

## CULTIVATION OF LIVE FOOD ORGANISMS—STATUS AND SCOPE IN INDIA

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### ABSTRACT

In the hatchery production of cultivable shell-fishes and fin-fishes, a reliable and continuous supply of live food of appropriate size is of importance. The organisms cultured as live feed presently in India are the microlagae, turbellarians, tanaidaceans, rotifers, annelids, brine shrimps, cladocerans and copepods. Their culture methods and yield are briefly summarized. Suggestions have been made for the cultivation of other organisms which have potential for use as live feed.

### INTRODUCTION

An important factor in the success of aquaculture is the availability of a suitable feed at a reasonable cost. In the traditional aquaculture operations such as paddy-cum-prawn culture in India, the prawns are allowed to grow on the naturally available detritus and no supplement feed is provided. In intensive culture, supplementary feeding is necessary to optimise the yield. This can come in two forms : compound dry feeds and live feeds. Compounded feeds have the advantage of stability and easy availability but are usually suited for adults only. On the other hand, live feed is essential for the growth of larval forms in hatcheries, and these can also be used for adults if generated in sufficient quantities. Besides, live feed are superior to compounded feed, because they are readily ingested (Kinne, 1977), digested more easily (Jirasek *et al.*, 1977), does not affect the water quality (Watanabe *et al.*, 1978) and have essential growth factors. Many organisms ranging from bacteria to the brine shrimps qualify as potential food sources, however, not all of them can be readily cultivated. The objective of this paper is to summarise the present knowledge of the cultivation of live food organisms in India and examine the scope for extensive use in aquaculture.

### CULTIVATION OF MICROALGAE

Microalgae such as *Dunaliella*, *Skeletonema*, *Chaetoceros*, *Thalassiosira*, *Nitzschia* and others are known to be good feeds for larval stages of commercially important crustaceans (Kinne, 1977; Silas & Muthu, 1977), as well as for other live feed organisms such as copepods, rotifers and *Artemia*. *Isochrysis galbana*, *Pavlova sp.*, *Dicrateria sp.*, *Chromulina sp.* and *Chlorella* are extensively used as larval food for many of the cultivated molluscan species (CMFRI, 1984). Among the microalgae, *Spirulina* has excellent scope for mass cultivation. At the National Botanical Research Institute, Lucknow (NBRI) an appropriate technology has been developed to culture *Spirulina* in raw and settled sewage using sodium bicarbonate as a carbon source. Biomass production was in the range of 7-8 g/m<sup>2</sup>/d (Venkataraman, 1983). Mass production of *Spirulina platensis* was made possible under optimal conditions of light, pH, agitation and nutrients at the Central Food Technology Research Institute (CFTRI), Mysore. An yield of 8-12 g/m<sup>2</sup>/d has been obtained. Preliminary studies have

shown that tilapias and carps can be grown successfully with algae in integrated systems. CFTRI's work on mass outdoor cultivation of *Spirulina* is very exhaustive covering culture methods, biochemical evaluation, nutritional assessment, processing and storage (Becker & Venkataraman, 1982). The Murugappa Chettiar Research Centre (MCRC) at Madras, initiated mass cultivation of *Spirulina fusiformis* in 1977. PVC lined ponds formed the basis of algal culture and algae were cultured on a continuous basis. An attempt to reduce nutrient inputs, biogas effluent, bone meat, etc. have also been used. Yield of *Spirulina* upto 12 g/m<sup>2</sup>/d has been reported (Venkataraman, 1983).

At the National Institute of Oceanography (NIO), Goa the microalgae *Tetraselmis gracilis* and the natural phytoplankters have been cultured in secondary treated sewage-sea water mixture. The cultivated algae were meant for larval feed for *Perna viridis* (Devassy, 1981).

The research on the culturing of *Scenedesmus obliquus* were launched at CFTRI, Mysore as a collaborative project between Government of India and west Germany in 1973. Highly useful technological and nutritional data were generated with respect to Indian conditions. Optimal growth rates of *S. obliquus* were obtained with CO<sub>2</sub> and molasses as carbon sources. The results of the algal project at CFTRI demonstrated that under given conditions an average yield of 15-20 g/m<sup>2</sup>/d can be obtained for *S. Obliquus* (Becker & Venkataraman, 1982).

