



LIVE FEED

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P R E F A C E

INDAQUA is India's prestigious, international aquaculture show organised every two years with the intention of creating awareness about the vast unexplored potentials of Indian aquaculture and focusing on the latest developments in the field. It is also intended to serve as a medium for projecting the developments in Indian aquaculture scene to the global markets, thereby serving to attract more and more buyers for our aquaculture products.

On the occasion of the first INDAQUA in March 1993, MPEDA had brought out 15 handbooks on aquafarming highlighting the culture techniques of almost all the important candidate species for aquaculture in India. It was gratifying to note that the publications were well received by the farmers, entrepreneurs, students and others interested in aquaculture. Responding to the persistent demand for copies of these publications from different quarters, we are bringing out reprints of these handbooks.

I hope that these handbooks will be found very useful by all.



Cochin - 682 036
January, 1995

(K. B. PILLAI)
Chairman, MPEDA

FOREWORD

Live Feed such as diatoms, artemia, rotifers, etc., play a vital role in the hatchery production of seeds. Without these live feeds, it is not possible to achieve good survival rate. India has immense potential to produce many kinds of live feeds and unfortunately the commercial production on a regular basis is yet to make a beginning. Therefore, the Organising Committee of the "INDAQUA" - the first Aquaculture Show in India decided to bring out this handbook for the benefit of hatchery operators. The sincere effort put up by Dr. C.P. Gopinathan, CMFRI, Shri. B. Vishnu Bhat, MPEDA and Dr. Peter Marian of IART deserves special appreciation. The publication work was coordinated by Dr. G. Santhana Krishnan and Shri. M. Viswakumar, of MPEDA. The financial assistance extended by the Ministry of Food Processing Industries - Fisheries Division, Government of India is deeply acknowledged. I am of the opinion that this handbook will be very useful to all hatchery operators in India.



M. Sakthivel
Chairman
MPEDA

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PART I
MICRO-ALGAL CULTURE

MICRO - ALGAL CULTURE

By

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The floating microscopic plants or the phytoplankton are the micro-algae which form the basic food of almost all the animals in aquatic ecosystem. Most phytoplankton organisms are unicellular and are the primary producers of organic matter in aquatic habitats. The scope of micro-algae as a possible source of protein food was recognised by the researches in the middle of the 20th century. In the past, the main attention has been on Single Cell Protein (SCP) production for human consumption and later many new applications have been evolved including waste-water treatment, water renovation, nutrient recycling, closed life-support systems, aquaculture and the bio-conversion of solar energy.

In recent years, there has been renewed interest in producing Single Cell Protein by mass culturing the unicellular micro-algae such as diatoms (species of *Chaetoceros* and *Skeletonema*) and nannoplankters (species of *Isochrysis*, *Tetraselmis* and *Chlorella*) for feeding the larvae of Crustaceans (prawns), Molluscs (oysters and clams) and fishes. As is well known, the success of any hatchery operation depends mainly on providing the required species of micro-algae. The larvae of prawns and fishes prefer the diatoms as the basic food while the molluscs live on the nannoplankton flagellates measuring less than ten microns, during the critical larval stages. Hence the culture of micro-algae is an essential pre-requisite for the rearing operations of shell-fishes and fin-fishes in a hatchery system.

Present status of knowledge

Realising the importance of diatoms and other nannoplankton flagellates as the essential food of the larvae of crustaceans, molluscs, fishes and sea cucumbers, the isolation and identification, maintenance of stock culture, laboratory and outdoor mass culture and large-scale open tank culture of these

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micro-algae are being carried out in various hatcheries in Japan, Taiwan, China, Philippines, Indonesia and India. The Molluscan hatchery at Tuticorin is one among them where rearing operations of oysters, clams, mussels and sea cucumbers are being carried out at present. Here separate rooms are provided for keeping the stock culture and mass culture of micro-algae. In the stock culture room, which is smaller than the mass culture room, maximum cleanliness is looked after to avoid any contamination. Even the chokes of the tube lights are kept outside to maintain the optimum temperature (23°C) in uniform levels. One air conditioner (2 ton capacity) is used in the stock culture room while 2 air conditioners are fitted in the mass culture room. Since the oyster larvae can ingest nothing larger than 10 μ and appear to rely for food on phytoflagellates belonging to the algal Classes, Chrysophyceae, Haptophyceae and Cholophyceae, only these forms have been isolated and maintained in good condition in the stock culture room.

Isochrysis galbana and species of *Pavlova*, *Dicrateria* and *Chromulina* are being used as food for the larvae of Pearl and edible oysters, clams, mussels and sea cucumbers upto the spat stage. Once the larvae settle as spat, they can be fed with mixed culture of micro-algae comprising mostly of diatoms, dominated by the species of *Chaetoceros* and other nannoplankters.

The various aspects of the micro-algal culture are : the isolation of required species, identification, preparation of culture media, stock culture maintenance, mass culture and harvest and preservation of the culture.

Isolation :

Isolation of the micro-algae can be done by one of the following methods.

1. *Pipette method* : Large organisms can be pipetted out using a micro-pipette under microscope and transferred to culture tubes which are having suitable culture medium.
2. *Centrifuge method* : By repeated centrifuging of the sample and by inoculating the deposits, we may get different organisms.

3. *By exploiting the phototactic movements* : By this method, most of the phytoflagellates can be isolated. Make a dark chamber with a small hole on one side and keep a candle outside, near to the hole. Pour the water sample in a beaker and keep inside the dark chamber, near to the hole. Since the flagellates have a tendency to move towards the light, after sometime, it is visible that these organisms could be crowded near to the candle light. By pipetting, we can separate these organisms and by culture tube method, can be raised to a pure culture.

4. *By agar plating method* : For preparing the agar medium, 1.5 gm of agar is added to a litre of suitable medium or even natural seawater. This agar solution is sterilized in an autoclave for 15 minutes under 150 lbs pressure and 120°C temperature. Now this medium is poured in sterilized petri-dishes and keep for 24 hrs. For the isolation, the required species can be picked up by platinum needle under microscope and streaked on the surface of the agar plate. After inoculation, these petri-dishes are placed in an incubation chamber for 7-8 days providing 1000 lux light and constant temperature (25°C). Within this time, the required species, if it has grown into a colony, could be removed by platinum loop and transferred to culture tubes. On further transfer from the culture tubes to small conical flasks and larger flasks, the algae can be grown on a mass scale.

5. *Serial dilution culture technique* : This method is used mainly for the isolation of phytoflagellates. In this method, 5 dilution steps (the inocula corresponding to 1, 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} or 4 steps - 0.001, 0.01, 0.1 and 1 ml) are involved for the isolation of the required species. For the serial dilution technique, nearly 40 culture tubes (15 ml) are required. After filtering the seawater through 10 microne seive, the filtrate has to be inoculated in five series of culture tubes in various concentrations. This has to be kept under sufficient light (1 k lux) with uniform temperature (25°C) conditions. After 15 days, some discolouration can be seen in the culture tubes, due to the growth of micro-algae. Further purification of this culture can be done by sub-culturing it in 50 ml conical flasks and then in 500 ml and one litre conical flasks. Once the culture is fully

purified, it can be transferred into a 3 or 4 litre Haufkin Culture Flasks and maintained as stock culture.

After the isolation of the required species in culture tubes, it may be sub-cultured again in few 50 ml test tubes. These test tubes are the base from which the algal food starts producing and from where the continuous supply of non contaminated algal food is obtained for the operation of the large-scale culture systems. Once the system is started, the test tube culture can be transferred to small culture flasks and in bigger flasks by adding 2-3 ml of the stock culture. Thereafter, every two weeks a new set of 10 test tubes for each species should be inoculated from the previous set. The filtration of water and medium enrichment should be done not earlier than 2 days, prior to inoculation.

Culture Media or Culture Solutions

For successful culturing of micro-algae, either diatoms or nannoplankters, various chemical culture media have been used depending on the type of organism cultured and their growth phases. Since the micro-algae in any water body require the nutrients such as nitrates and phosphates roughly in a ratio of 10 : 1 (N : P) for its normal growth and reproduction, the culture media used in the laboratory should have sufficient quantities of these elements besides other growth promoting substances including trace metals and vitamins. The absence of one or two growth promoting agents would definitely reflect on the growth of micro-algae especially in a culture system.

Though in earlier periods, Erd-Schreiber's and Miquel's medium were found to be very effective for culturing the micro-algae, several other media also came into existence with the addition of trace metals, vitamins and other organic and inorganic salts. Since the diatoms require silica for building up the cell walls, the culture media have a silicate compound besides the nitrate, phosphate, chlorides and trace metals. Similarly, for the nannoplankton flagellates, trace metals and vitamins are required for their growth and multiplication. Usually for culturing the flagellates, 'Conway' or Walne's medium is used in the laboratory for the maintenance of stock

culture as well as mass culture. Since this culture medium has got various chemicals, trace metals and vitamins, the phytoflagellates such as species of *Isochrysis*, *Dicrateria*, *Chromulina*, *Pavlova* and *Tetraselmis* are being cultured by using this medium alone. Still, the technique of culturing different algae require a clear understanding of their nutritional requirements, especially during the various phases of growth. The important culture media used for the culture of micro-algae are :

1. Miquel's medium

A. Potassium nitrate -20.2gm	B. Sodium orthophosphate-4gm
Dist. water-100ml	Calcium chloroxide-2gm
	Ferric chloride-2gm
	HCl-2ml
	Dist.water - 100 ml

Add 0.55 ml of 'A' and 0.5 ml of 'B' to one litre of filtered and sterilized seawater. This medium can be used for culturing various types of micro-algae.

2. TMRL (Tung Kang Marine Research Lab.) medium

Potassium nitrate: 10 gm/ 100ml of	dist. water
Sodium ortho phosphate:1gm	" " "
Ferric chloride: 0.3gm	" " "
Sodium silicate: 0.1 gm	" " "

Keep the chemicals separately in 100 ml reagent bottles. Add 1 ml of each to 1 litre of sterilized seawater. This medium can be used for the mass culture of diatoms such as *Chaetoceros* spp. and *Skeletoanema costatum*.

