered through pressure sand filter and subsequently sterilized either by steaming or by allowing it to pass through membrane filters, cartridge filters and ultra violet radiation. In the case of non-availability of the above-mentioned filters, the sea water can be treated with 10 ppm chlorine and subsequently neutralized with equivalent amount of sodium thiosulphate. The glass containers and accessories also should be sterilized in an autoclave or dry oven.

ENRICHMENT

The sterile medium has to be enriched with nutrients, which may be of organic or inorganic nature. Though organic enrichment is possible by utilizing a soil extract, the enrichment by inorganic chemicals is widely followed because of its easy availability and simplicity in preparation and manipulation. In addition to inorganic nutrients, the enrichment is continued by adding trace metals and vitamin mixture. Thus, the medium is enriched by mixing inorganic nutrients, trace metals and vitamin mixture, depending upon the nutrient requirement of the diatom species to be cultured. The important culture media, used for the culture of diatoms, are Walne's Conwy medium (Table 1), Guillard and Ryther's modified 'F' medium (Table 2), Silas *et al.* modified 'F' medium and Liao and Huang's modified TMRL medium (Table 3).

4. INOCULATION

In the enriched medium, a small quantity of the diatom to be cultured has to be either inoculated or introduced as a starter. To obtain the starter culture, a sample of phytoplankton which contains several species including the desirable ones, is to be collected from the sea shore water. The isolation, separation and purification of the desired diatom species from the wild phytoplankton collection are essential steps for inoculum preparation which are achieved by several methods depending upon cell size and characters of the particular diatom. These methods are i) biological isolation, ii) serial dilution, iii) repeated subcultures, iv) capillary pipette method, v) streak plating and vi) spray plating. Purification of the separated

diatom is necessary for its production as axenic culture and it is accomplished by repeated micropipette washing, physical separation from the adhering contaminants by ultrasonic vibration, concentrating the cells by centrifugation and subsequently transferring them to new medium, repeated streak plating on agar plate with or without antibiotics and killing or inhibiting growth of contaminants with chemical methods. When chemicals or antibiotics are used, it is necessary to initially determine the tolerance level of each diatom by carrying out preliminary tests.

5. SCALING UP THE CULTURE

The isolated, separated and purified diatom is initially cultured in 10 - 15 ml test tubes and 125 - 250 ml conical flasks. In due course, inoculum may be developed to required quantity. Stock cultures in 125 ml flasks are generally transferred once in a week. This transfer is usually done before half the life span of a culture has passed. Stocks in tubes or 125 ml flasks are inoculated by using a Pasteur pipette with half to 2 ml of culture depending on the species. Diatoms generally need a little amount of inoculum. Size of the inoculam can be adjusted, so that the crop in the flask will peak at 5 - 7 days. For a large scale culture, 10 - 20 % of the total volume is the suggested amount of inoculum.

6. GROWTH CHARACTERISTICS

The diatom growth is characterized by a sigmoid curve and is divided into 4 distinct phases. They are lag phase, logarithmic or exponential phase, stationary phase and death phase.

7. REGULAR MAINTENANCE

To maintain the stock culture, suitable physical conditions are to be provided. Stock cultures of 2 litres and above, which are maintained in glass flasks and carboys, should be provided with aeration. For small volumes of stock cultures, which are maintained in agar slants or in test tubes of 10 - 15 ml volume or in 125 - 250 ml conical flasks, aeration is not required. "Cool-white" fluorescent light, fitted above or around the culture-containers giving a light intensity of about 4750 lux, are used to maintain the stock cultures. The room temperature, should be kept at 20 - 26°C by providing central or window units of air-conditioning. Thus, the stock cultures are generally maintained in an air-conditioned and illuminated room with infrastructure facility for adequate aeration.

8. MASS CULTURE OF DIATOMS

As the hatcheries require diatoms in large scale to feed the larvae, diatoms are mass-cultured in indoor or outdoor tanks by making use of the inoculum generated.

INDOOR CULTURE

Large scale culture is carried out under controlled conditions in an air-conditioned room, where a series of glass carboys having a capacity of about 10 - 20 litres are kept in a rack or shelf. Transparent plastic containers of 30 to 480 litres capacity are also used. Sea water, enriched with either Modified 'F' medium or Walne's Conwy medium, forms as growth-medium. 10 to 20 % of the total volume is taken as inoculum. Adequate aeration, with air containing 1 to 2 % CO_2 and flowrate of 5 - 15 litres/minute, light with a minimum of 4750 lux and pH of 7.8 - 8.2 are essential to get optimum growth. If cell density is about 2 x 10^5 cells/ml after inoculation, diatoms will grow and reach a harvestable density ranging from 25 x 10^5 to 30 x 10^5 cells/ml within a period of 5 - 7 days. Indoor cultures are normally produced and harvested in batches in which one culture will be followed by a fresh new culture.