### CULTIVATION OF ZOOPLANKTERS

Though many zooplankters can qualify as potential source as food for larval forms, in reality only a few such as rotifers, brine shrimps and copepods serve this purpose. The reason for this lies in the fact that most of the zooplankters have longer growth period, occur in sizes not readily suitable for ingestion, do not have nutritional quality to support a wide spectrum of larval forms and are difficult to maintain in culture. However, attempts have been made to cultivate a variety of zooplankters and these are summarized below.

#### **Turbellarians :**

Mass cultivation of the Rhabdocoelid turbellarian was carried out using extracts of organic fertilizers prepared out of foodgrains, both dicot and monocot. Shirgur (1980) found that the wheat (*Triticum durum*) extract and Matki extract (*Phaseolus aconitifolius*) in equal proportions with addition of superphosphate yielded maximum production (1 million/m<sup>3</sup> culture media). Reproductive behaviour under different feeding conditions and varying salinities of *Macrostomum orthostylum* have been studied for mass cultivation (Shirgur & Ingole, 1983). However, the importance of turbellarians as food sources for cultured animals remains to be explored.

#### **Tanaiidaceans :**

The tanaiidaceans *Apsuedes chilensis* Chilton, a detritus feeder was found to form highly preferred food item of shrimps and fishes in the Cochin Backwaters (Gopalan *et al.*, 1980). Therefore, laboratory experiments have been conducted to elucidate the life cycle and evolve suitable mass culture methods to use them as live feed. It was possible to culture

the tanaidaceans, using filtered estuarine water and substratum enriched with artificial detritus. Cultures inoculated at the rate of 600 individuals/m<sup>2</sup> was found to multiply upto 25,000/m<sup>2</sup> in 45–50 days. Occasional mass mortalities were reported due to infestation of unidentified microsporidean and peritrich ciliates.

#### Rotifers:

Among the rotifers, the most widely cultivated species is *Brachionus plicatilis* Muller. Following its successful culture (Ito, 1960) it was introduced into mariculture. A technique for the continuous culture of *B. plicatilis* has been perfected at the Narakkal Prawn Culture Laboratory (NPCL) Cochin of CMFRI using groundnut oil cake, superphosphate and urea as fertilizers and *Chlorella* culture as feed. It resulted in a harvest of 200–250 million rotifers everyday. After processing and preservation, rotifers are frozen into blocks for future use (Manisseri & Palanichamy, 1983). *Brachionus mulleri* Pallas serves as an important food item in the culture of several aquatic animals. They were (Nandy *et al.* 1977) mass cultured under yard conditions. Culture media containing a combination of mahua oil cake, cowdung and urea gave maximum density of rotifers in 22 days time. The limiting factor in rotifer culture is their dependence on unicellular algae as food. Effective mixture of dry feed for growth of rotifers are available and it is now possible to raise rotifers with extracts of organic and inorganic manures. Lybzens *et al.* (1980) have induced production of resting eggs by transferring the rotifers to 25% sea water. Eggs could be stored and used as and when required. This situation could profitably be utilized for developing rotifer culture in India.

#### Annelids :

Several oligochaete worms such as the white worm *Enchytraeus*, the earthworm *Lumbricoides* and the small red worm *Tubifex* are acceptable as food for many marine invertebrates and fishes. While mass cultivation of annelids are relatively easy, very little information about the culturing is available. Marian & Pandian (1984) found *Tubifex tubifex* as one of the best candidate species for mass cultivation as it has shorter generation time of 42 days and can be easily grown on a single medium containing organic waste like cow dung and coconut mesocarp; it occurs in a wide range of habitats and tolerates a spectrum of environmental variables; it has high fecundity of 92–340 eggs and reproduces in a temperature range of 0.5 – 30°C. They cultured *T. tubifex* in outdoor cement culverts (150 x 15 x 15 cm). The substratum contained 75% cow dung and 25% fine sand and running water flow through the tanks at the rate of 250 ml/minute. The oxygen concentration in the running water was maintained at or above 3 mg/l. Fresh cow dung was added once in 4 days at the rate of 250 mg/cm<sup>2</sup>. It resulted in a harvest of 125 mg/cm<sup>2</sup> every 30 days, maintaining the worm density at about 180 mg/cm<sup>2</sup>. Interference of *Chironomus* occurs in such open system but this can be prevented by providing tanks with proper meshed nets to avoid insects laying eggs (Marian & Pandian, 1985). Thus mass culture and harvesting technique for this annelid has been perfected.

