

## Micro Algae Culture as Live Feed

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The early life stages of every cultivable organism in a hatchery system depend on the feed provided. Micro algae form the basic food for all the larval stages of crustaceans, molluscs, sea cucumber and finfishes. Even though, large number of micro algae is identified, only a few are used as live feed. Identification, isolation and the maintenance of stock culture and mass culture of required micro algae as feed is the prior task in every hatchery system. Moreover, the preference of the organisms varies with species and in its different stages of life cycle. Here, we try to discuss the present status, methods and protocols adopted for the production of micro algae as live feed, for the benefit of aquaculture.

**Key words:** Culture media; feed protocol; isolation; indoor and out door mass culture; micro algae

In recent years rearing techniques for economically important brackish water and marine organisms and method of mass culture of micro algae as live feed have advanced markedly to keep pace with ever increasing commercial fish production. In India, the need for culture of micro algae is increasing day by day, since it play a significant role in any type of hatchery operation. It is an established fact that the micro algae, especially nanoplankton forms the basic food of almost all the larval stages of crustaceans, molluscs and fishes. In nature, the larvae feeds on whatever the micro algae present in their environment. But in a hatchery, the required species of micro algae have to be identified, isolated, maintained in laboratory conditions and cultured in indoor and outdoor facilities and supplied in required concentration to feed the rearing larvae.

The success of aquaculture depends on a host of factors of which the techniques for production of micro algae in hatchery system for the production of quality seeds are vital. The prime requirement of aquaculture practice is the production of appropriate nutritionally balanced, non-polluting, economically feasible and readily acceptable micro algae which will ensure maximum survival and optimum growth of the cultivable organisms (Pandian and Peter Marian, 1991). During their development, the larvae of prawns and fishes require different sized micro algae. The prawn larvae prefer diatoms (species of *Chaetoceros*, *Skeletonema* and

*Thalassiosira*) as primary food while the molluscs (oysters, clams, mussels, etc..) feeds on nanoplankton flagellates (species of *Isochrysis*, *Pavlova*, *Dicrateria*, *Chromulina* and *Tetraselmis*) and other nanoplankters (species of *Chlorella*, *Nannochloropsis* etc.). It is therefore necessary to develop methods for isolation, maintenance, and indoor and outdoor mass culture of a variety of micro algae.

Research related to the mass culture of micro algae presently under way in many Universities and Research institutions are directed towards the solution of several key problems such as: (i) isolation and maintenance of selected species of micro algae (Table 1) (ii) small and large scale culture of micro algae under controlled conditions and its constant supply in different phases of growth (iii) developing viable methods for the intensive culture of micro algae as well as its harvest and preservation during optimum and peak densities and (iv) developing viable methods for the production of economically feasible micro algae feed which will provide the nutritional needs of the rearing larvae (Table 2) in a hatchery system.

Table 1 — Micro algae culture- important species used as live feed

Algal Classes	Microalgae
Chlorophyceae	<i>Chlorella</i> spp., <i>Nannochloropsis salina</i> , <i>Tetraselmis</i> spp., <i>Dunaliella</i> spp.
Bacillariophyceae (Diatoms)	<i>Chaetoceros</i> spp., <i>Skeletonema costatum</i> <i>Thalassiosira subtilis</i>
Chrysophyceae (Golden yellow flagellates)	<i>Dicrateria inornata</i> <i>Chryptochrysis</i> sp
Haptophyceae (Golden brown flagellates)	<i>Isochrysis galbana</i> , <i>Pavlova lutheri</i> <i>Chromulina frieburgensis</i>
Cyanophyceae (Blue green algae)	<i>Spirulina</i> spp (fresh water forms) <i>Synechocystis</i> spp.
Mixed culture (for feeding post larvae and spat)	Phytoflagellates, Nanoplankters, Centric and Pennate diatoms

Table 2 — Feeding protocol of micro algae in various hatcheries

Growth stage of animal	Microalgae feed
<b>Prawn Hatchery</b>	
Zoea I to Mysis I	<i>Chaetoceros</i> spp, <i>Skeletonema costatum</i>
Mysis II to post larvae V	<i>Chaetoceros</i> + <i>Tetraselmis</i> + Rotifer + <i>Artemia</i> cysts
PL V to PL XX	Mixed culture + Rotifers + <i>Chaetoceros</i> spp + <i>Artemia</i> cysts
<b>Fish Hatchery</b>	
Larvae to post larvae	Phytoflagellates + Diatoms + <i>Artemia</i> cysts + <i>Spirulina</i> powder (for fresh water forms)

Contd.