3. 'Conway' or Walne's medium

A. Potassium nitrate	: 100	gm
Sodium ortho-phosphate	: 20	gm
EDTA (Na)	: 45	gm
Boric acid	: 33.4	gm
Ferric chloride	: 1.3	gm
Manganese chloride	: 0.36	gm
Dist. water	: 1	litre

for the mass culture of micro-algae. In open tanks for economy purposes, the following fertilizing medium can be used:

Urea 46	:	10 mg/l
16-20-0	:	100 mg/l
20-0-0	:	10 mg/l

Growth phases of the algal culture

The usual way of the laboratory culture of micro-algae is one in which a limited volume of medium containing the necessary inorganic and organic nutrients is inoculated with a relatively small number of cells and these exposed to suitable conditions of light, temperature and aeration. Increase in cell numbers in such a culture follows a characteristic pattern in which the following phases of growth may usually be recognised.

1. *Lag or induction phase* : The cells taken from the stock culture room and inoculated to a new flask have to acclimatise the surroundings or to the new medium. So there will be no cell division for a few hours and this stage is known as lag or induction phase.
2. *Exponential phase* : Once the cells are acclimated to the surroundings, it starts multiplication and grow rapidly. It is assumed that within 12-18 hrs, the cells will divide into two and further these cells will continue to grow till the culture reaches its maximum concentration. This growing phase is known as exponential phase.
3. *Declining phase* : Once the cells reached its maximum concentration, the growth and multiplication of the cells will be arrested and slowly the cells show the symptom of decline. This stunted growth of the cells in the culture is known as declining phase.
4. *Stationary phase* : After the arrested growth, the culture will be stationary for a few days without any further cell division. Actually, stationary phase is prolonged in the case of flagellates. For this they may develop some cover or matrix around its body for thriving the unfavourable conditions. In the stationary phase, if the cells get a new

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environment, they may start further growth and reproduction.

5. *Death phase* : After a long period in the stationary phase, the cells may lose its viability and start to die and thus the culture will become useless, either for reculturing or for feeding.

Determination of algal cell densities

Regular counts of the algal cells must be made in order to schedule inoculation of the various cultures as well as mass culture containers, to monitor growth of the algal cultures and to determine the quantity of algae to be fed to the rearing larval organisms.

In sampling the mass or tank cultures, cut a length of rubber tubing comfortable to work with and place it on the mouth piece end of a sterile serological pipette. Place the other end of the rubber tubing in mouth and the pipette in the culture. To get a representative sample, move the pipette around the tank withdrawing algae upto the mark on the pipette and then place it in a flask. This is the sample to be counted.

Since most of the nanoplankters measure less than 10 microns, we have to use a haemocytometer for counting. For this, the sample has to be prepared for counting. First of all the sample has to be treated with a drop of eosin or formalin to kill the cells and after stirring well, one drop has to be taken with sterilised pipette. After placing the cover-slip on the haemocytometer, the pipette should be brought to the edge of the haemocytometer and touch it. The sample will run inside and thus we will get a thin film of the culture in which the cells should be equally distributed. Since the 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. To put it in equation form :

Average counts per chamber $\times 10^4$ = Total number of cells/ml

Stock culture maintenance

Stock culture of all the micro-algae are maintained in a special air conditioning room adjacent to the mass culture room. The autoclaved or heated seawater after cooling poured to the Haufkin culture flasks and required nutrients will be added. Walne's medium enriched with vitamins is the ideal one suitable to maintain the stock of all the phytoflagellates. About 10 ml of the inoculum in the growing phase is transferred to the culture flasks and place the same in front of 2 tube lights (1000 lux). After 8-10 days, when the maximum exponential phase was reached, the tube light can be reduced to 1 for further growth. The time required for the maximum cell concentration will vary depending on the species. However, it was noticed that all the flagellates required 2 weeks for the completion of growth phases before entering into the stationary phase. In the stationary phase, the micro-algae can be kept for a period of 2 months in the stock culture room, under controlled conditions of light and temperature, with or without aeration. The colour of the culture will turn into brown, when the culture enters into the stationary phase. It is assumed that the cells of most micro-algae form a thick matrix or cyst around it for the stationary phase and if we inoculate the same, these cysts will break and the flagellates or spores will emerge out from it for its further growth and multiplication. A minimum of 5-6 Haufkin culture flasks were required for keeping the stock culture of each species.

Mass culture of micro-algae

Large-scale culture of micro-algae, especially nannoplankton flagellates and selected species of diatoms are necessary for feeding the rearing larval forms in a hatchery. Since the molluscan larvae can feed organisms measuring less than 10 microne, these organisms have to be isolated from the seawater, maintain them as stock culture and utilising the inoculum, mass culture can be done in the laboratory conditions as well as in the outdoor 1 tonne tanks.

The containers used for the mass culture of micro-algae are 10 litre capacity polythene bags, 20 litre glass carbuoys,

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100 litre perspex tanks for the indoor culture and 250 litre or 1 tonne fibre-glass tanks for the outdoor culture. For the indoor culture, the containers are kept in wooden racks with light and aeration facilities. Fully grown culture from the stock culture room is used as inoculum for the mass culture in these containers. About 100 ml of the inoculum is used for the polythene bags, 250 ml for the glass carbuoys and 2 litres for the 100 litre perspex tanks which are properly lighted and aerated. 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 haemocytometer, the culture is supplied to the hatchery for the rearing operations of the larval organisms. Leaving 2 litres of the same culture, fresh enriched medium can be added for further culture in the same container.

Equipments and glasswares

For the identification of the micro-algae as well as for the determination of cell concentration of the culture, a good microscope is necessary. Since the flagellates are identified by noting the number of flagellae and other cell characteristics, a powerful microscope is advisable.

For the stock culture maintenance, the glasswares required are : micro-pipette, dropper with teats, reagent bottles, culture tubes, conical flasks, Haufkin culture flasks, haemocytometer etc. For the mass culture : 10 litre polythene bags, 20 litre glass carbuoys, 100 litre perspex tanks and for the outdoor culture, 250 litre 500 litre and 1 tonne fibre-glass tanks are used for the culture of micro-algae in a hatchery.

Sterilization of glasswares

The glasswares for the isolation, maintenance, laboratory culture and mass culture should be cleaned thoroughly prior to sterilization either by steam or by autoclaving. The cleaning procedure are :

1. Treat with con. hydrochloric acid and keep the same for 15 minutes.

2. Rinse 3 times with hot tap water
3. Rinse 2 times with cold tap water
4. Pour few drops of suitable soapwater (T-poll or Labolene) and rinse 6 times till the froth is completely removed
5. Rinse 2 times with distilled water.

After drying in the sunlight, the glasswares have to be kept in an oven at temperature 100°C for an hour. The culture tubes, conical flasks and Haufkin culture flasks have to be plugged with cotton before keeping in the oven.

Illumination of algal cultures

One of the most important factor in determining successful culture of micro-algae is the type of illumination. Most of the flagellates requires less light during the stationary and declining phases. Too much of light will cause the culture for the early declining. Comparing the low light levels found in natural waters, successful cultures of these micro-algae in the hatchery also requires relatively low levels of light to achieve maximum growth and optimal densities of cells.

For maintaining the stock cultures of all micro-algae during the declining and stationary phases of growth, one tube light (500 lux) is essential while for the mass culture containers, 2000-3000 lux is necessary. Twelve hours of light and 12 hours of darkness is ideal for maintaining the stock as well as mass culture which can be controlled by auto-timer control switch mechanism.

Temperature control

Normal room temperature (28-30°C) is not ideal for the maintenance and culture of micro-algae. Hence A.C. rooms are used for keeping the stock culture as well as mass culture. Both the rooms should have 23-25°C during day time. Since there is no light during night hours, A.C. can be switched off for few hours.

Aeration distribution system

Similar to light and temperature, aeration is also important for developing and maintaining healthy cultures, as well as to enhance the exponential phase of growth of micro-algae for few days more. It was noticed that if aeration has given to the mass culture tanks, culture will remain in the growing phase 3-4 days more than the tanks where there is no aeration. Moreover, aeration is more required during day time when there is light when compared to night hours. Aeration not only help the culture for oxygen deficiency but also disturb the culture always in suspension. Also, aeration will help the nutrient salts to distribute uniformly in the medium and also supplying CO₂ required for photosynthesis. Lastly, aeration will prevent the settling out of the cells at the bottom of the culture tanks and causing death due to the lack of supply of CO₂.

Anti-contamination procedure

In working with the various species of micro-algae, the most important aspect is the cleanliness of all surfaces. All work with algae should be done with one species at a time. Neither samples from, nor inoculation of culture containers containing different species of algae should be done at the same time. In addition, personal should wash their hands after working with one species and before starting to work with another species of algae. Transfer of tube cultures should take place where there is a minimal movement of air to reduce chance of contamination. All regular pipettes, glass tubing and other glasswares should be sterilized before use.

Harvest of the culture

The fully grown 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.

Preservation of the culture

The maintenance of the culture and constant supply of the same whenever required is a problem in the hatchery, especially during adverse weather conditions. In this case, the preservation of the algae either by freezing or by sun-drying could be done in the sense that during scarcity of the food, the rearing operations may be successfully controlled. For the method of freezing, the culture has to be flocculated either by adding lime or by adjustment of pH using sodium hydroxide. Usually, the addition of lime will not give satisfactory results, but manifestation of pH with sodium hydroxide is the advanced technique conducted in the laboratory. After knowing the quantity of the culture to be flocculated, measure the volume of sodium hydroxide solution needed to flocculate to get one degree raise in pH. Suppose the pH of the culture is 8.4, raise to 9.4 by adding sufficient quantity of sodium hydroxide solution. After vigorous stirring, leave the culture for one hour. After one hour, the algal mass deposited at the bottom of the tank, has to be collected in a bucket by decanting the clear water. Then bring the pH of the culture to the original level by slowly adding dilute hydrochloric acid. Now the algae is ready for freezing or sun-drying. Drying of the algae can be done by pouring the mass in white enamel trays and keeping the same in bright sun-light. If the algae has dried up thoroughly, scrap the powder from the enamel tray and keep it in glass bottles. Before freezing of the algal mass, some protective reagents like dimethyl sulphoxide or glycerol (a few drops) can be added. Then pour the concentrate into polythene bags after measuring. Label the polythene bags and keep the same in deep-freezers. The frozen algae may not have the same protein content as in the live condition. Whenever adverse condition arise, the frozen food can be used for rearing the larval organisms.