#### Brine Shrimp, Artemia :

Due to the availability of dry cysts, the hardy anostracan branchiopod *Artemia* sp. has become a most familiar animal with mariculturists. *Artemia* has been found to be a suitable food for the most diversified groups of organisms. More than 85% of the marine

animals cultivated so far have been offered *Artemia* as food source either together with other feeds or more often as a sole diet (Kinne, 1977). During the past five years efforts to mass culture *Artemia* in India has been encouraging.

James (1980 a) mass cultured *Artemia* in 108–118‰ salinity using a combination of rice bran extract, yeast and vitamin B complex as feed. The efficiency of plate separator for effective isolation of eggs and nauplii from mass culture tanks of *Artemia* has proved useful (James, 1980 b). Effects of different temperatures, salinities, light condition for optimal hatching of the Tuticorin strain of *Artemia*, its nutritional value and growth in relation to feeds such as *Spirulina*, rice bran and yeast have been studied (Royan, 1980). Dwivedi *et al.* (1980) have attempted mass cultivation of brine shrimp under controlled conditions in cement pools. They have demonstrated the low input technology in culturing *Artemia* suitable for rural conditions in India and for commercial prawn hatcheries. It has been demonstrated that it is not only possible to cultivate the Indian strain of *Artemia* but also other geographical strains successfully in a new locality leading to local production to meet the evergrowing demand for *Artemia* (Royan, 1981). In the NPCL of CMFRI, continuous mass culture of different strains of *Artemia* are maintained (Vedavyasa Rao, 1983). In order to minimise the cost of production mass culture of *Artemia* under laboratory conditions using renewable organic wastes has been attempted (Basil & Marian, 1985). Basic studies related to ecology, hatchability and nutritional assessment of *Artemia* from Didwana salt lake and rearing in a 2 ha. salt pan near Sambhar Lake have been successfully carried out by Bhargava *et al.* (1985 a, b & c). Hatchability, cyst characterization, evaluation of different feeds for optimal survival and growth and use of adult *Artemia* (Saurashtra strain) as live feed for commercially important shrimp juveniles have been studied with a view to mass culture, (Royan *et al.*, 1987 a, b & Sumitra Vijayaraghavan *et al.*, 1987). Marine Products Export Development Authority (MPEDA) at Cochin has developed a simplified indigenous continuous culture technique for mass production of *Artemia* to meet the feed requirement of prawn hatchery (Bhat & Ganapathy, 1985)

#### **Cladocera:**

These small crustaceans are useful as food for young and adult fishes. Grey mullets in culture were observed to consume *Moina* readily. However, moinids do not survive in the sea water. It has been shown that it is possible by gradual acclimatization to salinity from 4.5‰ to 5.26‰ to avoid mortality of *Moina* upto 96 hours (Ghosh & Kowtal, 1980). At the NPCL of CMFRI, Cochin *Moina* sp. obtained from local freshwater ponds are mass cultured in out-door plastic pools containing fresh water fertilized with groundnut oil cake juice. Densities upto 30,000-40,000 nos/l has been achieved in 6-7 days. They are harvested with 60  $\mu$ m mesh nets and frozen into small blocks containing 0.5-0.75 million animals. Methods for obtaining dry cysts of *Moina* with considerable shelf-life has been reported (Vedavyasa Rao, 1983). Naik & Shirgur (1980 a) used different organic and inorganic fertilizers for cultivating *Alona taraporevalae* and found that maximum production was obtained with groundnut oil cake and phosphate. Their observations on fertilizer activity in culture media showed that groundnut oil cake decomposes steadily and generated biomass of the bacterium *Pseudomonas acriginosa* and other microorganisms. Thus, in the culture media combination of *P. acriginosa* and suitable suspended algal cells constituted an ideal food for maximum production yielding 6-7 lakh/m<sup>3</sup>. Tolerance of *A. tarapore-*

*valae* to different temperature, salinity and oxygen has been studied by Naik & Shirgur (1980b) and observations on the reproductive biology have yielded valuable information for culture (Naik & Shirgur, 1980 c).

Nandy *et al.* (1977) screened feeds such as brewer's yeast, unicellular algal culture (*Chlorella vulgaris*) and poultry excreta for culture of *Daphnia lumholtzi* Sars in plastic pools (0.6 x 0.5 m<sup>2</sup>). Their studies revealed that media containing 0.1% uniform suspension of brewer's yeast yielded 12,650 - 15,000 nos/l in a seven day period.

