A REVIEW OF THE STUDIES ON LARVAL NUTRITION IN CULTIVABLE PENAEID AND PALAEMONID PRAWNS

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Introduction

The technological developments on large scale culture of prawns have been significant all over the world since the successful rearing of *Penaeus japonicus* by Hudinaga (Fujinaga) in 1942. In the initial phase of its development, efforts were mainly directed to develop appropriate technologies of breeding potential of cultivable species under controlled conditions and to delineate their larval development. Of late, however, there is great awareness of the importance of knowledge on the nutritional requirements of these species and the development of a feed technology for their successful culture operation. There is also a thinking that the triumph of future aquaculture of these animals largely depends on a sound understaning of these aspects as the diet in prawn culture is probably the most expensive input representing about 50-60% of the running cost of intensive culture operation from larvae to marketable size.

Information on prawn nutrition is relatively less as compared to those available on finfish nutrition. Prawns, as other crustaceans, derive nutrients directly from the media in which they live and from the ingested material. This makes the entire process of nutrition and its physiology, complex. The first review on the subject of feeding and nutrition, digestion and metabolism, and vitamins in crustaceans including certain cultivable species was made by Marshall and Orr (1960), Vonk (1960) and Fisher (1960) respectively. Subsequently, New (1976) offered an excellent review of the literature available on dietary studies with prawns and shrimps. This was followed by another comprehensive review by Kinne (1977). In an useful publication, Biddle (1977) covered various aspects of nutrition in fresh water prawns of cultivable interest. Apart from these, the books published by Imai (1977), Hanson and Goodwin (1977) and Stickney (1979) treat some aspects of nutrition of the candidate species dealt with by them. More recently New (1980) provided a bibliography of prawn and shrimp nutrition covering 494 literature published upto 1980, while Castell *et al.* (1981) presented the advances made in nutrition research on protein and amino acids, vitamins, lipids, minerals, carbohydrates and energy requirements and feed technology of crustaceans, molluscs and finfishes at the World Conference on Aquaculture and International Aquaculture Trade Show held in Venice in September, 1981.

Much of the work on prawn nutrition has been carried out on juvenile and adult forms. In the field of larval nutrition, most of the investigations carried out so far relate to identification and production of live feed and other natural material, and recently to artificial diets. Studies on quantitative aspects of food taken, total nutritional requirements of different larval stages, and those relating to their metabolism are few. In this paper an attempt is made to summarise the present knowledge of the food, feeding and nutrition of larvae of cultivable penaeid and palaemonid prawns as available from the published works.

Food and feeding of larvae

Qualitative as well as quantitative data on the nature and amount of food consumed by the larvae in the wild are scarce. This is mainly because of the constraints involved in studying the gut contents by the classical methods due to the delicate nature of the larvae, and the predominence of unrecognisable food in the gut. Structurally, the digestive tract of the larvae is simple and relatively short. However, it is observed that the Prawn larvae feed on a wide range of material of suitable particle size present in the water table available to them. This includes the phytoplankton, zooplankton and non-living particles. It is also observed that, in nature, they eat frequently and rapidly.

The type of food ingested and the feeding mechanisms are correlated with the characteristics of the different larval stages of penaeid and palaemonid prawns (New, 1979) and the functional morphology of their appendages (Muthu, 1980). In the penaeid prawns, the first larval stage, namely, the nauplius, does not feed and lives by utilising the internal yolk. After 2-3 days, the nauplius metamorphoses into protozoea stage and begins to feed on phytoplankton of approximately 3-10 μ size. After about 5 days, the protozoea transforms to mysis stage and feeds on particulate food of ten times the size of the food of protozoea. The mysis metamorphoses into postlarva after another 5 days and feeds mainly on particulate food of still large size and changes from an omnivorous to carnivorous feeding depending on the species. In palaemonid prawns, the eggs carried in the pleopods hatch out as zoea larvae and commence feeding on particulate food of the size of *Artemia* nauplii (0.4 mm) and even larger particles from the first or second day.