Yield per unit area

An elementary analysis of the economics of large-scale culture indicates that the total cost of the algae produced will be approximately proportional to the growth area. Hence, as a first approximation, the unit cost is inversely proportional to the yield per unit area. The yield per unit area and the cost

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per unit area are the two fundamental parameters in planning for large-scale culture.

The yield per unit area is a measure of the efficiency with which light energy is utilized by the culture. The volume and depth of culture and the concentration of algae do not have the fundamental significance that attaches to the areas illuminated. In growth experiments performed in flasks, bottles and tubes, it is often difficult to determine the exact area illuminated. Hence, many investigators have expressed yield in terms of concentration, for example, 'grams of algae per litres of culture'.

Economics

For the production of a small scale unit of micro-algal culture, the initial investment and the operational cost per month are given below :

A. Initial Investment

1. Air condition room for stock culture (12' x 18' size)	Rs. 75,000.00
2. Asbestos-cum-transparent fibre-glass roofed room (24' x 36') for mass culture	Rs. 85,000.00
3. Office-cum-lab rooms - 2nos. (12' x 18')	Rs. 50,000.00
4. FRP tanks -250 l (4 nos.) and one tonne tanks (4 nos.)	Rs. 30,000.00
5. Persepex tanks (100 l capacity) 4 nos.	Rs. 6,000.00
6. Glasswares, PVC pipes, aeration tubes etc.	Rs. 5,000.00
7. Lighting arrangements & electrical fittings	Rs. 5,000.00
8. Wooden racks (4 nos.)	Rs. 8,000.00
9. Air compressor	Rs. 15,000.00

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10. Pump and motor 1 HP (2 nos.)	Rs.	10,000.00
11. Microscope - 1 no.	Rs.	6,000.00
12. Miscellaneous items	Rs.	5,000.00
Total	Rs.	300,000.00

B. Operational Cost per month

1. Wages for 3 labourers	Rs.	3000.00
2. Monthly salary for 2 Technicians	Rs.	4000.00
3. Expenditure for electricity	Rs.	2000.00
4. Cost of chemicals for the culture medium	Rs.	3000.00
5. Other expenses	Rs.	1000.00
6. Interest for the investment (15%)	Rs.	4500.00
Total	Rs.	17500.00

The Operational cost per month for producing 250 litres per day of the pure culture of micro-algae (about 1 million cells/ml) in the laboratory conditions and 1 tonne of the mixed culture (about 0.5 million cells/ml - dominated by the diatoms) developed in outdoor conditions per day will cost about Rs. 17,500/-. The initial investment of the micro-algal culture unit is about Rs. 3 lakhs.

The above mentioned quantity of micro-algal feed can be used in the hatchery to produce about 1 million bivalve seed per month.

PART II

1. ARTEMIA

ARTEMIA

By

B. Vishnu Bhat

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1.0 INTRODUCTION

Aquaculture is a dynamic, rapidly expanding industry worldwide. Annual world production through aquaculture was about 15 million tonnes in 1990 and expected to reach 20 million tonnes by 2000 AD. Domestication of aquaspecies is difficult as it involves a complex interaction between technological and biological parameters for developing a healthy simulated culture system. Feed is an important input in aquaculture operations and accounts for about 50-60% of the recurring investment. The brine shrimp *Artemia* is given as live feed to over 85% aquacultured species around the world, although several artificial feeds have been formulated and compounded. But, none have equalled and or given better performance than *Artemia*. Because of its unique nature and role it has revolutionised the aquaculture industry especially prawn culture and salt production in many advanced countries. The recent developments in aquaculture production has further resulted in increased demands for *Artemia*.

2.0 BIOLOGY & ECOLOGY OF ARTEMIA

2.1 Classification

The brine shrimp *Artemia* is a crustacean closely related to shrimp belonging to phylum Arthropoda. The systematic classification is as below.

Phylum	:	Arthropoda
Class	:	Crustacea
Sub Class	:	Branchiopoda
Order	:	Anostraca
Family	:	Artemiidae
Genus	:	<i>Artemia</i>

The speciation in *Artemia* is not clearly understood and only the genus designation is used in classification.

2.2 Life History and Distribution

In nature, *Artemia* are normally found in hypersaline lakes, brine ponds and lagoons and man made salterns. Brine shrimp thrive very well in natural seawater and can tolerate salinity ranges from 3 to 300 parts per thousand. Due to lack of any anatomical defense mechanism against predation, they are always in danger in salinities which are tolerated by other aquatic species. However, *Artemia* have very efficient ecological defense mechanism and physiological adaptation to live in very high salinity, where predation is minimum. Different geographical strains have adapted to widely fluctuating conditions with regard to the temperature (6-35°C) and the ionic composition of the medium. The distribution of *Artemia* is limited to biotopes where salinity always are sufficiently high to keep out predators or where low temperatures during winter time. The cysts are at the origin of the worldwide distribution of *Artemia*. Wind and waterbirds like seagulls, ducks, flamingos are the most important natural dispersion vectors. In recent times, man has also been responsible for several *Artemia* transplantation to Australia and South America either for salt improvement or for aquaculture uses.

The life cycle begins by hatching cysts, which are metabolically inactive encysted embryos. Dormancy can persist for several years as long as the cysts are kept dry. Sooner they are placed in seawater the embryos resume development and the cysts burst and young larva exits the shell after 15-20 hours. Inside the hatching membrane the nauplius completes development, its appendages begin to move and it emerges free-swimming. The first larval stage (Instar 1) is 400-500 μm in length and about 2 μg in weight and is brownish orange in colour because of its yolk reserves. During Instar 1, nauplii do not feed and after 12 hour, molts into II nd instar, which feeds on 1 to 40 μm particles mainly microalgae bacteria and detritus. Nauplii grow and progress through juveniles, sub adults and adults in 15 molts in 10-15 days. Sexual dimorphism is observed in bisexual strains from the 10th larval stage. In the males, the 2nd antennae develop into hooked claspers which will become functional during copulation, while in the females the antennae degenerate into sensorial appendage. In some

Artemia strains parthenogenesis occurs, i.e., no males are present and embryos develop from fertilized eggs in females. Adults average 8-10 mm long in bisexual strains and upto 20 mm in some polyploid parthenogenetic strains. The weight is around 1 mg. which represents a 20 fold increase in length and 500 fold in biomass in a matter of a fortnight from naupliar stage.

Two main modes of reproduction are found in *Artemia*. In ovoviviparous reproduction, the embryos develop to free swimming nauplii which are released by the mother while in oviparous reproduction, eggs develop to the gastrula stage, become surrounded by the thick shell and are deposited as cysts. Such reproduction is predominant in hypersalinities

Brine shrimp can live for several months, grow from nauplius to adult in less than 2 weeks time and reproduce at a rate of upto 300 nauplii or cysts every 5 days. At low salinities and optimum food levels, females produce free swimming nauplii. Above 150 ppt salinities and low oxygen levels, cysts are released which float in the brine water. The floating cysts are eventually blown ashore where they accumulate in large masses and dry. Development is resumed when the cysts are rehydrated and the life cycle begins again.

3.0 RESOURCES AND EXPLOITATION

3.1 Resources

Brine shrimp *Artemia* have discontinuous distribution in more than 300 natural biotopes in temperate and tropical regions of the world. Due to the geographical isolation, the population of *Artemia* has diversified to more than 150 strains. Over 250 findspots of *Artemia* have been reported in scientific literature of which, only few are exploited for aquaculture purposes. Most of the others have not been surveyed for potential exploitation. The recent findspots of *Artemia* are scattered throughout the tropical, subtropical and temperate climatic zones along coastlines as well as inland, sometimes at hundreds of miles from the sea. *Artemia* occurs in biotopes, which are small salt ponds to very large salt lakes and from very shallow stratification to relatively deep stratified lakes. In India, over 14 findspots have been located in the States of Tamil Nadu, Maharashtra, Gujarat and Rajasthan.

3.2 Exploitation

Artemia are exploited in the form of cysts and/or adult biomass, mainly for use in aquaculture and aquariology. Adult *Artemia* are mainly collected from shallow salt ponds with conical nets mounted in a raft or boat. Best catches are made in the morning. *Artemia* cysts are harvested at many places in the world. They are collected either directly in the water or after being thrown ashore where they accumulate in reddish-brownish layers, several centimeters thick and many metres in length. In the 70's the commercial supplies were mainly from the two natural sources in the United States and Canada to the extent of 30-50 metric tonnes/year but later improved the production to 100 metric tonnes as a result of commercial aquaculture.

In 1982, the consumption of cyst was 60 metric tonnes, which increased to 80 to 90 tonnes in 1985 harvested from only a few biotopes. This is expected to increase upto 150-170 tonnes annually. The estimated world stock in 1986 was about 4000 tonnes and the harvested stock was over 200 tonnes. The exploitation and utilisation of biomass was 1000 tonnes in late seventies but increased to 3500 tonnes in 1987 and the demand is expected to rise to at least 20,000 tonnes by the year 2000 AD. Japan imports about 40% of the total world supply of *Artemia* cysts. In India, commercial exploitation has not been carried out in right earnest and the small quantity harvested (over 100 kgs) in Gujarat was being utilised in the petfish industry. There is, however, no commercial exploitation of *Artemia* biomass. In the early seventies, a kilo of dry cysts was approximately US\$ 10. This went upto US\$ 80 by mid seventies and came down to US\$ 30-40 by early eighties. (If the quality is good, a kilo of cyst can fetch upto US\$ 80).

3.3 Production and exploitation of *Artemia* from natural habitat

Natural populations of *Artemia* are found in large salt lakes and coastal salinas. The distribution of brine shrimp over such habitats is seldom homogenous and very low and as a consequence it is extremely difficult to estimate exact productions. In salt works, *Artemia* is found only in the evaporation

ponds at intermediate salinity levels from about 100 ppt onwards upto 200-250 ppt. At high salinity levels depending on the local strains as well as hydrological conditions in the ponds, cysts are eventually produced and are driven by the wind and accumulate on the shore of the ponds. The quality of *Artemia* differs from strain to strain and location to location. Adults as well as cysts are contaminated with high levels of heavy metals and chlorinated hydrocarbon. It is better to assess the quality of the adult *Artemia*/cyst before it is commercially exploited from such areas. Most of the present exploitation of *Artemia* cysts and biomass, which is utilised by the commercial aquaculture comes from such large production system distributed in the USA, China, Canada, etc. Great salt lake produces 75 per cent of available brine shrimp eggs harvested from the wild. Annual sales is around 600 metric tonnes worth upto \$ 10 million. They are harvested by several companies which ship much of the production abroad.

