Live algal feed and their culture

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Live feed algae are the microscopic plant life of any water body which forms the primary producers in an aquatic ecosystem. They form the basic live feed to all the zooplankters and larval forms of fin fishes and shell fishes. Indian coastal waters contain diverse groups of microalgae or phytoplankton such as diatoms, dinoflagellates, blue green algae, silicoflagellates and cocolithophores together constitute nearly 95% of primary production in the marine ecosystem. A handful of diatom and dinoflagellate species are toxic to marine animals and humans. Species such as *Pseudo- nitzschia* is known to cause Amnesic Shellfish Poisoning (ASP), *Pyrodinium bahamensei* and *Gymnodinium spp* are known to cause Paralytic Shellfish Poisoning (PSP) and *Prorocentrum lima* and *Dinophysis* spp are known to cause Diarrehetic Shellfish Poisoning (DSP). In the seawater, the diatoms and dinoflagellates are the most obvious representatives of phytoplankton in terms of both cell size and availability.

In mariculture these microalgae form the main live feed organisms during the early developmental stages of commercially important marine fin fishes, Crustaceans and Molluscs. With the rapid increase in aquaculture production, there is an ever-growing interest in live feed culture. Hence marine hatcheries maintain stock cultures of diatoms and flagellates for producing mass cultures for feeding requirements. The marine hatchery complex of Calicut Research Centre of CMFRI maintains the stock cultures of following species of live feed organisms.They are:

Sl no.	Group	Species
1	Chlorophyceae	*Chlorella vulgaris*
2	do	*Dunaliella salina*
3	do	*Nannochloropsis oculata*
4	do	*Nannochloropsis salina*
5	do	*Chlorella sp* (freshwater strain)
6	Bacillariophyceae	*Chaetoceros calcitrans*
7	Haptophyceae	*Isochrysis galbana*
8	do	*Dicrateria gilva*
9	Prasinophyceae	*Tetraselmis gracilis*

Basic biochemical constituents in some live feed organisms

Protein, aminoacids, fattyacids and carbohydrate composition of microalgal species are qualitatively similar but are markedly different quantitatively. It is observed that when the cultures of *Isochrysis, Tetraselmis, Dunaliella, Chlorella, Nannochloropsis* and *Chaetoceros* were maintained in Walne's medium in sterilized seawater of 30-32 ppt with a light intensity of 1500 lux from fluorescent lamps for a photoperiod of 14/10 hours of dark and light regime, *Isochrysis* recorded higher level of protein, *Chaetoceros* recorded higher levels of soluble sugars and lipids and *Chlorella* registered higher level of free aminoacids than the other strains.

Table to show levels of basic biochemical constituents (mg/g dry weight) in six cultures of live feed organisms

Species	Protein	Soluble Sugars	Lipids	Free Aminoacids
Isochrysis	59.62	26.52	14.43	2.57
Tetraselmis	51.54	17.15	10.54	2.73
Dunaliella	42.16	12.28	07.20	2.04
Chaetoceros	56.37	33.62	16.71	3.05
Chlorella	54.11	30.65	10.23	4.03
Nannochloropsis	49.67	15.20	11.28	3.11

These levels are subjected to vary with the culture conditions and age of the growing cells. A ratio of protein: carbohydrate: lipid approximately 4:3:1 is suitable live feed for zooplankton and bivalve larvae. Normally flagellates form the basic live feed for very early larval stages and diatoms for the post larvae of fin fishes and shell fishes.

Culture methods

In the sea microalgae being autotrophs, grow and multiply by availing the nutrients dissolved in the seawater such as nitrates (N) and phosphates (P) in the ratio of 10:1. However, in cultures as their number in unit volume is more, extra nutrients (essentials) should be supplemented. This nutrient rich solution diluted in filtered and sterile seawater is known as culture medium. Besides these essential nutrients, growth promoters such as trace elements and vitamins (optional) are also required.

