SUMMER INSTITUTE IN

CULTURE OF EDIBLE MOLLUSCS

HELD AT

TUTICORIN RESEARCH CENTRE OF

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE

From 26 May to 24 June 1980

Central Marine Fisheries Research Institute

P.B. 1912, COCHIN - 682018, INDIA

Indian Council of Agricultural Research

September, 1980

THE CULTURE OF PHYTOPLANKTON

D.C.V. EASTERSON

The bivalves during development depend mainly on lipids available in the egg for their energy requirements, while as adults rely chiefly upon carbohydrates. The oyster larvae at the time of hatching have some quantity of lipid left to meet the initial metabolic requirements. With the initiation of feeding on carbohydrate rich algae they switch over to carbohydrate based energy metabolism. The smooth switching over of the energy source and the easy availability of the choice food algae are the two vital factors which determine the survival of the larvae.

The bivalve larvae are pelagic, fine particle filter feeders having opposed ciliated bands. They feed on very small sized unicellular algae. Therefore in the hatchery production of molluscan seed, culture of unicellular algae come to occupy a pivotal position.

Choice of the algae

Not all unicellular algae suit the purpose of being a larval food organism. It depends on the following factors. The phytoplankters in general are known to release certain chemicals as metabolic bye products into the medium called extra cellular metabolites or exocrines. The exocrins of many algae contain toxins, the quantity of which depends on the density of the algae. Apart from this, the algal cell boundaries become infested with toxic bacteria, whereby even otherwise non-toxic algae may acquire toxic quality. The cell wall of some of the algare are very thick and resist digestion. Therefore algae with thin or no cell wall are preferred. Another important factor is the size of the cell. The algae should be very small so that the larvae are able to swallow them. In the following table the algae and their food value for the bivalve larvae have been compiled.

Table 1. Food value of various algae to the bivalve larvae.

Algae Chlorophyceae	Food value	Remarks
Coccomyxa sp.	None	Thick cell wall
Chlorella stigmatophora	"	"
C. marina	"	"
Nannochloris atomus	Low	
Dunaliella tertiolecta	Low	
D. euchlora	Medium	
Prasinophyceae		
Pyramimonas grossi	Good	Difficult to culture
P. ovata	High	
Tetraselmis suecica	High	
T. marina	Medium	
Micromonas pusilla	High	
Haptophyceae		
Isochrysis galbana	Very high	No cell wall
Dicrateria inornata	"	Difficult to culture
D. gilva	"	"
Chrysochromulina spp.	High	"
Prymnesium parvum	Poinsonous	
Chrysophyceae		
Chromulina pleiades	High	Difficult to culture
Monochrysis lutheri	Very high	No cell wall
Cryptophyceae		
Crytochrysis rubens	Medium	Difficult to culture
Cryptomonas acuta	"	
Hemiselmis rufescens	Medium	
H. virescens	Low	
Cyanophyceae		
Synechococcus elongatus	None	
Bacillariophyceae		
Phaedactylum tricornutum	Low	
Chaetoceros calcitrans	Very high	Difficult to culture
Cyclotella nana	"	"
Skeletonema costatum	Medium	"

The nutritional value of algae is not always uniform, it is a function of culture conditions. Further it is also found that mixture of different algae often give better larval growth. In order to keep the concentration of the exocrines low the algal density should be maintained at an optimum and further a constant flow of water is also helpful.

Glassware

The glassware used in phytoplankton culture should be of borosilicate (Corning or Pyrex) and neutral in reaction. First the new glassware are cleaned in tap water to remove spores and dust from the packing materials and then soaked in 1% hydrochloric acid to remove any free alkali present. Afterwards washed in laboratory detergents (teepol or lab wash), many times with tap water and finally rinsed in distilled water for a number of times. Since copper is toxic, the distilled water prepared from stainless steel or glass still should be used. It is found that chromium ions are toxic and get absorbed on to the glasswares. Therefore chromic acid should not be used to clean the glass vessels. Instead concentrated H_2SO_4 saturated with L.R. grade sodium nitrate or hot nitric acid should be used. Finally the culture vessels are allowed to dry well and it is preferred to heat them at 70°C for atleast 1 hr. For large scale culture we use Hoffkins flasks and glass carbouys.

Sea water

Though artificial sea water is used in many laboratories natural sea water is preferred. Sea water collected from the offshore regions and allowed to age is the best suited. The sea water is first filtered through cotton wool supported on a nylon mesh and then through a whatman No. 1 filter paper. Whatman G.F.C. filter paper can also be used along with suction. For better results Millipore membrance filters are recommended. The filtered sea water is autoclaved at 2 atm for 1 hr.

ENRICHMENT MEDIA

For special purposes culture media are used. But generally sea water to which nutrients have been added, known as enrichment media are used. There are so many. In our laboratory the following are being used.