4.0 **ROLE OF ARTEMIA IN AQUACULTURE AND SALT PRODUCTION**

4.1 **Use in Aquaculture**

Artemia has several characteristics, which make it ideal for aquaculture use. It is easy to handle, adaptable to wide environmental conditions, non selective filter feeder and capable of growing at very high densities. It also has high nutritive value, high conversion efficiency, short generation time, high fecundity rate and considerable long life span. All the life stages of *Artemia*, i.e., cysts (after decapsulation), nauplii, juveniles, sub adults and adults are used as feed in aquaculture operations according to the feed size requirement of the predator. Live *Artemia* nauplii and/or adults are currently used virtually in all commercial aquaculture hatcheries many as a sole diet. Frozen adult *Artemia* are widely used by aquarists, fish breeders, and aquaculturists. Utilisation of live adult *Artemia* is not as familiar to aquaculturists as live nauplii or frozen adults. Live adult *Artemia* provide a high quality, complete protein diet which yields better survival, faster growth rate and fuller color development than most other diets. Live *Artemia* are preferable to frozen because of their higher

nutritional value and live foods tend not to degrade water quality. *Artemia* biomass harvested from natural population are quick frozen and utilised by aquaculturists and pet fish dealers. *Artemia* biomass has also been used as a food additive for domestic livestock, for extraction of pharmaceutical products in protein rich food products and is even used for human consumption in Africa and Thailand. The exploitation, utilisation and trade in *Artemia* is a growing business for use in aquaculture and other industry all over the world.

4.2 Role in Salt Production

Though *Artemia* is a by product from the salterns and solar salt operations, it has its own role in salt production. The crucial role played by *Artemia* in improving the quality and purity of salt has been recognised by intensive scientific studies. Under the conventional system of salt production, the quality of salt is lowered by the presence of micro algae and algal mats in the salt pans. *Artemia* being a non-selective filter feeder feeds on these particles. In doing so, it swallows algae, particulate calcium carbonate and calcium sulphate impurities from the salt water. This process purifies the salt water and improve the quality of salt produced. In nature, red coloration of crystallizers is due to bloom of halobacterium which *Artemia* metabolites as essential nutrients. This can replace expensive dyes normally used in the salterns for increasing solar absorption and evaporation in the production of salt.

5.0 AQUACULTURE PRODUCTION OF ARTEMIA

The culture practices for *Artemia* are mainly two types, considering the use of technology, management, etc.

5.1 Production in saltwater ponds

For cysts and biomass production, *Artemia* can be cultured in large scale in manmade saltwater ponds and temporal salt works. Such a production system can also be termed as extensive culture. Several thousand hectares of salterns in tropical and subtropical belt, the production of salt combine with *Artemia* yields good production of biomass and cysts. Several areas in Brazil, China, Philippines, Thailand, Mexico

etc., have been inoculated with good quality *Artemia* cysts for biomass and cyst production. Such production system also provides unique opportunities for vertically integrated aquaculture projects. Brine ponds with salinities in the range of 100 to 150 ppt are utilised for *Artemia* production.

The area selected for *Artemia* culture should have suitable climatic conditions with temperature 25°C to 35°C and salinity 30 ppt to 200 ppt. It should have high evaporation rate with little rainfall and be closer to sea for water source.

The water source should be free from pollution and the pond should maintain the water level without having any seepage or leakage. In saltpan operations, the water flows from a series of evaporation ponds to become brine, i.e., saturated with sodium chloride and this brine is then introduced to crystallising ponds. *Artemia* can be intensely cultured in evaporation ponds with minimum inputs.

The basic principle for *Artemia* production in temporal salt works is the inoculation of small quantity of freshly hatched nauplii (1 to 10 per litre) after deepening the ponds to 40-50 cm and ensuring that predators are excluded. The ponds are fertilized for enough food production to maintain continuous ovoviviparous mode of reproduction to facilitate fast increase in population density to harvest biomass and further exposing *Artemia* to higher salinity levels to give stress to the population for induction of oviparity to produce cysts (above 150 ppt). The liberated cysts will float and due to wind action, they will accumulate on the shore from where they can be harvested. Such methods of culture is practiced in Thailand, Philippines, Brazil, Mexico, etc. A production of 15 gm live weight/m³/day and 60 kg dry cysts/ha/5 months is usually achieved which shows an average production of 10 to 20 kg dry cysts/ha/year and a few metric tonnes of live biomass in salt pans. The abandoned land or unsuited for classical agriculture due to salinity problems can be utilised to produce *Artemia*

5.2 *Artemia* Production in Controlled Systems

Decades of research on the culturing biology of brine shrimp has resulted in reliable production technique for con-

trolled high density culture of *Artemia*. In outdoor pond system brine shrimp densities of upto 100 animals/litre are considered high. In intensive culture more than 15,000 animals per litre can be operated. Although still in R & D and pilot scale stage the system offers unique opportunity for industrial application. Over a culture duration of only 2 weeks, 25 kg live weight (biomass) can be produced in a tank of 1 cm³ capacity. Due to its tolerance for qualitative and quantitative water composition and various feeds, *Artemia* can also be cultured in a wide variety of thermal effluents (power stations, desalination plants etc.) which also will help recycling of local waste products.

Biomass production under controlled condition (intensive culture of *Artemia* from nauplius to adult stage) can be carried out either in batch or in flow-through culture systems. In both the culture systems, provisions are made to maximise oxygenation of the medium and ensuring food availability to all the larvae, while culturing in high density.

5.2.1 Batch Culture System

In batch culture system, nauplii are reared upto adult stage, without any water renewal in air water lift (AWL) raceways at 50-100 ppt water salinity which provides continuous aeration, homogeneous circulation and uniform distribution of feed.

Artemia raceway consists of a rectangular tank with curved corners and a central partitioning. Water depth should not exceed 1 metre to ensure optimal water circulations with the help of blowers. PVC pipes and elbows are used to construct AWL. Aeration lines are connected to the AWL through polythene tubes

Batch culture in raceway system at 50-100 ppt salinity ensures removal of competitors and predators. Freshly hatched nauplii is stocked at a rate of 10,000/litre and feeding is maintained at 15-20 cm transparency with rice bran. As *Artemia* is a non-selective filter feeder, it can be cultured by feeding with a wide range of feed both live and inert materials of less than 50 μ . Adequate food must be available in the medium at all times as *Artemia* is a continuous filter feeder. Faecal pellet and exuviae have to be removed regularly from the culture

medium from the 4th day of culture onwards as they affect water quality. pH of the water should be maintained above 7.5. Though culture period varies with temperature and strain of the *Artemia*, it is generally about 2 weeks. Harvesting is done by stopping the aeration, by scoop net. If proper environmental conditions are provided, a biomass of 5 kg/cubic metre can be obtained in 2 weeks.

5.2.2 Flow-through culture systems

More intensive *Artemia* culture can be achieved with flow-through system in which continuous renewal of culture water will be maintained and in all other aspects, it resembles the batch culture system. Continuous inflow of fresh culture medium with food (micro algae or inert diet) to the culture tank is maintained. The continuous water change results in removal of all metabolites and hence *Artemia* culture can be carried out with intensive stocking, i.e., double the stocking rate practiced in batch culture. In such systems, the animals can be reared by stocking 20,000 nauplii per litre to achieve a production rate of 25 kg/m³/2 weeks. Also, efforts are underway for induction of cyst production under intensive culture systems by giving stress to the animals. Usually salinity or oxygen stress is given and within a week, eggs will be formed in the ovaries and coated with secretion of shell gland in the ovisac. The cysts liberated will be collected in 11 µm filter bags.

6.0 PROCESSING AND STORAGE OF BIOMASS AND CYSTS

6.1 Harvesting and Processing of cysts

In naturally occurring environments, *Artemia* cysts are harvested in winter months. The floating cysts form slicks on water surface, formed by wind and water currents. The technology used for harvesting is derived from the methods and equipments used to clean up oil spills. Hundreds of feet of floating boom manoeuvred to entrap and consolidate the egg into a workable thickness. The egg is then pumped into large, porous bags on deck of the harvesting fleet. Cysts from the salt pans are harvested by using a double screen dipnet from the corners of the ponds preferentially in the morning. Har-

vested cysts should be stored in saturated brine before processing, cleaning and drying.

Raw cysts are dried and cured. Processing essentially consists of careful screen-washing, disinfection, separation of viable cysts from dead and empty ones, drying and dry screening to remove all foreign matter. After quality testing, the cysts are packed under vacuum/nitrogen atmosphere.

6.2 **Harvesting and Processing of Biomass**

Adult *Artemia* can be manually harvested with a dipnet or conical bag net with less than 100 μ mesh. It is better to harvest *Artemia* live. In order to assure optimal product quality *Artemia* must be frozen, when still alive for future feeding. The nutritional quality of *Artemia* is greatly reduced when drying the biomass in the sun or in the oven.

6.3 **Quality evaluation of *Artemia* cysts**

The quality of *Artemia* cysts varies according to strain or commercial brand. Lack of information on the hatching performance of cysts could lead to uneconomical hatching. Determination of the cyst quality is essential to maximise the use of *Artemia*. Cyst quality can be assessed on the basis of its moisture content, hatching efficiency, hatching percentage and hatching output.

6.3.1 *Cyst Hatching*

Quality evaluation is mainly carried out by hatching the cysts. At least five conditions are essential for restarting the embryological development in cysts leading to the hatching of the nauplii. They are 1) hydration of the cysts in seawater 2) oxygenation of the medium 3) illumination of the hydrated cysts 4) pH above 8.0 and 5) temperature of 26-30°C. Hatching can be carried out in salinities ranging from 5 to 75 ppt. Continuous moderate aeration which keeps the cysts in suspension is beneficial to hatching. Hatching efficiency is considerably higher in light as compared to dark, to obtain good hatching, the eggs must be exposed to light after hydration in order to assure and trigger embryo development and the medium must be continuously oxygenated and the eggs must

be kept in suspension. Cylindroconical hatching containers and a stocking density of upto 10 gms cyst per litre give good results. Usually after 24 to 36 hours, the separated nauplii are collected. BY taking advantage of the positively phototactic behaviour of the animal, the nauplii are separated from the hatching debris. Usually a hatching yield of 70 to 90% is obtained for various strains.