For the **stock culture** of micro algae, various culture media are prevalent such as:

Miquel's medium

A.	Potassium nitrate	20.2 gm
	Distilled water	100 ml
B.	Sodium orthophosphate	4.0 gm
	Calcium chloride	2.0 gm
	Ferric chloride	2.0 gm
	Hydrochloric acid	2.0 ml
	Distilled water	100ml

0.55 ml of solution A and 0.50 ml of solution B are added to one litre of filtered (0.45 μ) and sterile seawater.

Schreiber's medium

Potassium nitrate	0.1 gm
Sodium orthophosphate	0.02 gm
Garden soil extract	50 ml (1 kg garden soil boiled in one litre freshwater, Supernatant is decanted)

Volume made upto one litre by adding filtered and sterile seawater.

TMRL medium (TungKang Marine Research laboratory)

Potassium nitrate	10.gm dissolved in 100 ml distilled water
Sodium orthophosphate	1.0gm dissolved in 100 ml distilled water
Ferric chloride	0.3 gm dissolved in 100 ml distilled water
Sodium silicate	0.1 gm dissolved in 100 ml distilled water

One litre of this medium is prepared by adding one ml each of the above solutions to filtered and sterilized seawater.

Walne's or Conway medium

A.	Potassium nitrate	100 gm
	Sodiumorthophosphate	20 gm
	EDTA (sodium salt)	45 gm
	Boric acid	33.4 gm
	Ferric chloride	1.3 gm
	Manganese chloride	0.36 gm
	Distilled water	1 lit
B.	Zinc chloride	4.2 gm
	Cobalt chloride	4.0 gm
	Ammonium molybdate	1.8 gm
	Distilled water	1 lit
C.	Vitamin B_1 (Thiamine HCL)	200mg dissolved in 100ml dist water
	Vitamine B_{12}(Cyanacobalamine)	10 mg dissolved in 100ml dist water

One litre of medium is prepared by adding together one ml of solution A, 0.5 ml of solution B and 0.1 ml of solution C and final volume made up to one litre with filtered and sterilized seawater.

Mass culture

While stock cultures of phytoplankton is maintained in indoors and in controlled conditions in Haffkine flasks, large volumes of the mass cultures are maintained in open condition utilizing sunlight in fiberglass tanks and raceways.

Culture medium for mixture of live feed algae

Potassium nitrate	-	1.20 gm dissolved in 25 ml distilled water
EDTA (Na)	-	0.66 gm dissolved in 25 ml distilled water
Sodium silicate	-	0.66 gm dissolved in 25 ml distilled water
Sodium orthophosphate	-	0.66 gm dissolved in 25 ml distilled water

Mix together all the above solutions in 100 litre of filtered seawater and give continuous aeration.

Similarly cowdung, vegetable- oilcakes and inorganic fertilizers such as urea, potash and superphosphate can be used to prepare media suitable for mass culture of live feed organisms (Natarajan *et al.*, 1997. *Mar. Fish Infor Serv.*, T & E *Ser.*, Vol.149).

Culture systems

There are three major culture systems of phytoplankton as described by Coutteau (Coutteau, P., *FAO Fishereis Technical Paper*, 361: 7-47) such as:

Batch culture system	Entire culture is harvested and used as feed. Cultured in small containers.
Semi-continuous culture system	Only a part of the culture is harvested for feeding and the quantity harvested is replaced with fresh medium and the process is repeated every 3rd or 4th day when the culture density improves.
Continuous culture system	Here the number of growing cells in the culture is constantly monitored. As the culture progresses a control system keeps the culture density at a preset level by dispensing fresh culture medium

Growth phases of culture

From the stock culture of live feed species, an inoculum is introduced to large volume of culture medium and is exposed to suitable conditions such as light, temperature, pH and aeration. The cells start to divide once they come in to contact with the minerals and nutrients present in the fresh medium. Hence their growth pass through four basic growth phases such as Lag phase, Log phase, Stationary phase and Death phase.