1. Erdschreiber medium

Sodium nitrate ($NaNO_3$)	0.100 g
Disodiumhydrogen phosphate ($Na_2HPO_4 . 12H_2O$)	0.020 g
Soil extract	50 ml
Sea water	900 ml
Distilled water	100 ml

Soil extract is prepared as follows: Good garden soil is collected, allowed to dry, the large stones are hand picked and crushed well. The crushed sample is finely seived. This fine powder is autoclaved at 120°C for 20 minutes with twice its volume of distilled water and allowed to sediment. The supernatant yellow-brown coloured water is decanted and stored in a refrigerator for use.

In preparing the media, sea water along with sodium nitrate and acid phosphate is autoclaved, in which the salts may precipitate, and the addition of distilled water the precipitate dissolves. To this cool solution soil extract from the regrigerator is added.

2. Miquel's medium

Solution - A

Potassium nitrate (KNO_3)	20.2 g
Distilled water	100 ml

Solution - B

Sodium acid phosphate monobasic($Na_2HPO_4.12H_2O$)	4 g
Calcium chloride ($CaCl_2.6H_2O$)	4 g
Conc. hydrochloric acid (HCl)	2 ml
Ferric chloride ($FeCl_3$)	2 g
Distilled water	98 ml

Culture medium:

Solution A	0.55 ml
Solution B	0.5 ml
Sea water	1 litre

3. Modified Miquel sea water- Shiraishi's medium

Solution -A - as given above

Solution -B

Sodium acid phosphate ($Na_2HPO_4.12H_2O$)	4 g
Calcium chloride ($Ca\ Cl_2.6\ H_2O$)	4 g
Hydrochloric acid	2 ml
Distilled water	80 ml

Solution-C (Mineral mixture)

Disodium ethylene diamine tetra acetic acid(EDTA)	300 mg
Ferric chloride ($Fe\ Cl_3$)	8 mg
Manganese chloride ($Mn\ Cl_2.4H_2O$)	12 mg
Zinc chloride ($Zn\ Cl_2$)	1.5 mg
Cobatous chloride ($Co\ Cl_2.6H_2O$)	0.3 mg
Copper sulfate ($Cu\ SO_4.5H_2O$)	0.12 mg
Ortho boric acid (H_3Bo_3)	60.0 mg
Distilled water	100 ml

Solution-D (Vitamin mixture)

Vitamin B_{12}	0.1 mg
Thiamin	10 mg
Biotin	0.1 mg
Distilled water	100 ml

Store in a refrigerator

Culture medium

Solution A	2 ml
Solution B	1 ml
Solution C	2 ml
Solution D	1 ml
Sea water	1 litre
Tris buffer	50 mg

4. Walne's enrichment medium

Solution-A

Ferric chloride ($FeCl_3.6H_2O$)	2.6 g
Manganese chloride ($MnCl_2.4H_2O$)	0.72 g
Orthoboric acid (H_3BO_3)	67.20 g
Sodium EDTA	90.00 g
Dibasic sodium acid phosphate ($NaH_2PO_4.2H_2O$)	40.00 g
*Potassium nitrate (KNO_3)	200.00 g
Distilled water	2 litres

Solution-B

Zinc chloride	2.1 g
Cobalt chloride	2.0 g
Ammonium para molybdate $(NH_4)_6 Mo_7O_{24}.4H_2O$	0.9 g
Copper sulfate ($CuSO_4.5H_2O$)	2.0 g
Distilled water	1 litre

Acidify with HCl to obtain a clear liquid

Solution-C

Vitamin B_{12}	10 mg
Thiamine	200 mg
Distilled water	2 litres

To be stored in a refrigerator

Culture medium:

Solution A	10 ml
Solution B	1 ml
Solution C	1 ml
Sea water	10 litres

*Dr. Walne has given $NaNO_3$

Chelaters

Chelaters are used in the culture medium to avoid heavy precipitation of metals whereby avoiding toxicity. The addition of a chelating agent such as EDTA to the sea water sets up equilibria in which cations compete for available EDTA bonds, while enough ions are released to meet the needs of the growing cells.

ISOLATION OF ALGAE

At the onset autoclaved 1.5 - 2.0% agar solution prepared in desired culture medium is poured in autoclaved petridishes and kept ready. The heat liable nutrients are to be poured after cooling. The algae from the sea water suample is concentrated either by sedimentation or by centrigugation and examined for the presence of the desired alga. In case the wanted alga is present a drop of algal concentrate is taken in a platinum wire loop and streaked over the agar in a zigzag pattern. Then the petridishes are inverted and kept under or above cool white fluorescent light. Dust free air conditions rooms are preferred. The petridishes are periodically examined for growth. Different algae are distinguishable in the form of colonies. Each clean patch or algal cells are picked up with a sterile wire loop and second agar plating is done. This process is continued until a single species of algal cells are obtained. Finally this pure culture is transferred to the liquid media. Instead of petridishes slant cultures can also be done. Other methods of isolation and purification are pipette method and by using antibiotics.