7.0 PROSPECTS FOR AN AQUABUSINESS IN INDIA

In India, there has been no systematic efforts at exploitation or scientific culture of *Artemia* so far, although, it has been the subject of experimental studies in many laboratories for several years. However, some attempts have been recently made by M/s. Tata Chemicals Ltd. and M/s. Ballarpur Industries Ltd. in Gujarat.

Baseline information on the culture aspects collected both at laboratory and field studies have revealed that the Indian strain of *Artemia* is parthenogenetic and the size of the cyst and nauplii are bigger. A great market potential and demand exists for *Artemia* cysts and biomass in many countries of the world not only for their use in aquaculture but also from scientific workers. The demand is very high and a kilogram of cyst produced in India costs Rs. 1,000 to 1,500. Culture of organisms and the trade for cyst and biomass would earn money from domestic and export markets. *Artemia* farming in India seems to be expanding only very slowly due to lack of information on culture technique. By the turn of the century, India is aiming to produce 1,00,000 tonnes of cultured shrimp for which seeds have to be produced in large scale hatcheries, which may be requiring additional 10 to 15 tonnes of cysts in another couple of years if the present growth in shrimp hatcheries materialise as envisaged. According to one estimate, by 2000 AD, about 100 tonnes of *Artemia* cysts would be required to meet the increased demand. Higher production and sales are expected as a result of fast development in shrimp and prawn aquaculture industry and further diversification of the *Artemia* products. In view of the fast expanding aquaculture industry for which *Artemia* biomass has proven to be a very valuable food, it is predicted that the demand for biomass will increase very significantly in the coming years. Short supply of

Artemia would seriously impede this kind of development of aquaculture activities because high priced cysts have to be imported to cater to the increasing demand from hatcheries and nurseries.

India with a long coast line of over 6000 km has about 76,000 ha of salt pans and brine ponds, which offers excellent sites for culturing *Artemia* for cysts and biomass. Solar salt works are suitable biotopes for the integrated exploitation of salt, *Artemia* and eventually fish or shrimp. Intensive biomass production plants are economically feasible when set up close to metropolitan areas (for petfish market) or when integrated into aquaculture plants for the production of juvenile and reproductively active brine shrimps. High yields of biomass and cyst can be achieved by seasonal inoculation and production in fertilized salt pans. It assumes more importance in the new concept of 'Satellite Shrimp Farming' as all products of the *Artemia* business can be effectively utilised. It assumes significance as it will obviate the necessity to import costly and vital *Artemia*.

A detailed survey of saline lagoons and salt pans along the east and west coast of India for the occurrence of *Artemia* is necessary. This will help the exploitation at the optimum level and ensure sustained yield. A study of their density, potential egg production and cyst evaluation would be essential along with the susceptibility of *Artemia* to natural and manmade changes in the habitat. Production of high quality *Artemia* eggs can be enhanced or augmented by inoculating quality *Artemia* to new areas of salt pans/biotopes as variation exists in nutritional quality among various sources and strains of *Artemia*. The Indian strain of *Artemia* can more advantageously be used in production of biomass compared to other strains for intensive culture by virtue of its parthenogenetic and oviparous life history. Import of technology may also be considered for production of cysts and biomass from experienced countries like Thailand, Philippines, Brazil, etc., in order to develop *Artemia* business in the country for 1) increasing income for the salt production with improved quality of the salt, 2) Import substitution and 3) Employment generation in the rural areas.

8. LITERATURE FOR FURTHER READING

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2. Manual for the culture and use of Brine shrimp *Artemia* in Aquaculture, 1986, by Patrick Sorgeloos, Patrick Lavens, Philippe Leger, Wim Tackeret, Danny Versichale, State University of Ghent, Belgium.
3. *Artemia*, Research and its applications. Proceedings of the second international symposium on the brine shrimp *Artemia*. Edited by Patrick Sorgeloos, David A Bengten, Walter Declair and E. Jaspers. Universal Press Wettern, Belgium, 1987, (3 volumes).

Annexure

List of Artemia cyst suppliers

A. Overseas

1. Argent Chemical Laboratories
8702, 152nd Ave. N.E.
Redmond, WA 98052, U.S.A.
Tel : (206) 885-3777
Tlx : 269161 ACLI UR
Fax : 206-885-2112
2. Aquafauna-Biomarine Inc.
P.O. Box 5
Hawthorne
California 90250
U.S.A.
Tel : (213) 973-5275
Tlx : 3718812 BIOMARINE
Fax : (213) 676-9387
3. Bonneville Artemia International Inc.
P.O. Box 511113
Salt Lake City, Utah 84151-1113
U.S.A.
Tel : 801-972-4704
Fax : 801-972-4795
4. Ocean Star International Inc.
P.O. Box 643
Snowville, Utah 84336
U.S.A.
Tel : 801-872-8217
Tlx : TWX 910-971-4002
Fax : 801-872-8272
5. Prime Artemia, Inc.
1042, East Fort Union Blvd.
Suite 400, Midvale
Utah - 84047, U.S.A.
Fax : (801) 562-8728

LIVE FEED

6. San Francisco Bay Brand, Inc.
8239 Enterprise Drive
Newark, CA 94560, U.S.A.
Tel : (415) 792-7200
Tlx : 5101000165 Bay Rand

7. Aquaculture Research/Environmental Associates, Inc.
P.O. Box 1303, Homestead
FL 33090, U.S.A.
Tel : (305) 248-4205
Fax : (305) 248-1756

8. Marine Feed(s) PTE Ltd.
10 Benoi Place, Singapore 2262
Tel : 861545 (Shines)
Tlx : RS 39500 MAFEED
Fax : 8622252

9. Utah Pacific
5415 South, 7500 West, Hooper
Utah 84315 U.S.A.
Tel : (801)-773-3283

10. Sanders
Brine Shrimp Company Inc.
3850 South 540 West
Ogden, Utah 84405-1500 U.S.A.
Tel : 801-393-5027
Fax : 801-621-3825
Tlx : 453041

11. Prime Artemia Inc.
1042 E.Fort Union Blvd.
Suite 400
Midvale, Utah 84047, U.S.A.
Fax/Phone : 801-562-8728

LIVE FEED

12. Artemia Systems
Inve nv-Oeverstraat 7
9200 Dendermonde, Basrode
Belgium
Tel : 32-52-331320
Tlx : 24986 Premix b.
Fax : 32-52-341205
- B. Indian
1. Ballarpur Industries Ltd.
Singach Salt Works
Singarch (Post)
Jamnagar - 361 010
Gujarat
Tel : 77642-8529-Vadinar Exchange
2. Tata Chemicals Ltd.
Mithapur, Okhamandal - 361345
Gujarat State
Tel : 21
- C. Indian Suppliers of Foreign Brand
1. S.R.V. & Co.
6, South Sivan Koil Street,
Vadapalani
Madras - 600 026
Tel : 862742
Tlx : 041-8866 No. 601; 041-6902 No. 601
Fax : 044-842822 SHARMA; 044-82 53588 No.
448
2. Hi-tech Systems
80, Hardwar Road, P. O., I.I.P. Mokhampur
Dehradun-248 005
Tel : (0315) 25853, 26939, 25939
Tlx : 0585-270 HLSC IN
Grams : ATMA

LIVE FEED

3. Southern Scientific Corporation
47, Seventh Street
Shenoy Nagar
Madras - 600 030
Tel : 612532
Tlx : 24065 RIA IN

4. Samurai Techno Trading Pvt. Ltd.
P.B. No. 678, Kings Buildings
Subramaniam Road
Wellington Island
Cochin - 682 003
Tel : 69486
Tlx : 885 6436 KING IN

5. Southern Indian Aquarists
No. 8, Giri Road
T. Nagar, Madras - 600 017
Tel : 864007
Tlx : 41-7558-PCO-IN-MSM-1
(Attn - Southern India Aquarists)
Fax : 91-44-856332

6. The Aquashop
Plot No. 9, 11/1, Beach Home Avenue
Besant Nagar
Madras - 600 090
Tel : 4916450/4916070
Fax : 91-44-415867

7. Marine Technologies
Temple Trees, Suite D, Ground Floor
20, Venkatanarayana Road
T. Nagar, Madras - 600 017
Tel : 444061
Tlx : 041-6447 JOTI IN
Fax : 91-44-442179

PART II

2. ARTEMIA CULTIVATION TECHNOLOGY

ARTEMIA CULTIVATION TECHNOLOGY

By

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1 Construction of Culture Systems

1.a Selection of Site :

- * Site should be having the access to sea water or brackish water.
- * Ponds must be protected from flooding.
- * High salinity water of about 100-150 ppt is needed. This level of salinity is found in condenser ponds of the salt works.
- * A water depth of about 50 cm is necessary to prevent high temperature. There should be possibilities of increasing the height of the dike, or excavating the pond bottom.
- * Regular water supply. There should be possibilities of taking in water without affecting the operations of the saltworks.
- * Water supply should be free from pollution as Artemia could bio-accumulate them in the cysts making it toxic as a food organism.
- * The ponds should be free from leaks and seepages to be able to maintain salinity and water depth.
- * The existing saltworks layout should involve the least quantum of work to transform condenser ponds into Artemia ponds.

1.b Design of the Culture Pond :

- Artemia ponds should be positioned in such a way so as to facilitate cysts collection. The longer dike side of the pond should be parallel or diagonal to the

prevailing wind direction so as to allow the cysts to accumulate in the corners of the shortest bank.

- The ponds should have access to high saline water and to fresh sea water for manipulation of water salinity to a desirable level during culture is considered as a key product of the salt-making process.

1.c *Ponds Layout and Construction :*

- The condenser ponds could be deepened to increase water depth. Also dikes could be increased by excavating a perimeter trench in the pond and putting the soil materials on the dike.
- Condenser ponds to be used for *Artemia* production should have the facility to draw seawater or low salinity water from a water supply canal or from a reservoir and high saline water from an adjoining condenser pond.
- PVC pipes could be used to control water in the ponds. It could also be installed at a certain height along the dike to drain off surface freshwater during sudden rain. Pipes should be fitted with screens to prevent loss of *Artemia*.