**Molluscan Hatchery**

'D' shape to spat

*Isochrysis, Pavlova, Dicrateria, Chromulina  
Chaetoceros spp, Skeletonema, Thalassiosira,*

Spat to juvenile

*Tetraselmis + other flagellates*

Juvenile to adult

Mixed culture dominated by diatoms, *Chlorella,*  
*Tetraselmis etc.***Sea cucumber Hatchery**

Larvae to auricularia stage

Phytoflagellates + Nannoplankters

Auricularia to pentacula

*Chaetoceros + Mixed culture*

Pentacula to young one

Algal powder + detritus

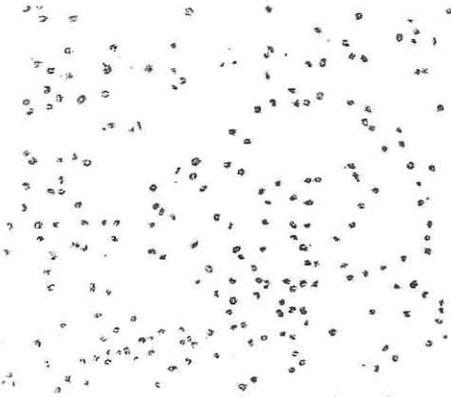
In India, micro algae culture was developed during 1950's by the workers of Madras University under the guidance of Prof. M.O.P. Iyengar. Initially the culture experiments were related to purely academic interest. Later when aquaculture activities were developed, micro algae culture was an essential item for all types of rearing/feeding experiments. When mariculture programmes were initiated by Central Marine Fisheries Research Institute (CMFRI) during 1980's, almost all the Research Centers were having a small unit of micro algal culture depending on the animals available in the locality. Nowadays, culture of micro algae, as live feed is a pre-requisite in all the mariculture activities of this Institute.

**Background Information**

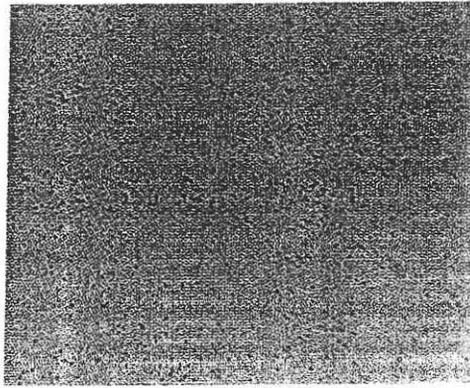
Mass culture of micro algae has been in prevalence in many Research Institutions, Universities and hatcheries world over, since the past 50 years. For many years, this has been experimented by plant physiologists, algologists and bioengineers as an alternate means of producing protein. But in the late 60's the enthusiasm was dampened when it was found that the production of protein was uneconomical because of the technical problems involved especially in the recovery of the algal product and its subsequent conversion to a human food supplement (Fogg, 1975). Recently, there has been renewed interest in producing single cell protein (SCP) by mass culturing the unicellular algae for feeding the larvae of crustaceans, molluscs, sea cucumbers and fishes. In recent years, many other potential applications for large scale culture of micro algae have been advanced including waste water treatment, production of extractable commercial chemicals, pharmaceuticals, life support systems, aquaculture and the bioconversion of solar energy.

**Methods**

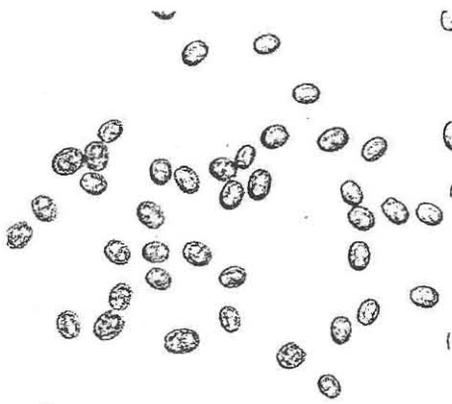
The various aspects of micro algae culture are: identification and isolation of required species (Plate 1), preparation of suitable culture media, maintenance of stock culture, indoor and outdoor mass culture (Plate 2), supply of the culture in required quantities after determining the cell concentration and harvest and preservation of the culture for future use.