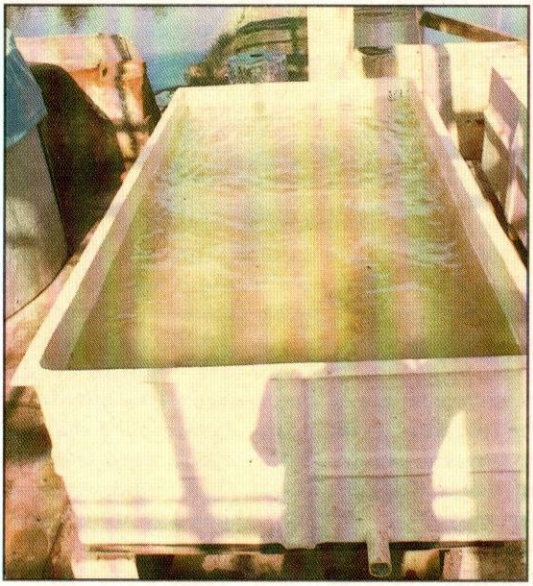
OUTDOOR CULTURE

In order to produce enormous quantity of diatoms, at cheaper cost, culture is carried out in outdoor tanks, for which, 1 ton and above capacity FRP / cement tanks are used. These tanks should be shallow and their inner surface should be white in colour. Vigorous aeration has to be provided.

Only one diatom species or a mixture of several species can be cultured in outdoor tanks. If a single species is cultured, it is known as monoculture. When more than one species are cultured as a mixture, it is called as mixed culture. When the diatoms reach harvestable stage, three-fourth portion of the culture is drawn as harvest. One-fourth volume of the culture which is retained in the tanks, acts as inoculum and fresh enriched sea water is added to original culture-volume. The culture is thus a continuous process.

OUTDOOR MONOCULTURE

For outdoor monoculture, the sea water medium is thoroughly filtered by pressure sand filter, cartridge filters and UV filters as done in indoor culture and enriched with Modified 'F' medium (Flow Chart - 1). Harvested sterile carboy cultures from air-conditioned algal room are used to inoculate to a level



Outdoor monoculture - Enriched sea water

Outdoor monoculture - Full bloom after 24 hours of enrichment

of 10 - 20 % of the total volume. Within 24 hours, density of 4 - 6 x 10^5 cells/ml will be attained from an initial density of 0.5 - 0.6 x 10^5 cells/ml if sunlight is more than 1 lakh lux and water temperature is above 26°C.

OUTDOOR MIXED CULTURE

Instead of monoculture, several diatom species may be cultured together as mixed culture. Silas *et al.* have developed a simple technique to produce a mixed diatom culture dominated by *Chaetoceros* spp. As per the above technique (Flow Chart - 2), sea water is filtered through 50 micron net and fertilized with sodium/potassium nitrate, sodium dihydrogen orthophosphate, sodium silicate and ethylene diamine tetra acetic acid at the rate of 12 ppm, 3 ppm, 6 ppm and 6 ppm respectively. Although several phytoplankton species are originally present in the sea water, *Chaetoceros* spp. will become the dominant diatom forming about 75 % of the cells in the culture after 24 - 48 hours at a water temperature range of 28°C to 35°C and sunlight of 20 x 10^3 to 120 x 10^3 lux. The other diatoms such as *Thalassiosira* spp., *Skeletonema* spp., *Navicula* spp. and *Nitzchia* spp. (Fig.1) will account for the remaining 25 %. The mixed diatom culture will be dominated by *Thalassiosira* spp. if the water temperature ranges between 26°C and 28°C. *Skeletonema* spp. will dominate the mixed culture if the water temperature goes below 26°C. In this method, the cell density will attain a maximum of 4 x 10^5 cells/ml from an initial density of 2 x 10^4 cells/ml within a culture period of 2 days.

9. HARVEST OF DIATOM CULTURE

When the diatom is in the exponential phase of growth and attains a maximum density, it should be harvested and utilised as feed for penaeid shrimp larvae. Appropriate quantity of diatom culture will be added to larval tanks as feed and the volume of feed is usually determined by measuring the cell density with haemocytometer or other counting devices.

Diatom cells can be concentrated and frozen prior to the actual feeding to the larvae, by using a dairy cream separator and a standard home freezer. When treated in a cream separator, diatom cells accumulate along the outer wall of the centrifuge bowl. The concentrate is removed from the cream separator and frozen in polythene containers and preserved at - 4°C to - 20°C, in a freezer. Whenever necessity arises, frozen diatom block is suspended in sea water after removing the polythene cover and used as feed.