Studying the structure of the mouth parts and feeding appendages, Muthu (1980) classified the larvae of penaeid and caridean prawns into three broad categories, namely, the filter feeding type, the mixed feeding type and the carnivorous type. Accordingly, the protozoea and mysis of penaeids belong to filter feeding type with well developed maxillary filters; the postlarva of penaeids and zoea larvae of palaemonid prawns to carnivorous type with strong mandible, chelae of the first or more posterior pair of walking legs and raptorial setae, and the zoea larvae of the caridean prawns belonging to Hippolytidae, Processidae, Pandalidae, Crangonidae, Hoplophoridae and Atydae to mixed feeding type.

Dealing with the feeding habits of of crustaceans in general, Marshall and Orr (1960) grouped them into filter feeders, scavangers, predators and parasites. Among the filter feeders, they further observed, on the basis of the functional involvement of various mouth

parts and trunk limbs and the essential feeding mechanism, the trunk-limb filters, modified thoracic limb filters, maxillary filters, maxillular and mandibular filters and antennal and antennular filters.

Larval food in culture system

Live food organisms

Inadequacy of knowledge on the nature of food consumed by the larvae in the wild formed a constraint for a long time in successful rearing of prawns from egg through different larval stages. The development of pure culture of *Skeletonema costatum* by Hudinaga (1942) for feeding the protozoea of *Penaeus japonicus* paved way for the first time to rear the species through different larval stages. Besides *S. costatum*, Hudinaga offered *Artemia* nauplii for the late mysis and early post larval stages. For a long time *S. costatum* served as a classical feed for rearing of the early stages of penaeid prawns. In 1962, Fujinaga and Miyamura found that *Chaetoceros ridigus* is also suitable for the culture of early larvae of *P. japonicus*. Later studies showed that while *Skeletonema* cannot be cultured in high temperature, *Chaetoceros* spp. can withstand relatively high temperature and are found to be easily digestable by the larvae.

In the initial phase of the larval rearing history, it was thought that pure culture of suitable diatom was essential for larval feeding. However, in later attempts Fujinaga and Kittaka (1967) and Fujinaga (1969) used mixed cultures of diatoms in outdoor tanks with appreciable survival rate of larvae of *P. Japonicus*. Since then several diatoms of either pure culture or in combination were developed and used to feed the larvae by different workers (Table 1).

When the larvae are reared employing cultures of diatoms, generally a concentration of 5,000 – 20,000 cells/ml is used. It is reported that the larvae can be successfully reared through the different stages by feeding entirely on monoculture of the diatom, *Chaetoceros* at a higher concentration of 30,000–100,000 cells/ml (New, 1979). However, the success of diatom culture depends on several factors, and to achieve the maximum rate of survival and growth of larvae in the event of failure of diatom culture during the course of larval rearing, a series of studies were made on the use of combination of diatom with other foods. Mock (1972), Brown (1972) and Furukawa (1973) prescribed the use of preserved algae and yeast as supplemental feed when live algae are scarce. Bread yeast was offered with diatom to the larval stages in Philippines (Anon, 1976). Villegas *et al.* (1980) reported highest survival of *P. japonicus* larvae fed with a mixture of *Chaetoceros* and Baker's yeast.

The mysis and postlarva of penaeid prawn are normally fed with *Artemia* nauplii. Though it is a nutritious and convenient food, its availability and large-scale use in hatchery production of larvae are restricted due to high cost and variations in quality and food value of the different strains. In an attempt to finding out an economic alternative to *Artemia*, investigations were carried out on the use of rotifers, *Brachionus* spp. (Platon, 1978; Muthu, 1980), free living nematodes, such as *Panagrellus* (Samocha and Leweinsohn, 1977) and cladocerans like *Moina* spp. It was found that *Brachionus* and *Moina* offered