### Copepods :

Copepods are important groups and serve as a major food source for numerous organisms including fishes and crustaceans. Among benthic copepods, *Tigriopus* and *Tisbe* have been mass cultured. The *T. japonicus* is considered as the most promising food organism (Kitajima, 1973, Iwasaki, 1973). Effect of various food items such as detritus from decaying *Salvinia* weed; *Chlorella* cultured in the laboratory; shrimp headmeal and compounded diet (trash fish 45% ; tapioca 45% and rice bran 10%) have been evaluated for experimental mass culture of harpacticoid copepod *Nitocra spinipes* Boek by Gopalan (1977). He observed that the rate of reproduction was highest at 25‰ salinity and shrimp head meal as food resulted in highest production of 11,100 individuals/l. Goswami (1977) cultured *Laophonte setosa* (Boeck) as food for shrimp larvae and found that the maximum growth occurred between 25-27°C at 20‰ salinity. The copepods were raised on natural detritus. Mass cultivation of euryhaline harpacticoid copepod *Amphiascoides subdebilis* (Willey, 1935) under phased fertilization was undertaken in view of the brackish water fish seed production (Shirgur & Ingole, 1983). Cultivation of *Pseudodiaptomus amandalei* on experimental basis was done in out-door tanks (James & Thompson, 1980).

### REMARKS AND SCOPE

The foregoing account indicates that though efforts have been made, except for *Spirulina*, *Tubifex*, *Artemia* and rotifers most of the mass culture trials have been on an experimental basis and only used to a limited extent. This is partly due to the various constraints and certain areas of investigation need more extensive efforts. Microalgae constitutes the major source of food in the controlled rearing of shrimps and its cultivation is generally restricted to monospecies. Success in copepod culture has been achieved on experimental basis and have been used to a limited extent in mariculture hatchery systems. Major problems in copepod culture relate to maintaining adequate density involving different physico-chemical factors and the other organic waste products in the culture medium (Vedavyasa Rao, 1983). Sufficient information on the biology and ecology of the live feed organisms are required before one can design effective culture systems. Information on the nutritive quality, inclusive of biochemical constituents, amino acid and fatty acid composition are necessary to select a suitable species. This has been neglected by many workers. While some information is available on the sporozoan infestation in commercially important penaeid prawns, diseases encountered in eel *Anguilla* sp. and parasitization by *Bucephalopsis* and spirochaete infestation in live *Crassostrea madrasensis* (CMFRI, 1984), research on diseases in live feed culture and methods to overcome them need to be strengthened. Technology for proper preservation and storage needs to be perfected.

There appears to be scope in attempting to culture other live feeds - thus, the euryplastic protozoan *Fabrea salina* provides excellent food for a variety of invertebrates and fishes - they are known to thrive at high temperature 37-40°C; could be harvested using simple techniques; their dormant stages (cyst) and excysting can be produced at will by environmental changes and nutritional value controlled (Kinne, 1977).

It has been made possible to culture ciliates in large numbers (Sreedharan, 1986) which could be used for aquaculture purposes. Molluscs, echinoderms and fishes are known to feed on sponges while cultivated animals have been offered Cnidarians as food. The importance of Porifera and Cnidarians as food organisms is yet to be fully explored. Nematodes such as *Penagrellus* sp. and *Caenorhabditis elegans* and *Chironomids* are small; can be easily grown; known to withstand sea water salinity for several hours and can serve as an adequate food source for small fishes (Kahan & Appel, 1976, Ivelva, 1969). Several molluscs *Mya arenaria*, *Mytilus edulis*, *Cardium edule*, *Carssostrea virginica* have been fed to cultivated animals. Gastropods, bivalves and other molluscs deserve more attention in regard to the role they play as food organisms (Kinne, 1977). Eggs of larvae of echinoderms specially asterioids and echinoids have also been used as food for cultivated animals. With expansion of aquaculture programmes in India more and more hatcheries are being set up. Cultivation of groups of animals which hitherto have not been given importance may open new avenues in live feed culture.

The production cost of live feed is the crucial point on which the entire viability of culture technique depends. Information on production cost analyses of live feed is very scanty in India due to the fact that the culture experiments are based on limited studies on a pilot scale with small cultivation area. Cost can be worked out only when the scale of operation and utilization is known for certain.

Table I summarises the way in which microalgae are utilized in aquaculture. The major problem encountered with the use of natural phytoplankton is the lack of control of production and the composition of algal populations. Production cost of microalgae is given in Table II. Depending on the technology used it may cost upto \$ 200/Kg dry weight for monospecific algal cultures used in hatcheries to \$ 0.2 for harvested sewage-grown algae (De pauw *et al.*, 1983). They have suggested means of reducing the cost in small scale as well as large scale systems which could be profitably used in future for development of microalgal production in India.