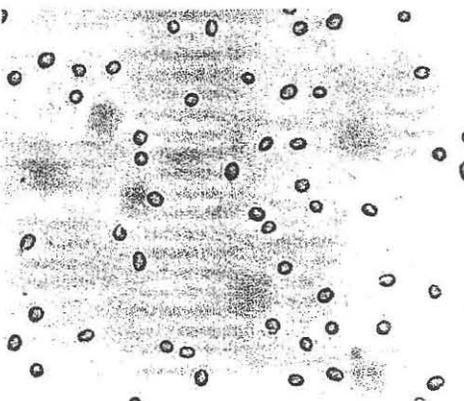
*Chaetoceros calcitrans*



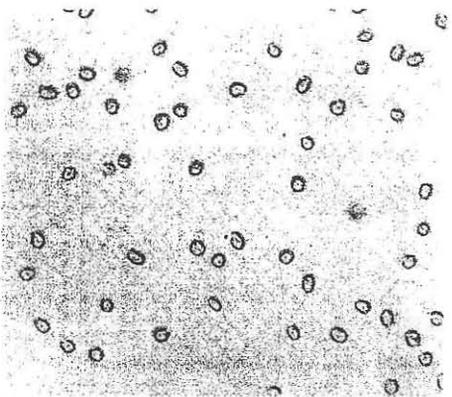
*Chlorella marina*



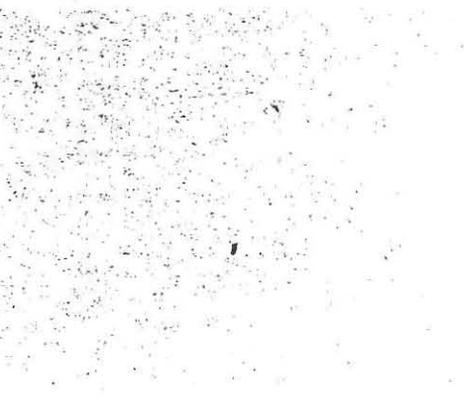
*Tetraselmis gracilis*



*Dicrateria inornata*

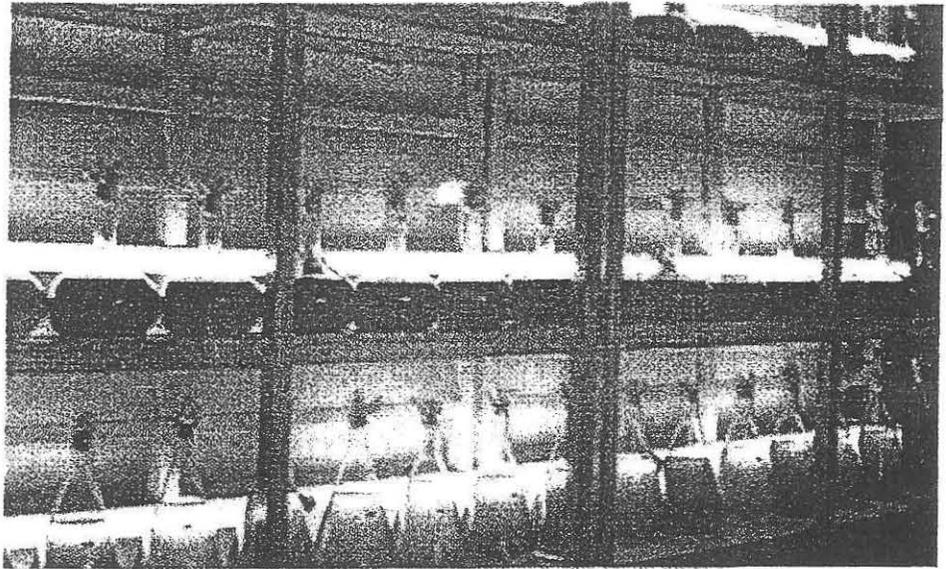


*Isochrysis galbana*

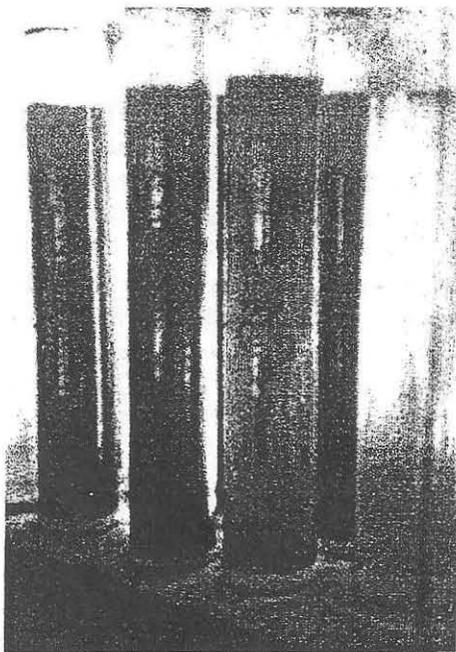


*Nanochloropsis salina*

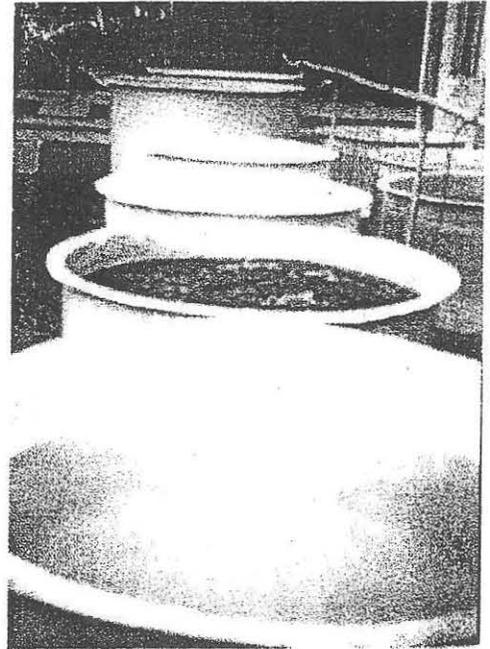
Plate 1 — Photographs of different microalgae used as live feed



Stock Culture



Indoor mass culture



Outdoor mass culture

**Plate 2 — Maintenance of stock culture, indoor and outdoor mass culture of microalgae**

### ***Isolation***

Isolation of suitable species of micro algae can be done by (i) Pipette method (ii) Centrifuge or washing method (iii) by exploiting the phototactic movements (iv) by Agar plating method and (v) by Serial dilution culture technique (Sournia, 1971; Gopinathan, 1982, 1996). After the isolation of the organisms in small culture tubes (15 ml), it is sub-cultured again in 50 ml, 250 ml conical flasks for its further purification. Once the cultured is purified in 250 ml conical flasks, slowly increase the volume by transferring into 1 litre and later into 3 or 4 litre Haufkin culture flasks for keeping as stock culture. Filtered and sterilized seawater should be enriched with suitable culture media not earlier than 3 days prior to inoculation.

### ***Culture Medium***

The culture of planktonic micro algae to be used as food for oysters and other filter feeders have initiated in the late 19<sup>th</sup> century. Miquel (1892) grew a few diatoms in his laboratory in the early 1890's, making the observations that water from lakes, ponds and the sea were unable to support much growth unless they were enriched with mineral solution. Continuous cultures of micro alga, i.e., culture that are partially harvested while new medium is added, were first attempted in Czechoslovakia during the early 20<sup>th</sup> century (Landau, 1991).

For the successful culture of the micro algae, various culture media have been used depending on the organisms to be cultured and their growth phases. Although most algae are photoautotrophic and can grow in purely inorganic media, other prefers organic compounds and for many, they are stimulatory. Though Erd-Schreiber's and Miquel media were found to be very effective for culturing diatoms and other nanoplankters, several other media have come into existence with the addition of trace metals, vitamins and other organic and inorganic salts. Conway or Walne's medium (Walne, 1974) and Guillard 'f' medium contain all ingredients and are widely used in the laboratory maintenance and mass culture programmes (Gopinathan, 1996). Besides, the laboratory prepared chemicals, which serve as nutrients; it is economic to use commercial fertilizers also for the mass production of nanoplankters in outdoor open tanks.