Table I. Important diatoms used in the rearing of Penaeid larvae

Diatom	Species of prawn	Reference
Skeletonema costatum	*Penaeus japonicus*	Hudinaga (1942)
Chlamydomonas sp. *Dunaliella* sp.	*P. duorarum*	Dobkin (1961)
S. costatum *Thalassiosira* sp. *Cyclotella nana* *Phaeodactyium tricornutum* *Dunaliella* sp. *Exuviella* sp. *Gymnodium splendens* *Isochrysis galbana*	*Penaeus* spp.	Cook and Murphy (1969)
Skeletonema costatum *Nitzschia closterium*	*P. japonicus* *P. monodon*	Liao and Huang (1973)
Coscinodiscus grandi *C. centralis*	*P. kerathurus*	FAO (1974)
Synachosystis sp. *Tetraselmis gracilis*	*Metapenaeus affinis* *M. dobsoni*	Thomas *et al.* (1976 a, b)
Cylindrothica sp. *Tetraselmis* sp.	*P. merguiensis* *P. japonicus* *P. aztecus* *P. semisulcatus* *M. ensis*	AQUACOP (1978)
Tetraselmis sp.	*P. monodon* *P. merguiensis*	Platon (1978) Beard *et al.* (1977)
Chaetoceros gracilis	*P. stylirostris* *P. vannamei* *P. japonicus*	Simon (1978) Villegas and Kanazawa (1980)
Skeletonema sp. *Thalassiosira* sp. *Melosira* sp. *Nitzschla* sp. *Tetraselmis* sp.	*Penaeid* spp.	New (1979)
Chaetoceros, sp.	*P. japonicus* *P. indicus* *P. monodon*	Kurata and Shigueno (1979) Muthu (1980)

in frozen form serve as effective food for the advanced larval stages.

In the culture of palaemonid prawns like *Macrobrachium rosenbergii* mixed algal culture developed from the wild plankton following the techniques of Fijimura (1966) was used for rearing the larvae in certain instances. These cultures were predominantly composed of *Chlorella* at concentrations ranging from 5×10^5 to 2×10^6 cells/ml (Sandifer *et al.* 1976). This rearing technique is mainly encountered in the static larval culture system using 'green water'. Successful rearing of larvae of *Macrobrachium* using single celled algae was reported by Fujimura (1966) and Minamizawa and Morizane (1970). In this connection, monoculture of selected species of algae such as *Chlamydomonas, Chlorella, Dunaliella, Isochrysis, Nannochloris, Phaeodactylum, Pseudoisochrysis, Skeletonema* and *Tetraselmis* was tested (Wickins, 1972; Maddox and Manzi, 1976; Manzi and Maddox, 1977). Manzi and Maddox (1977) obtained a production average of 59 post larvae/litre using *Phaeodactylum tricornatum* at densities of 340,000 cells/ml in the larval rearing tanks.

Although the larvae of *Macrobrachium* were successfully reared using the above algae, it was observed that phytoplankton was not an essential requirement for their culture. Sick and Beaty (1974) have shown that the algae have no nutritional value for the developing larvae of *Macrobrachium*. Nevertheless, there is evidence that the presence of phytoplankton in larval rearing tanks is beneficial as they are capable of converting the excretory products toxic to larvae to less harmful nitrates, thus improving the quality of water (New, 1979).

Macrobrachium larvae can be reared with a good survival rate on a diet of *Artemia* alone. Ling (1969) raised the larvae of *M. rosenbergii* on live zooplankton such as rotifers, cyclops, copepods, insect larvae, and chopped fish, shellfish and steamed chicken egg. Live feeds such as *Daphnia* and oyster larvae were also used. As better growth and survival of larvae were obtaind with a combination of food during the later half or two-thirds of larval development of this species, many feeds such as pulverised fish flesh, cooked egg custard, fish eggs and frozen adult *Artemia* are used to supplement the *Artemia* diet. Recently, Subramanyam (Giant fresh water prawn culture, CIFRI Technology, published by the Director, CIFRI, Barrackpore, year of publication not indicated) successfully reared *M. rosenbergii* through all the larval stages using different particle size of *Tubifex*. Besides the type of food used in larval rearing, Sick and Beaty (1974) reported that appropriate density and size of *Artemia* nauplii influence the growth and survival of the larvae to a great extent. They recorded that among several stocking densities, groups of larvae stocked at 20 to 40 animals/litre showed the best growth and survival rates and that only *Artemia* nauplii larger than 0.7 mm were ingested in adequate amounts to allow maximum rate of growth.