2. **Preparation of the Culture Pond**

- Newly excavated ponds should be properly conditioned by drying and flushing. Soils with pH values lower than 6.5 need to be limed to neutralize the acidity. For newly excavated ponds usually CaO or Ca(OH)₂ are used while for old ponds CaCO₃ is used. Normally, to raise soil acidity by 0.1 pH unit, about 500 kg CaCO₃/ha is used.

Calculation for the Lime Requirements

i) Type of Lime :

- CaO (quick lime or unslaked lime) 173% efficiency
- Ca(OH)₂ (hydrated lime) 135% efficiency
- CaCO₃ (agricultural lime) 100% efficiency

ii) Dosage :

500 Kg CaCO₃/ha would raise 0.1 pH unit.

LIVE FEED

Inorganic fertilizers are added on a combination of 10 Kg/ha monoammonium phosphate (16-20-0) and 50 kg per ha. ammoniam nitrate (30-0-0). The fertilizers are usually dissolved in a bucket of water and are evenly broadcasted over the water surface for better effect. When the water turns greenish brown in colour and transparency readings are 30 cm and less, then inoculation could be conducted.

4.a Inoculation of Artemia

- In general Artemia nauplii are inoculated as they are more resistant to salinity changes than the adults.
- Nauplii are inoculated at the rate of 80-100 nauplii per liter pond water.
- Hatching is best done near the pond itself so that inoculation could immediately be done. Hatching containers should be placed in shaded area to prevent the rise of temperature of the hatching medium. Proper aeration should be provided. In the absence of electricity, portable aerators could be used.

4.b Determination of the Amount of Artemia Inoculum

Example : Area of the pond = 5000 m²
Water depth = 0.4 m
Inoculation Density = 40 nauplii/liter
Hatching efficiency* = 4g/1.0 x 10⁶ nauplii

i. Obtain volume of pond water = Area x depth
= 5000 m² x 0.4 m
= 2000 m³ x 1000 $\frac{\text{litre}}{\text{m}^3}$
= 2,000,000 litres

* Hatching efficiency is the weight of the cysts needed to produce one million nauplii.

ii. Obtain number of nauplii needed = inoculation density x volume
= $\frac{40\text{N}}{\text{L}}$ x 2,000,000
= 80,000,000 N

- When hatching is completed, the aeration is stopped to allow the empty cysts to float. The nauplii could be siphoned from the bottom of the container into a filter net of not over 100µm mesh. These could be placed in a bucket of clean seawater or pond water and inoculated.
- Inoculation is done when the temperature in the pond is low preferably late evening or early morning. So when hatching is started in the morning, inoculation could be done in the evening of the next day.

5. **Water Quality Management**

- Water is taken into the pond daily following the routine water management of the saltworks. Water level is maintained.
- The inoculated Artemia is allowed to grow and reproduce.
- Transparency readings are taken daily to be able to fertilize the water when transparency is 30 cm or more. To maintain a good transparency fertilization may be done weekly using (50 kg/ha) ammonium phosphate 16-20-0 and ammonium nitrate 33-0-0 or 500 kg chicken manure/ha. The rate of fertilization could however be adjusted depending on the food level in the pond.
- Salinity should be maintained at around 100-110 ppt in the first pond for biomass production and about 120-150 ppt in the second pond for cysts production. If salinity increases, fresh seawater or water of lower salinity should be taken into sustain the growth of the population in the first pond.

Calculation of Water intake

Since water of different sources and of different salinities might have to be mixed in the ponds at first filling or during later management, following formula to calculate proportionate volumes may be useful :

$$d_1 = \frac{S_2 - S_f}{S_2 - S_1} \times d_f \quad d_2 = d_f - d_1$$

d_1 = Water height (in cm) to take from the first source.

d_2 = Water height (in cm) to take from the second source.

d_f = Final depth (in cm) in culture pond.

S_1 = Salinity of the first source.

S_2 = Salinity of the second source.

S_f = Salinity wanted.

Example :

The water depth in the culture pond has to be 60 cm (d_f) with a salinity of 110 ppt (S_f) and the available water sources have a salinity of 35 ppt (S_1) and 150 ppt (S_2).

$$d_1 = \frac{150 - 110}{150 - 35} \times 60 = 21 \text{ cm water depth to be taken in from the 35 ppt pond.}$$

$$d_2 = 60 - 21 = 39 \text{ cm water depth to be taken in from the 150 ppt pond.}$$

— When a considerable population has developed in the first pond, biomass can be harvested and some can be allowed to flow through the second pond to increase the biomass population. There would be no population recruitment in second pond as the organism would only be producing cysts. Adjustments have to be done to balance harvest and the population in the ponds.

3. HARVESTING OF CYSTS

— Cysts must be harvested regularly from the pond to ensure good quality. The cysts would float in the water and could be harvested simply by gathering them with a scoop net.

— Collection of the floating cysts free from adult *Artemia* is done with a double-screen dipnet.

- If water is agitated and much foam develops, cysts get trapped and lost in the air-born foam. In this case, wave-breakers (floating bamboo) should be installed in 2 or more rows parallel with the cyst barrier.
- Harvested cysts are collected in large buckets filled with brine. When a considerable quantity has been collected, these are processed by cleaning, dehydrating and packing.
- Biomass (adults or nauplii) may also be harvested regularly from the pond. These are washed with seawater and packed at high densities in plastic bags filled with oxygen.

4. PROCESSING OF CYSTS

- The cysts are placed in solutions with different specific gravity to separate the cysts from the debris. This process is based on a bi-phase floatation principle.

4.1 Cleaning

- The cysts stored in brine shall float on the surface while the heavy debris will sink to the bottom.
- When considerable quantity has been collected, the floating cysts can be scooped out and transferred to another bucket with brine. A stick could be used to mix the water half way through the container to remove other debris that may be sticking to the cysts. Aerating the container at half its height would also be beneficial. This brine transfer could also effect further dehydration of the cysts.
- After 24 hours the cysts could be scooped out and washed with fresh water just to remove the salt. This should take only about 5 minutes.
- The cysts are then placed in a bucket with freshwater. This would separate the cysts from light debris and some empty cysts shells. The full cysts would sink in freshwater. This process should not take longer than

15 minutes otherwise the cysts would hydrate. The cysts are then collected and finally washed with fresh-water and allowed to dry by squeezing water out of the filter bag.

4.2 **Drying**

- The cysts are spread out on trays and are either sun-dried (without direct sunlight) on outdoor racks under the shade or in drying boxes.
- Drying is complete when the cysts lumps collapses into powder when touched and when it does not lose further weight.

4.3 **Storage**

- Dried cysts could be stored in tightly closed containers for storage of about a year.
- Commercial quantities should be packed in vacuum sealed or nitrogen flushed cans for longer storage and ease of transport.
- Cysts could also be stored in brine for shorter durations. This method would not necessitate drying the cysts.

5. **ECONOMICS OF FARMING (10 ha Saltpan)**

A.	Capital Cost	(Rs. in lakh)
	1. Pond renovation/excavation	2.5
	2. Equipments/Materials for Inoculation and harvest activities, processing, etc.)	1.5
B.	Operating Cost	
	(Artemia Inoculum, Lime, Fertilizer, Power, Repair and Maintenance, etc.)	1.2
	Manpower (Manager/6 labourers, 10CLR)	

LIVE FEED

Manager	-	0.42	
Labourer (6)	-	0.86	
CLR (10)	-	0.20	1.48

C. Gros Income/year (Rs. in lakh)

1. Sale of 500 kg Cyst (Rs. 1000/kg)		5.0
2. Sale of 20 ton Biomass (Rs. 40/kg)		8.0
3. Sale of salt (1400 tonne) (200 Rs/tonne)		2.8

D. Income 15.8

PART III
BRACHIONUS

BRACHIONUS

By

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2.1 CULTURE TECHNOLOGY

Brachionus culture system may be divided into three stages, viz., 1. Raising appropriate food organism and 2. Raising *Brachionus* culture and 3. Mass production of rotifers.

1.1 Raising food organism

Many types of food organisms such as baker's yeast, marine yeast, *Chlorella* and *Tetraselmis* have been used to raise *Brachionus* in mass cultures. But most commonly *Chlorella* is used.

Chlorella requires inorganic nutrient. The concentrations of nutrients (per litre) in the stock solutions are shown in the table 1. To make one litre of working *Chlorella* medium, add one ml each of stock solutions, except potassium nitrate and sodium dihydrogen phosphate which should be added 5 and 2 ml respectively, to one litre of distilled water (or tap water).

For raising pure stock *Chlorella*, propagation is to be done through agar slants as described below :

- To 1 g of agar add 100 ml of working *Chlorella* medium.
- Boil and distribute agar into 10-12 rimless Borosil or Corning test tubes.
- Insert non-absorbant cotton plugs and cover them with aluminium foil.
- Autoclave the agar tubes for 20 minutes.
- Place them in slanting position while they are still hot.
- Transfer agar tubes into a UV chamber and turn on light for 45 minutes.

LIVE FEED

- Switch off the UV light and introduce stock *Chlorella* into the chamber.
- Inoculate the agar tube with stock *Chlorella* over flame using platinum loop.
- Transfer agar tubes into B.O.D. incubator set at 20-25°C with continuous fluorescent illumination.

Table 1. Nutrients for *Chlorella* culture

To make stock solutions, dissolve separately the undermentioned quantity of each of the nutrients in 1000 ml of distilled water.

Chemicals	Quantity (g)
Potassium nitrate	202.0
Sodium dihydrogen phosphate	310.5
Sodium monohydrogen phosphate	89.0
Magnesium sulphate	246.5
Calcium chloride	14.7
Trace elements	*
Fe - EDTA complex	**

* Dissolve 61 mg of boric acid, 169 mg of manganous sulphate, 287 mg of zinc sulphate, 2.5 mg of copper sulphate and 12.36 mg of ammonium molybdate in 1000 ml of distilled water.

** Dissolve 6.9 g of ferrous sulphate and 9.3 g of disodium salt of EDTA in 800 ml of distilled water. Bring it to boil, cool it and make up the solution to 1000 ml by adding distilled water.