### ***Growth Phase***

Increase in cell numbers of micro algae culture follows a characteristic pattern of growth in which the culture may be recognized as (i) Lag or induction phase (ii) Exponential or growing phase (iii) Declining phase (iv) Stationary phase and (v) Death phase (Gopinathan, 1982). For feeding the larval forms, the culture should be given during its exponential phase only otherwise metabolites of the micro algae such as oil and fat excretory will be more and that will affect seriously on the rearing organisms.

### ***Microalgae Stock Culture Maintenance***

Stock culture of different species of micro algae is to be maintained in a special room adjacent to the mass culture laboratory. The heated or sterilized seawater after cooling may be poured into the Haufkin culture flasks and the required nutrients are added. Walne's medium enriched with vitamins and silicate is the best medium for maintaining the diatoms and without silicate for other micro algae. About 10 ml of the inoculum (1 million cells/ml) in the growing phase of the culture is transferred to the culture flasks and placed in front of 2 tube lights (1000 lux) for about 5-6 days at temperature of 25 °C. When the maximum growing phase has reached, only one tube light is necessary for further growth of the culture. In the stationary phase of most species of micro algae under controlled conditions of temperature and light, the stock culture can be kept for nearly 2 months.

### ***Indoor and Outdoor Mass Culture***

The containers for the indoor mass culture of micro algae are: Glass carboys, Polythene bags, transparent Perspex tanks, transparent cylindrical FRP tanks and one ton capacity FRP tanks (inside pure white). These containers can be kept in wooden racks and frames providing light and aeration facilities. For the outdoor culture, the containers are 1-5 ton capacity FRP tanks and 5-10 ton capacity concrete tanks. Fully grown culture from the stock culture room is used as inoculum for the mass culture in the containers. Required quantities of chemical/fertilizer may be added. These containers will have the maximum concentration of the cells with growing phase on 3-4<sup>th</sup> day and before going to the declining phase, it should be harvested. After estimating the cell concentrations, the culture can be supplied to the hatchery for feeding the rearing larvae (Figure 1 and 2).

### ***Harvest and Preservation***

The fully-grown culture should be harvested during the growing phase of the micro algae after determining the cell concentration with the help of a Haemocytometer or Sedwig-Rafter counting cell. If the culture has entered the declining or stationary phase of growth, the metabolites will be very high and the cells may not be in healthy condition. The culture can be preserved either by freezing or drying employing the technique of flocculation (Gopinathan, 1993) to overcome the unfavourable condition in the hatchery.