Mass Culture of live food organisms for larval feeding

a. Culture of microalgae

In the hatchery production of prawn seed, a reliable and abundant supply of

live food of appropriate size is of paramount importance. Although concerted efforts are put in to replace the live food organisms by artificial feeds, the former still play a major role serving as principal food during the part or whole of the larval development. Realising this, intensive attempts on large scale culture of microalgoe and zooplankton are carried out by several investigators (Ukeles, 1976; Shaw Watson, 1979; Kinne, 1977 and Kahan, 1982). As a resualt of these studies, considerable progress has been made on large scale algal production technology. Recently, de pauw and Pruder (1981) reviewed the various modes of use and production of microalgae in aquaculture, the biological and technical problems encountered, economic implications and the major research task to improving the system. Similarly the advances made and the constraints faced in large scale production and supply of *Artemia*, and of several non - *Artemia*, live food organisms were reviewed by Sorgeloos (1981) and Nellen (1981) respectively.

Generally, two methods are followed for mass culture of microalgae. In the first method the selected algae are grown in the culture media with or without the supply of nutrients or addition of fertilizers in outdoor tanks of various sizes and shapes using an external light source. Often the algae are cultured along with the consumer species. The second method entails the culture of algae under controlled conditions providing optimal growth conditions such as nutrients, pH, light and agitation. The former is an extensive approach, less expensive, but unreliable for culture of selected or preferred species. The algal culture raised by this system often gets contaminated with other species and production rate is also found to be relatively less than in intensive algal culture. However, the out-door algal culture is found to be suitable for short period, semi-intensive culture and as a valuable supplement to intensive culture system.

At the Narakkal Prawn culture Laboratory (NPCL) of the Central Marine Fisheries Research Institute (CMFRI), mixed culture of phytoplankton predominated by *Chaetoceros* is raised in filtered sea water with fertilisation in white one ton fibre glass tanks utilising solar energy (Muthu, 1980). Successful outdoor culture of valuable species in nurtient rich water from oceanic upwelling was reported by Yoneshigue-Braga and Rodriguez (1975) and Moreira dasilva (1976). Sea water from deep wells was also used for mass culture of algae in Hawaii, while Dunstan and Mensel (1971) and Mann and Ryther (1977) reported the use of sea water enriched with sewage effluent solar irradiated tanks for the dense culture of microalgae. Recently, Radhakrishnan *et al.* (1980) studied the effect of various cheaply available organic substances in the mass culture of selected brackishwater microorganisms under yard conditions. To the filtered brackishwater enriched with a dose of yeast, extracts of cowdung, ground nut oil cake, vegetable leaves, powdered blue green and green algae were added and it was found that the leaf extract alone and in combination with the other organic substances gave the maximum yield of microorganisms. In another study, Joseph *et al.* (1980) studied the utili-

sation of bottom slush from polluted estuarine areas for plankton production.

The intensive culture of microalgae is generally restricted to the culture of selected monospecies and their further upscaling for feeding on large scale. This system required energy intensive steps and careful monitoring of various parameters promoting the balanced growth conditions.

Important steps involved in algal culture are 1) separation of the desired species and maintenance of its stock culture in agar slants or as liquid culture, 2) inoculotion to culture vessels containing appropriate culture media, (3) cultivation with supply of nutrients and other congenial conditions of temperature, salinity, carbondioxide concentration, light, pH and agitation and (4) separation, concentration and harvesting of the cultured species. Ukeles (1977) described the different types of culture vessels, media used and culture techniques developed in indoor mass production of algae. The light source is either natural light or artificial source using flourescent lamps fitted above or around the culture vessels. Recently, transparent polythene containers of 30 to 480 litre capacities are used (Persoone and Sorgeloos 1975; Baynes *et al.* 1979). Devices have been developed for automatic harvesting and replenishment of the culture medium (Trotta, 1981) reducing the labour and construction cost. Helm *et al.* (1979) discussed the design, construction, operation and cost structure of algal culture in 200 l vessel, while Laing (1979) described the methods and media for the batch culture of *Chaetoceros calcitrans* in 2 l, 20 l and 200 l stages.

b. Culture of Artemia

Great strides have been made in recent years on the mass production of *Artemia* which serve as an ideal prepacked larval food (Kinne, 1977; Mohsen Al-Khares *et al.* 1980; Sorgeloos, 1981). It has a world-wide distribution occuring in super saline waters. It is either harvested from the natural source or cultured under controlled conditions in sea water by feeding with various material such as yeast, rice bran, dried algae, beef extract or organic detritus. In the NPCL of CMFRI continuous culture of 3 strains of *Artemia* are maintained in 6' diameter plastic pools feeding with the juice taken out of ground nut oil cake. The research results obtained on hatching quality and on the techniques of harvesting the cysts have appreciably enhanced the production of *Artemia* and its use. Recently, it has been shown that *Artemia* can be successfully transplanted and insulated to a new locality leading to the local production to meet the increasing demand.