Lag phase: This is an induction phase or acclimation phase as the cells introduced to new medium has to acclimatize in the new micro environment. This lasts for a few hours.

Exponential phase: This is an active growing phase and also known as *Log phase*. Once these cells got acclimatized they start divide and multiply till the nutrients added to the medium is exhausted, the culture conditions are altered or the culture reaches optimum density.

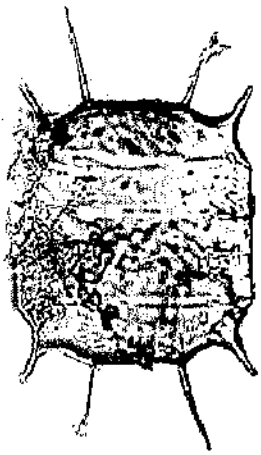
Stationary phase: This is the declining phase of the culture and is when cell death equals new cells and the metabolic activity of cells decline. Once the cell multiplication reaches a maximum and the available nutrients deplete growth of the culture declines as the cell division and multiplication

reaches a halt. During this phase, to tide over adverse culture conditions the cells may develop cyst or some protective cover around its body and remain inactive. The culture will go to the previous phase if nutrients or fresh medium is supplemented or the culture conditions turn favourable.

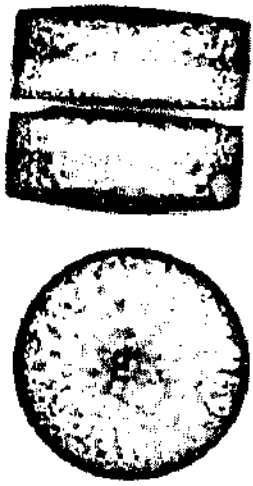
Death phase: If the stationary phase is prolonged for a long time they tend to lose their viability and start dying. Such cultures can't be revived.

References:

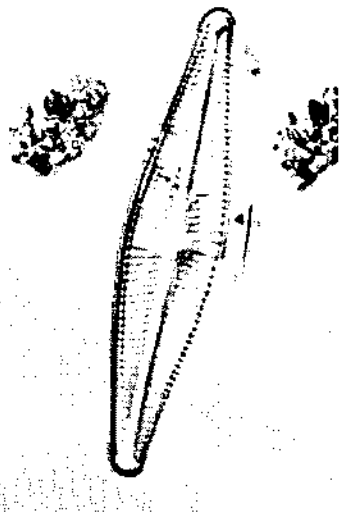
1. Coutteau, P., *FAO Fishereis Technical Paper*, 361: 7-47
2. Natarajan *et al.*, 1997. *Mar. Fish Infor Serv.*, T & E *Ser.*, Vol.149.