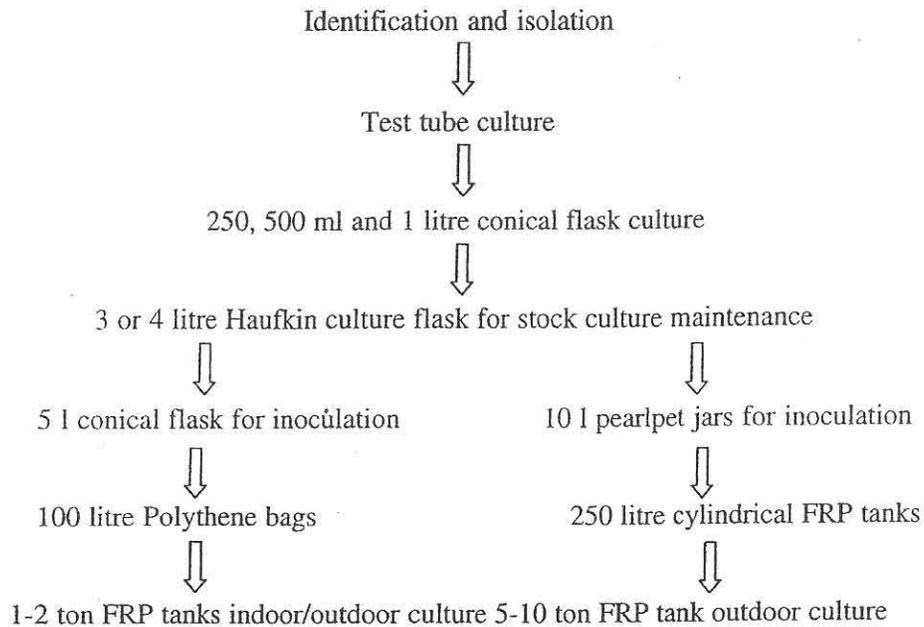


Fig. 1 — Procedure of Micro algae mass culture

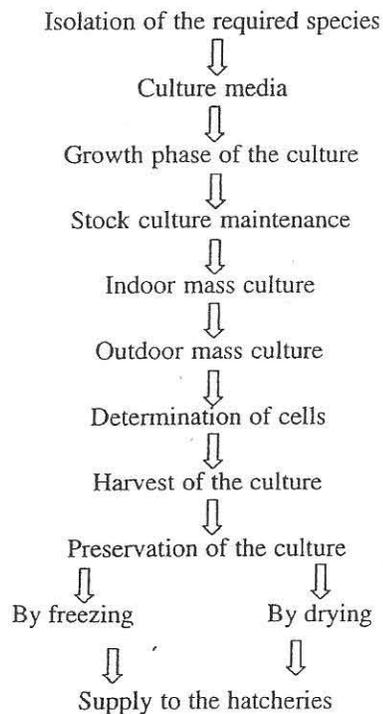


Fig. 2 — Production of micro algae culture. Various stages.

The important species of micro algae used as live feed, procedure for their mass culture and the feeding protocol in various hatcheries are presented in Table 1& 2 (Girijavallabhan *et al.*, 2000).

### ***Problems and Prospects***

Large-scale culture operations with micro algae and other live feeds have been carried out intensively in most of the hatcheries in India. Today, numerous algal waste water treatment systems are in operation in different parts of the world and a few commercial ventures exist that involve the production of high priced protein food derived from micro algae.

For the mass culture of micro algae (*Chlorella*, *Nannochloropsis* and *Isochrysis*) effluents from different marine grow out systems were tried on preliminary scale in CMFRI as culture medium. Based on the encouraging results, a well laid out experiment is in progress to arrive at the best medium for mass culture of the micro algae, which will in turn contribute, to its sustainable production with economic viability. This will substitute the costly inputs.

The basic problems faced in any type of hatchery operation are to supply enough feed of the best quality at the lowest cost. In this, three points can be considered; (i) Algal production is available to technological approach (ii) Product quality can be manipulated and (iii) Additives may improve utilization efficiency, increase growth rate and thereby decrease cost.

It is significant that the prime goal of all mass culturing ventures is to maximize photosynthetic conversion efficiencies and to optimize the production of plant material. To achieve this on a large scale, it is necessary to take advantage of available solar energy. However, algal mass culture system when industrialized would pose many bioengineering problems.

### ***Recent Technological Advances***

The high costs associated with algal production, the risks for contamination and temporal variations in the algal food value still pose problems for any aquaculture operation depending on the mass culture of unicellular algae. In order to overcome or reduce the problems and limitations associated with algal cultures, various investigators have attempted to replace algae by using different artificial diets as a supplement or as the main food source. Different approaches are being applied to reduce the need for *in situ* algal production including the use of preserved algae, micro encapsulated diets and yeast based feeds.

In recent years, the requirement of live algae in the mass production of prey organisms has been slightly reduced in this way. Baker's yeast, marine yeast and lipid enriched yeast diets are now routinely used as sole diet or in combination with *Chlorella* for rearing rotifers. In addition, considerable progress has been made in the replacement of live algae in the larval rearing of commercially important shrimp species. Partial replacements of live algae in the larval rearing of commercially

important species by using micro encapsulated and yeast based diets is now routine in hatcheries for penaeid shrimp.

### *Future Plans*

Looking into the future, we may forecast some of the long term effects of the culture of micro algae. Assuming that half of the per capita requirement of 65 gms of protein per day was to be obtained from micro algae, the total area required for algal culture would be less than a million acres for the present world population.

The development of the culture of micro algae offers immense scope and should run concurrently with mariculture programme. At the same time, it offers a challenge for the phycologists. Considerable research would be needed for elucidating the problems of growth kinetics of different species and also the period of economically viable harvest. In the matter of wastewater recycling, unicellular algae hold out much promise. Maximization of solar energy utilization under controlled conditions of light, temperature, pH and aeration, development of viable methods for intensive culture, maintenance, formation of extracellular products and their significance are promising lines of investigations.

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