c. Culture of Zooplankters

Besides *Artemia,* the most important zooplankton mass cultured for feeding prawn larvae, are rotifers, cladocerans and copepods. Among rotifers, the most widely cultivated species is the euryhaline rotifer, *Brachionus plicatilis* Muller. The technique of mass culture of the species is now well established (Kinne, 1977). In the culture of the species, they are fed with different algal diets such as *Chlorella, Dunaliella, Exuviella, Gymnodium, Monochrysis, Nannochrysis,* bacteria and marine yeasts. Recently, Trotta (1980) described a simple and inexpensive system for continuous culture of *B. plicatilis* fed on live *Chlorella,*

producing daily about 1 million individuals. The harvest concentration was 300/ml. The culture vessel was plastic bags. At the NPCL of CMFRI, continuous mass culture of *B. plicatilis* is carried out in 24' diameter outdoor tanks containing brackishwater. The medium is fertilised with groundnut oil cake which induces dense *Chlorella* blooms. Within 6–7 days high concentration of rotifers (0.5 to 0.6 million/l) is developed. The rotifers are harvested by specially designed silk nets and washed well. They are then frozen into small blocks containing 10–15 million rotifers and stored for use in larval rearing.

Several freshwater species of zooplankters such as *Chirocephalus,* daphnids and *cladocerans* were tried as larval food. Among these, *Moina* received considerable attention in our country. At the NPCL of CMFRI, the species obtained from the local freshwater ponds are mass cultured in out door plastic pools containing freshwater which is fertilised with ground nut oil cake juice. It is found that they develop very fast attaining a concentration of 30,000 – 40,000/l in 6–7 days. They are harvested in the same manner as for the rotifers and frozen into small blocks containing 0.5–0.75 million animals for furture use. Recently, a breakthrough was reported in obtaining dry cysts of *Moina* with appreciable shelf-life.

Copepods form an important food in the rearing of the advanced larval stages of prawns. Various aspects of copepod culture are reviewed by Kinne (1977), Paffenhofer and Harris (1979), Nellen *et al.* (1981) and Kahan (1982). Although, several workers have achieved considerable success in the culture of copepods, calanoids, harpacticoid copepods such as *Tigriopus* and *Tisbe* and recently, the brackishwater cyclopoids (James and Thompson, 1980) on experimental basis, they have been used only to a limited extent in mariculture hatchery systems. Major constraint encountered was to maintain an adequate density of the culture involving light, temparature, salinity, oxygen, pH and other organic waste products in the culture medium. Recently, Kahan *et al.* (1982) developed a simple method for cultivation of Harpacticoid copepod in a floating basket in the larval rearing tanks and discussed the advantages of the method in producing various size and stages of copepods for larval feeding.

Chemical composition of live food organisms

It is well known that the live food organisms serve as ideal food for the developing larvae. Chemical composition of several microalgae and other live food organisms (Table 2) provide the relative nutritive value. It is observed that the diatom such as *S. costatum, Chaetoceros* sp. and *Phaeodactylum* and *Artemia, B. plicatilis, Moina* sp. and *Tubifex* that are currently used in larval rearing possess appreciable levels of macro-nutrients. Although prominent synthesis of protein occurs in diatoms, increased synthesis of fats and carbohydrates at the expense of protein as well as variations in protein/carbohydrate ratio is reported in prolonged batch cultures of certain algae.