For inoculating into large solution bottles, high density *Chlorella* in large quantities (up to 50 ml at the density of 70×10^6 cells/ml) are required. Since it is not possible to directly inoculate *Chlorella* from agar slants into large solution bottles, the following procedure is to be followed :

- Prepare *Chlorella* medium in one litre of distilled water and distribute into 250 ml conical flasks, each with a volume of about 50 ml.
- Place cotton plugs, cover them with aluminium foil and autoclave.
- Using platinum loop, scratch *Chlorella* from agar tubes and inoculate into conical flasks over flame.
- Avoid scratching of agar.
- Add autoclaved sodium bicarbonate as carbon source to these flasks @ 1 g per litre.
- Shake the flasks gently and transfer them into B.O.D. incubator.
- Sub-culturing may be carried out in the following way when the stock cultures in 250 ml flasks are required in large numbers :
- Keep two 250 ml flasks with autoclaved medium in UV chamber and switch on the light for 25 minutes.
- Introduce one stock 250 ml flask containing densely grown *Chlorella* into the chamber after turning off the UV light.
- Transfer about half of *Chlorella* into the other flask.
- Add autoclaved sodium bicarbonate as mentioned earlier. Repeat the procedure for other densely grown stock *Chlorella* in 250 ml flasks.
- Mass algal cultures may be raised depending on demand. This may be performed as mentioned below.
- Prepare *Chlorella* medium in tap water and add to (10 or 20 liter capacity) Borosil solution bottles.
- Autoclave as described earlier.
- Keep the medium in bottle up to two-third level to avoid spilling over during autoclaving.

- Close the bottles with cotton plugs and aluminium foil.
- Some precipitate forms at the bottom of the bottle after cooling which may be siphoned off using a sterile tube.
- Inoculate the solution bottle using 250 ml flask containing dense *Chlorella*.
- Add autoclaved sodium bicarbonate @ 1 g/l every alternate day.
- Aerate vigorously using oil-free compressor.
- Maintain the bottles under constant fluorescent illumination and aeration.

1.2 Stock Culture of rotifers

For raising stock *Brachionus*, initial zooplankton samples may be collected from stagnant water bodies using mesh ranging from 50-100 μ m pore size. About 50-60 l of pond water when filtered yields sufficiently large number of individuals of *Brachionus* spp.

Live samples from field are to be examined under a stereozoom microscope. Using a fine dropper the desired *Brachionus* species whenever encountered is to be picked up and introduced into the glass cavity block containing 3.5 ml of distilled or dechlorinated tap water the pH of which is adjusted to that of the field sample.

Inoculation may be done in the following way :

- Adjust *Chlorella* pH using a pH meter to 7.5-8.0 by adding appropriate quantities of dilute sulphuric or hydrochloric acid. In case alkali is needed, add dil. sodium hydroxide solution.
- *Chlorella* may also be centrifuged at 3000 rpm for 10 minutes and resuspended in distilled water. In this case pH remains almost neutral and hence no addition of alkali or acid is needed.
- Estimate *Chlorella* density using haemocytometer or spectrophotometer or particulate counter.

- Prepare *Chlorella* density to about one million cells/ml by adding appropriate quantity of distilled water or tap water.
- Distribute the above *Chlorella* into 5-10 ml capacity glass cavity blocks each with about 3-5 ml of medium.
- Using narrow-bore dropper, transfer one individual of the desired rotifer species into one of these cavity blocks. The individual may be ovigerous, if fast rotifer growth is required.
- Serially transfer the isolated individuals through several cavity blocks to eliminate any associate organisms.
- Cover the cavity blocks with a glass plate and place in diffused light.

Stock culture initiation and maintenance :

The general procedure for initiation of culture and maintenance is as follows :

- Following isolation, replace the medium with fresh *Chlorella* (density-one million cells/ml) after every 12 h interval.
- Transfer adult rotifer along with eggs and neonates, if any.
- Avoid transfer of dead rotifers.
- Gradually increase the volume up to 25 ml and use 50 ml capacity beaker; change the cultures daily once.
- Individual transfer at this stage is laborious and hence use 50-70 μ m mesh to separate out rotifers.
- Proceed till the density reaches about 50 individuals/ml and the volume up to 500 ml.
- Increase *Chlorella* density upto 3-4 million cells/ml.
- When the density exceeds, remove about half of the quantity and add dechlorinated tap water or distilled water.

- Change the culture daily with fresh chlorella at the above food density.

1.3 **Mass Culture**

Various methods have been described for mass propagation of rotifers under both marine and freshwater conditions. These may be condensed into three types:

i) *Batch cultures*

Algae under low density are inoculated with low density of *Brachionus* and harvested once all the algal cells are consumed.

ii) *Semi-continuous culture*

A certain volume of water is replaced by fresh quantity of water with or without algae and

iii) *Feedback culture*

The accumulated particulate matter and faeces in the growing rotifer tanks are decomposed in a separate tank using bacteria, while the decomposed matter is used as fertilizer for algal cells.

Under marine conditions, the euryhaline rotifer *B. plicatilis* can be easily grown in batch cultures. In this case, filtered seawater is enriched with agricultural fertilizers, such as urea, ammonium sulphate and superphosphate and then inoculated with marine phytoplankton (such as *Chlorella*). The culture tanks may be maintained under greenhouse conditions and aerated. The culture is inoculated with rotifers, when the algae reach suitable density. The rotifers grow very rapidly and may be harvested if the density is between 100 and 300 indiv./ml. However, rotifer cultures maintained under this system usually contained other zooplankton and in such cases after harvest the entire batch is discarded and a fresh batch is initiated.

1.4 **Commercial Culture**

To grow the rotifer *B. plicatilis* on a large scale level (100 ton capacity), 6 concrete tanks with the size 7.2 x 7.2 x

2.5 m in size can be selected. For this the *Chlorella* must be in 300 ton capacity.

1.5 **Chlorella Culture**

Culture density	=	@ 2000 x 10 ⁴ cell/ml
Culture tanks size	=	22 x 17 x 0.8 m
No. of tanks	=	6
Total capacity	=	300 tons

4.2 **Culture Media**

Filtered Sea water

4.3 **Inorganic Fertilizers (g/ton water)**

Ammonium sulphate	-	100
Calcium superphosphate	-	20
Urea	-	5
Metal mixture (Cu, Zn, Mg)	-	3

4.4 **Preparation :**

- Stock *Chlorella* (200 x 10⁵ cells/ml) is pumped into these tanks. Normally, *Chlorella* reaches a maximum density of 2,000 x 10⁴ cells in 20 days.
- Large scale *Chlorella* cultures are developed at regular intervals and periodically pumped to the rotifer rearing tanks.
- An average of 50-100 tons of *Chlorella* cultures are taken every day to feed the rotifers.
- Intensive aeration must be given in culture tanks.
- An equal amount of fresh sea water is added after the harvest by proportionate addition of fertilizers for further development.
- 3-4 harvests are taken from each tank before the culture is started again.
- Tanks are cleaned between cultures with a high pressure water jet.

1.8 Water Quality Maintenance

Water temperature beyond 30°C may not be suitable for maintaining the culture. pH around 9.00 is good for a maximum growth of *Chlorella* and the density around 20 million cells/ml are normally expected at this pH. Initially, due to the effect of fertilizers, ammonia may range from 15 to 20 ppm, and it comes down to 5 ppm during the course of time by which time the *Chlorella* density reaches the maximum of 20 million cells/ml. The age old cultures developed bacteria or protozoans in summer and such cultures are discarded. The normal range for N₂ in the culture tanks is found to be between 2 and 5%. When N₂ exceed 1,000 ppm the pH falls and as a result *Chlorella* may perish. Dissolved oxygen content in the culture tanks varies from 9 to 10 ml/L during day time and it decreases to 6-7 ml/L at night.

1.9 Culture of Rotifers

Culture density	-	50 - 100/ml
Culture tank size	-	7.2 x 7.2 x 2.5 m
No. of tanks	-	6
Total capacity	-	100 tons

Stock of 4,000 million can be kept available for ready harvest and feeding. The excess rotifers are gathered and preserved in cold storage. The rearing tanks can be provided with pipeline inlets for the supply of *Chlorella* containing water, sea water, freshwater, harvesting pipelines and draining outlets. Through the translucent fibreglass roof of the building, adequate light must be obtained for the production of *Chlorella* and rotifers in rearing tanks.

The tanks are filled 3/4th with *Chlorella* (15-20 million cells/ml) water using the filter screen of 4 μ in pumping hose and the rest with filtered sea water. Large air stones are provided at 6 equally spaced points at the bottom for vigorous aeration and circulation.

2.1.9 Inoculation of Rotifer

Rotifers are added to give an initial density of 30-40/ml. During the active season, in addition to *Chlorella*, baker's

yeast at a rate of 1 g per million rotifers is also supplied daily. Rotifer consumes 1,50,000 cells of *Chlorella* per day.

2 HARVESTING TECHNOLOGY

Harvesting starts after about a week when the density is higher than 100 rotifers/ml and repeated every 3-4 days. Rotifers are harvested by draining the water through a 80 μ . nylobolt filter held on a wooden frame and suspended in a hapa like plastic container in a small cement tank constructed at the ground level. The size of rotifer is 150-220 μ . In the rotifer culture techniques, the thinning method is followed rather than the sub-culture method, the latter is more laborious. The density is estimated by counting the rotifers in 5 ml sample fixed in formalin. After thinning the stock, an equal volume of *Chlorella* water is added to the rotifer tank for further propagation, and the process is repeated. After the final harvest the tanks are drained and cleaned and a new culture is started. Normally the cultures are run for about 30 days.

2.1 Calculation of *Chlorella* requirements

1. The quantity of *Chlorella* water required to feed rotifers by keeping the optimum standard food ratio was found by using the following formula.

$$\text{Vol. of } \underset{\text{water}}{\text{Chlorella}} = \frac{\text{No. of rotifers in millions} \times \text{Std. rate of feeding of } \underset{\text{cells}}{\text{Chlorella}} \times \text{Density of } \underset{\text{tanks}}{\text{rotifer}} \times 100}{\text{Density of } \underset{\text{(to be taken)}}{\text{Chlorella of stock tank}}}$$

2. After adding the *Chlorella* water in rotifer rearing tanks, the density of the microalgae was determined by using the equation:

$$\text{Chlorella density} = \frac{\text{No. of cells already present} \times \text{Quantity of } \underset{\text{water added}}{\text{Chlorella}} \times \text{Density of } \underset{\text{water}}{\text{Stock Chlorella}}}{\text{Total volume of water}}$$

The consumption efficiency of rotifers can be determined by this method.