TABLE-1

WALNE'S CONWY MEDIUM

COMPONENT		QUANTITY	
STOCK - A			
$FeCl_3 6H_2O$	-	1.3	gm
$MnCl_2 4H_2O$	-	0.36	gm
H_3BO_3	-	33.6	gm
EDTA	-	45.0	gm
$NaH_2PO_4 2H_2O$	-	20.0	gm
$NaNO_3$	-	100.0	gm
Trace metal solution	-	1.0	ml
Distilled water to be made to	-	1.0	litre
Add 2 ml Stock - A per litre of sea water			
TRACE METAL SOLUTION			
$ZnCl_2$	-	2.1	gm
$CoCl_2 6H_2O$	-	2.0	gm
$(NH_4)Mo_7O_2 4H_2O$	-	0.9	gm
$CuSO_4 5H_2O$	-	2.0	gm
Distilled water to be made to	-	100.0	ml
Acidify with sufficient conc. HCl to obtain clear solution.			
STOCK - B			
Vitamin B_{12}	-	10.0	mg
Vitamin B_1	-	200.0	mg
Distilled water to be made to	-	200.0	ml
Add 0.2 ml Stock - B per litre of sea water			
STOCK - C			
$Na_2SiO_3 5H_2O$	-	4.0	gm
Distilled water to be made to	-	100.0	ml
Add 2 ml Stock - C per litre of sea water			

NOTE: $FeCl_3 6H_2O$ in Stock - A is 1.3 gm if autoclaved sea water is used
$FeCl_3 6H_2O$ in Stock - A is 3.25 gm if filtered sea water is used

TABLE-2

GUILLARD AND RYTHER'S MODIFIED 'F' MEDIUM

COMPONENT		QUANTITY	
$NaNO_3$	-	84.148	mg
$NaH_2PO_4 H_2O$	-	10.000	mg
$FeCl_3 6H_2O$	-	2.900	mg
Na_2EDTA	-	10.000	mg
$Na_2SiO_3 9H_2O$	-	12.000	mg
VITAMINS:			
B_1 (Thiamin HCl)	-	0.200	mg
B_{12} (Cobalamine)	-	1.000	mg
B (Biotin)	-	1.000	mg
TRACE METALS:			
$CuSO_4 5H_2O$	-	0.196	mg
$ZnSO_4 7H_2O$	-	0.440	mg
$CoCl_2 6H_2O$	-	0.200	mg
$MnCl_2 4H_2O$	-	3.600	mg
$NaMoO_4 2H_2O$	-	0.0126	mg
Sea water	-	1	litre

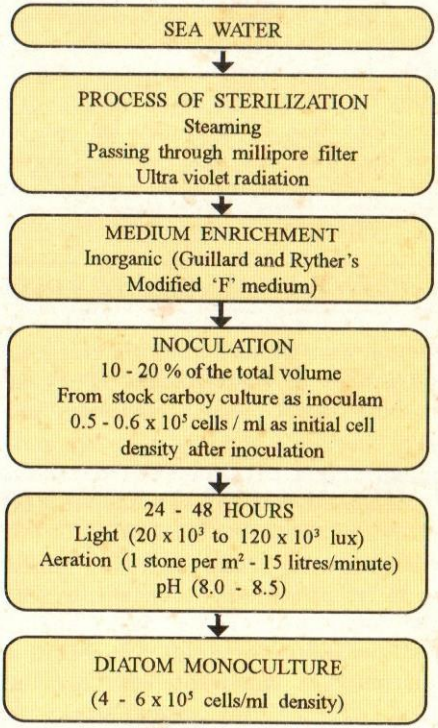
TABLE-3

LIAO AND HUANG'S MODIFIED TMRL MEDIUM

COMPONENT		QUANTITY
KNO_3	-	100.00 mg
$Na_2HPO_46H_2O$	-	10.00 mg
$FeCl_36H_2O$	-	3.00 mg
$Na_2SiO_39H_2O$	-	2.00 mg
Sea water	-	1 litre

FLOW CHART-1

MASS CULTURE OF DIATOMS OUTDOOR MONOCULTURE



FLOW CHART - 2

MASS CULTURE OF DIATOMS
OUTDOOR MIXED CULTURE

SEA WATER
Filtered through 50 micron net
Having less than 20,000 cells per ml
Salinity (30 to 35 ppt)

↓

FERTILIZATION
Silas *et al.* modified 'F' medium
Sodium / Potassium nitrate - 12 ppm +
Sodium dihydrogen orthophosphate - 3 ppm +
EDTA of sodium - 6 ppm +
Sodium silicate - 6 ppm

↓

PROVISION OF FAVOURABLE CONDITIONS
Aeration (1 stone per m^2 - 15 litre / min.)
pH (8.0 - 8.5)
Light (20×10^3 to 120×10^3 lux)

↓

AFTER 24 TO 48 HOURS

↓

MIXED CULTURE OF DIATOMS
(4×10^5 cells / ml)
Dominated by *Chaetoceros* sp. at water temperature above 28°C
Dominated by *Thalassiosira* sp. at water temperature between 26 - 28°C
Dominated by *Skeletonema* sp. at water temperature below 26°C

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