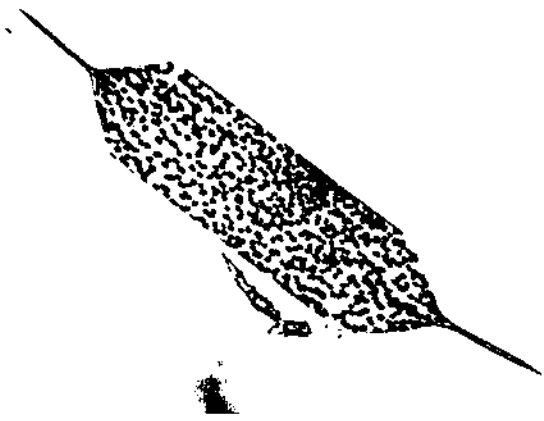
Biddulphia sp



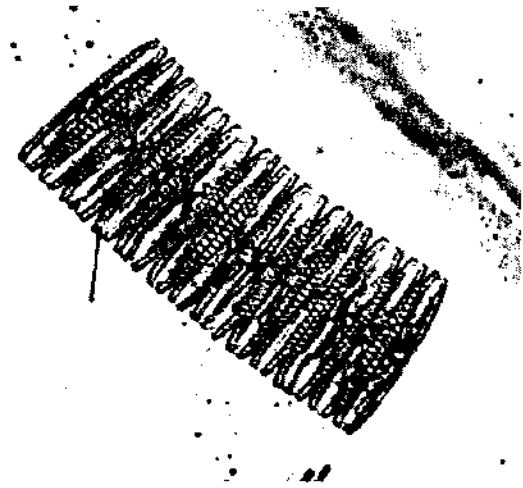
Coscinodiscus sp



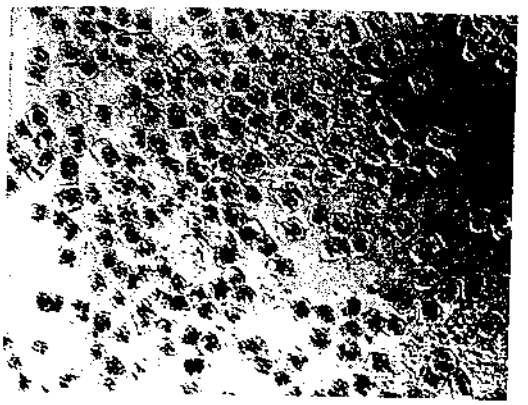
Navicula sp



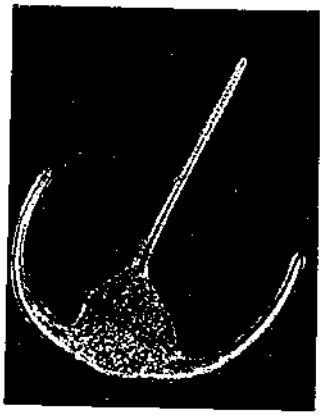
Rhizosolenia sp



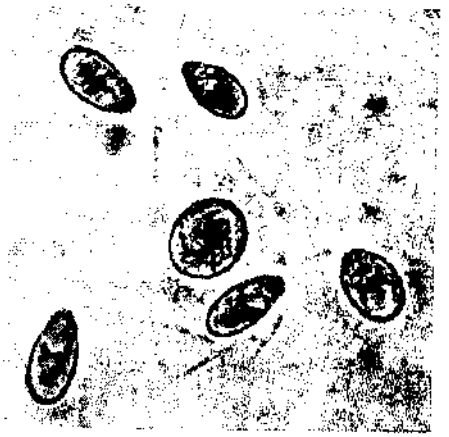
Fragillaria sp



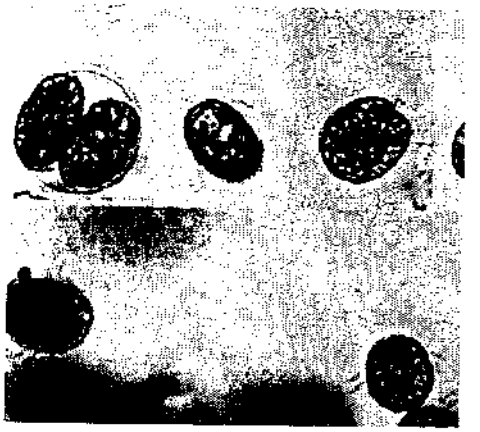
Isocrysis galbana



Ceratium sp

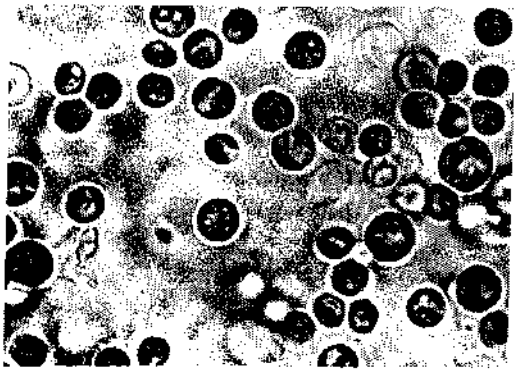


Dunaliella salina



Nannochloropsis sp.

Tetraselmis sp



Chlorella sp