3 PROBLEMS IN ROTIFER CULTURE

When the rotifer cultures are maintained under the conditions mentioned above, usually there would not be a problem with rotifer population growth. However, sometimes cultures tend to decline which may be due to many reasons. Some of the problems encountered in rotifer culture systems are mentioned below :

- Rotifers may suffer from ciliate contamination, which results in decreased growth of rotifer populations.
- Predatory rotifer *Asplanchna* when it invades *Brachionus* cultures, quickly destroys the cultures due to its exploitative feeding habits.
- Some cladocerans are known to interfere with *Brachionus* cultures leading to decline of rotifer population growth.
- Sudden changes in temperature and pH may cause the death of rotifer cultures.

It is usually desirable to periodically check all the cultures for any possible contamination. Once the contamination is detected in one culture, it should be separated from the rest and various treatments such as addition of dilute solution of sodium chloride or formalin are to be employed. If the contamination is very severe, a fresh batch is to be initiated.

4 COST OF BRACHIONUS PRODUCTION (RUNNING COST)

To produce 1 ton (wet weight) *Brachionus* the following quantities of algae and water are required.

Chlorella	:	3.50 ton (wet weight)
Water	:	2500 ton (reusable)

In general, culturing of *Brachionus* is a bit expensive. For a single species culture of rotifers, special in-door conditions are to be maintained. Since larval fish and prawn almost invariably require live food organisms of the size of rotifers, cost of *Brachionus* production alone will not become the main factor in aquaculture. However, once the culture system is stabilized, the running cost remains within a reasonable range.

PART IV
DAPHINA CULTURE

DAPHNIA CULTURE

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I CULTIVATION TECHNOLOGY

1.1 Construction of culture system :

The size of the culture system mainly depends on the magnitude and frequency of the production harvest of *Daphnia*. To produce 1 tonne (wet weight) of *Daphnia*/week, 2500 tonne capacity tank is required.

Preparation of Culture Tanks

- Sun dried (for 3 days) soil is added to each cement tank upto 5 cm depth and lime powder is also added at the rate of 0.2 kg/ton.
- Water is added up to 15 cm height.
- Poultry manure is added at the rate of 0.4 kg/ton on the 4th day as first dose.
- Water level is raised to 50 cm on the 12th day. Poultry manure is added 1.0 kg as second dose.

1.2 Field Collection :

Collection site

Daphnia spp. may be collected from temporary ponds and some sewage lagoons.

Materials required for collection

5 to 10 l buckets; 500 μ m mesh dip-net or zooplankter vertical sampler.

Collection Procedure :

- Fill the buckets with water from the collection site
- Sweep the dip net through the surface water near the shore, or take vertical tow sample.

LIVE FEED

- Keep the mixed zooplankton in a container.
- To avoid crowding, separate them into one or two buckets.

1.3 Isolation from other zooplankters

Materials required :

Plastic trough (10 l capacity); Microscope; Petri-dish; Plankton nets (200-800 μ m pore size); Pipettes or eye dropper; Aerator; Aquarium.

Procedure :

- Dilute the sample (5 times) by adding water.
- Aerate gently
- Sort out daphnids from the sample.
- Keep 200 daphnids/l.
- Sieve the sample with the plankton net until the daphnids are isolated from other zooplankters.
- Flush the net with water.
- Place the contents of the net in the petri dish containing water.

Using the pipette or eye dropper, transfer 10-50 daphnids to a wet net of a same pore size and flush 3 or 4 times with water.

1.4 Inoculation of Daphnia

- Inoculate mature Daphnia of same size and age group at the rate of 10 numbers/l on the 15th day in each tank.
- Before inoculation, confirm whether any predators are present in the culture medium.
- Maintain the oxygen content above 3 mg/l, water hardness 200 mg/l and pH between 7-8.

1.5 Water quality management :

Water quality management is essential in the cultivation of *Daphnia*. Maintenance of optimum levels of oxygen, water hardness, ammonia and pH are essential as these parameters decide the survival, growth and reproduction and ultimately the production and yield of *Daphnia*.

Oxygen : Oxygen level above 3.5 mg/l favours normal growth and development; oxygen levels between 2 mg/l and 3.5 mg/l retard feeding, growth and reproduction and oxygen level 1 mg/l is lethal to *Daphnia*.

pH : The optimum pH for *Daphnia* culture lies between 7.1 and 8.0. At pH ranging from 8.0 to 10 or 5 to 7, growth is retarded; and at the extreme basic or acidic conditions *Daphnia* succumbs.

Ammonia : *Daphnia* could tolerate a wide range of ammonia levels, but ammonia content of 1 mg/l; results in heavy mortality; the animals feed and reproduce well, when the ammonia content lies below 0.2 mg/l.

Water hardness : Water hardness results in high reproductive rate and shorter sexual maturation time. Hence, it is essential to culture the *Daphnia* in hard water. Water hardness 250 mg l (measured as CaCO_3) is the optimum level for rearing the animals.

To maintain the water hardness, lime is normally added. Since organic manures are used as nutrients to the daphnids, oxygen usually gets depleted as time proceeds. To maintain the suitable oxygen level, provision for aeration is made. In order to keep ammonia content at a lower level it is desirable to change water at the required quantity in different periods of time interval.

1.6 Application of manure :

a) **Poultry manure :** As application of untreated poultry manure results in release accumulation of ammonia. It is desirable to apply the anaerobically treated poultry manure. The recommended dosage is 4g/l week.

b) *Cow-dung substrates* : Cow-dung extract can be given as nutrient to Daphnia. Fresh cow-dung is dissolved at the rate of 10 g/l water, filtered through a fine mesh (100 μ m size) to set an uniform suspension and the extract is added rate of 10-20 l/ton of culture water everyday. In the first week 10 l extract is added/day, 2nd week 20 l/day and 3rd week onwards 30 l/day. Addition is made in the morning and in the evening.

c) *Microbial water* : Microbial water is prepared by keeping anaerobically treated (10 d) poultry slurry in the earthen pots (capacity : 100 l; concentration : 100 g/l water) for 2-3 days. The microbial water along with the slurry is filtered through the fine mesh and then added to the culture system.

(d) *Supplementary diet* : Green algae like Chlorella and other blue green algae can be mass cultured and at the rate of added 40-50 mg/l daily to the daphnid medium as supplementary diet.

1.7 **Supply of water**

Freshwater (hard water preferably) must be added replacing one-fourth of the water for maintaining water quality.

2 HARVESTING TECHNOLOGY

Harvesting the daphnids at regular intervals during culture period is essential for sustaining the production and yield. There are several techniques available for harvesting the daphnids. Before harvesting them the population density and biomass must be ascertained.

2.1 **Sampling of population density :**

Quantity of the daphnids to be harvested is decided by considering the harvestable biomass and the carrying capacity of the remaining population.

Materials required

Beaker, Mesh net, Balance, Trays, Graph sheet, Pipettes with bulb, etc.

Procedure :

- Take 100 ml of water samples randomly in 5-10 beakers.
- Separate the daphnid populations into different size groups (< 1 mm; 1 - 1.75 mm; > 1.75 mm).
- Count the number and estimate the biomass of the daphnids.
- Release the individuals after estimating the density.
- Continue the sampling process once every 5-7 days.
- Plot the population density/biomass against time in a graph sheet.
- Note the day of asymptote phase and harvest.

2.2 Non-selective harvest :

In non selective harvest method, the daphnids are harvested irrespective of size, age group or sex. Here, manpower and instruments involved in segregation of neonates, immature and mature individuals are minimum. Continuous and regular yield may not be possible due to imbalanced recruitment.

2.3 Selective harvest :

In selective harvest method, the animals are harvested selectively, i.e. the individuals of medium sized daphnids are harvested, leaving the neonates and matured individuals in the culture tank.

Since the mature and neonate individuals are released into the culture tanks, continuous and regular yield is possible. However this is a time consuming process.

2.4 Alternate harvest :

In this method, smaller and larger animals are harvested. This is done according to the density of the different age groups of the individuals in the population. Selective

LIVE FEED

harvesting helps to maintain the population according to our requirement of the different size groups of daphnids as it avoids intraspecific competition in the culture tank, as also supplies suitable size first feed for fish larvae.

3 SEPERATION TECHNOLOGY

The harvested matter from the culture tank contains the unfed substrates, exuvia and faecal material etc. Hence separation of daphnids from the rest of the material is preferred.

Materials required :

Round plastic troughs; Sieve; Continuous water flow provision; Buckets; Pipette or sucking pump.

Procedure :

- Keep the harvested Daphnia in the containers.
- Stop the aeration.
- Transfer the daphnid mass into round plastic troughs.
- Using sieves or hand, let the water move in a circular manner.
- Keep it for minute.
- Daphnia will move from the centre.
- The unfed, exuvia, and faeces will remain in the centre.
- Using pipette or sucking pump, remove the unfed, exuvia and faeces.
- The daphnid mass is further cleaned by passing water continuously.
- Separate the mass and keep in a container and aerate.

4 PROBLEMS IN DAPHNIA CULTURE

The major problems in Daphnia culture are the occurrence of rotifers and predators.

4.1 Occurrence of Rotifers :

Among the zooplankters invading *Daphnia* culture, rotifers especially *Brachionus*, *Conochilus* and some bdelloids are known to retard or even kill the daphnids. *B.rubers* is known to be epizoic on the Daphnids. It causes hindrance to the swimming and food gathering activities of the daphnid. Being smaller than the *Daphnia*, *Brachionus* may be easily be eliminated by flushing it out of water in the culture system using appropriate mesh.

Conochilus may be eliminated by adding cow dung to the culture. Bdellois are bottom creeping rotifers and very resistant to wide change in temperature, ph, dissolved oxygen or even drought. They may be eliminated by circulating water continuously so that bdelloids coming into water column are removed through filters. While flushing out with water, care should be taken that daphnids are not damaged.

4.2 Predators :

Several predators are known to consume the daphnids, e.g. *Calanus* and *Anisops*. *Calanus* sp. mainly comes through the inoculated population. Hence it is better to inoculate only *Daphnia* either by maintaining a pure stock culture or selecting pure *Daphnia* from the field population. If the insect predators occur in the culture system, the predators can be sieved out, or clean the culture system completely and start a new culture. The system must be closed completely by a sieve.