Demand for *Artemia* cysts (excluding that used by hobbyists) has risen on an average of 15% per year during the last five years. *Artemia* cyst requirement in 1975 was about 25 mt and by 1980, the demand had increased to 46 mt (Table III). However, the present demand, has almost touched 200mt (Sorgeloos-personal communication). During 1979 the world supply of *Artemia* cyst has been estimated to be 95 mt. of product with 50% hatchability. The supply of 70% hatchability stock was estimated at 35mt. (Table IV). In terms of total supply and demand for *Artemia* cysts, no shortage of product exists.

Thus, available information from elsewhere (Tables I-IV) on microalgae and *Artemia* shows knowledge on supply and demand is a very important aspect for mass cultivation of any live feed for its economic viability. This aspect needs to be given stress in future.

TABLE I Modes of microalgae production and uses in aquaculture (Source : Panw *et al.*, 1984)

Type of culture	Degree of control	Type of algae	Algae-consumer relation	State of algae	Inoculation	Enrichment	Water source & Type of enclosure	Volumes of cultures	Types of consumers
Extensive	Uncontrolled	Natural phyto-plankton	Grown together	Live	Natural	Natural Upwelling	Sea Lake Lagoon Pond	> 1000 m <sup>3</sup>	Bivalve mollusc larvae, postlarvae, grow out
		Natural phyto-plankton	Grown separately (pumping)	Live	Natural	Natural	Natural — Sea Lake Lagoon Pond	> 100 m <sup>3</sup>	Bivalve mollusc postlarvae
Semi-intensive	Semi-controlled	Induced blooms of natural phyto-plankton	Grown together	Live	Natural	Artificial	Natural — Tank pond	—	Bivalve mollusc postlarvae Zooplankton Fish
		Induced blooms of natural phyto-plankton	Introduction of consumer after bloom induction	Live	Natural	Artificial	Natural — Tank Pond	—	Penaeid shrimp larvae Fish larvae Abalone <i>Brachionus</i>
		Induced blooms of natural phyto-plankton	Separated	Live preserved	Natural	Natural	Natural — Tank Race-way Pond	litres > 100 m <sup>3</sup>	Bivalve mollusc larvae and Postlarvae, Penaeid shrimp larvae Zooplankton
Intensive	Controlled	Monospecific algal cultures	Separated	Live preserved	Artificial	Natural Artificial	Natural Artificial — Tube bag — Plastic Bottle — Tank	—	Bivalve mollusc larvae Penaeid shrimp larvae <i>Brachionus</i>

TABLE II Cost of microalgae

<i>Type of algal culture</i>	<i>Cost (approx.) US \$ Kg<sup>-1</sup> dry wt.</i>	<i>Reference</i>
Monospecific algal cultures produced indoors or in a green house	120 — 200	Walne, 1976 Loring, 1981 (personal commun.) (in De Pauw, 1981)
Induced blooms of marine phytoplankton species	4 — 23	De Pauw, 1981; De Pauw, <i>et al.</i> , 1983
<i>Chlorella</i> (dried)	9 — 11	Kawaguchi, 1980; Soong, 1980
<i>Spirulina</i> (dried)	5	Soasa Texcoco, 1980 (Sales price, personal commun.)
<i>Spirulina</i> (dried)	1 — 5	Berned <i>et al.</i> , 1980
<i>Scenedesmus</i> (dried)	1.5 — 1.9	Soeder, 1978; Becker & Venkataraman 1982
Wastewater-grown microalga (harvested-dried)	0.17 — 0.29	Berned <i>et al.</i> , 1980 Shelef <i>et al.</i> , 1978 Lincoln & Hill 1980

Table III World demand for *Artemia* cysts, 1980 \* (Aquaculture use only)

<i>Region</i>	<i>Tonnes required</i>
Americas	22.5
Asia, including Pacific Rim	13.5
Europe	4.0
Middle East	1.5
Africa	1.0
Australia	0.5
U.S.S.R.	N.A. (not available)
Total	46.0



Table IV World Supply of *Artemia* cysts in Metric tons, 1979\*

Source	Minimum 50% Hatchability	Minimum 70% Hatchability
Utah	27.3	11.4
San Francisco	22.7	9.1
Brazil	13.6	11.4
Australia	11.4	1.1
Canada	11.4	1.1
Argentina	5.5	0.7
China	3.2	0.3
	95.1	35.1

\* Source, Tunsutapanich, 1982

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