Non-living and non-conventional food

In recent years several non-living and non-conventional feed were found

useful in rearing of the larvae of prawns. Hirata *et al.* (1975) successfully used soy cake powdered to a particle size of less than 100μ for rearing protozoea stages of *Penaeus japonicus*. However, in subsequent experiments, they found that a combination of soy cake powder and diatom produced the best results. Powdered fat free rice bran (Ishida, 1967), activated sludge (Imamura and Sugita, 1972), marine yeast (Furukawa, 1973), washings of filamentous algae and Sargassum juice (Anon, 1976), fermented extract of vegetable refuse from kitchen and egg yolk (Anon, 1977) estuarine detritus (Qasim and Easterson, 1974) and decomposed mangrove leaves (Sumitra Vijayaraghavan and Ramadhas, 1980) are some of the non-conventional feeds used in larval and postlarval culture with varying results. More recently Hameed Ali (1980) and Hameed Ali *et al.* (1982) reported successful rearing of larvae of *P. monodon*, *P. indicus*, *P. merguiensis*, *P. semiculcatus*, *Penaeus* sp., *M. monoceros*, *M. affinis*, *M. dobsoni*, *M. brevicornis* and *Parapenaeopsis stylifera* from protozoea through postlarval stage feeding exclusively on a diet of crustacean such as *Acetes indicus*, *Palaemon tenuipes* and *Mesopodopsis* fresh tissue of suitable particle size maintained in a suspension state. Alikunhi *et al.* (1980, 1982) had also achieved success in the large scale rearing of larvae of penaeid prawn fed with tissue suspension prepared with juveniles of *M. dobsoni* and stomatopod crustaceans.

In the rearing of *M. rosenbergii*, Subramanyam (Vide, Giant fresh water prawn culture, CIFRI Technology) offered plant products such as soy beans, maize, sorghum, coconut oil cake and cotton seed cake as supplementary feeds alongwith rotifers, brine shrimp nauplii and tubificid worms.

Artificial diet

With the increasing commercialisation of aquaculture of prawns, the demand for suitable feed and their steady supply is ever increasing. Large scale production of microalgae and other live food organisms is found widely fluctuating and often contaminated by unwanted species. Besides this, a great amount of manual labour, large quantities of culture medium, and vessels and equipments are involved in their production, adding considerably to the running cost of the culture operation. In an attempt to overcome these constraints, several attempts have been made to rear prawns on artificial diets (Subrahmanyam and Oppenheimer, 1969; Kanazawa *et al.* 1970; Forster and Gabbott 1971; Cowey and Forster, 1971; Shigueno, 1975; Sick *et al.* 1972; Kitabayashi *et al.* 1971, a, b, c; Aquacop 1978, Goswami and Goswami 1982; Raman *et al.* 1982; Mohamed Sultan *et al.* 1982; Ahamed Ali 1982). However, most of the diets developed were used to growing juveniles in the nursery and grow-out systems. Shigueno (1975) compounded diet with squid meal, squid extract, petroleum yeast, activated gluten, alpha-starch and vitamin and mineral mix in particulate size to rear larvae of *P. japonicus* and reported some success. Later, Villegas and Kanazawa (1980) prepared an artificial diet (Diet B) composed of glucose (5.5% in dry weight), sucrose (10%), starch (4%) glucosamine (0.8%), lipid and vitamin-free casein (50%), Na-succinate (0.3%), Pollack residual oil (8.1%),

Table 2. Chemical composition of some important live food organisms offered to prawn larvae

Organism	Percentage dry weight				Reference
	Protein	Carbo hydrate	Fat	Ash	
Tetraselmis maculata	52	15.0	2.9	—	Parsons *et al.* (1961)
Dunaliella salina	57	31.6	6.4	—	
Chaetoceros sp.	35	6.6	6.9	—	
Skeletonema costatum	37	20.8	4.7	—	
Phaeodactylum tricornatum	33	24.0	6.6	—	
Exuviella sp.	31	37.0	15.0	—	
Chaetoceros sp. (unialgal culture growing exponentially)	48.6	9.2	9.5	—	Lewin and Guillard (1963)
S. costatum (unialgal culture growing exponentially)	60.6	34.71	7.7	—	
S. costatum (unialgal culture, grown 2-4 weeks)	43.52	34.55	21.93	—	
P. tricornatum (unialgal culture growing emponentially)	35.7	25.9	7.1	—	
P. tricornatum (fusiform cells from 16-d culture)	46.5	2.2	38.6	—	
P. tricornatum (oval cells from 16-d culture)	37.7	21.1	26.6	—	
Diatom *(Chaetoceros)* *	29.0	8 0	63.0	—	From Marshall and Orr (1960)
Diatom (Mixed) *	24.5	14.2	61.3	—	
Mixed zooplankton *	46.0	6.0	23.0	25	
Artemia nauplii	55.60	—	15.20	15.25	Gallagher and Brown (1975)
Brachionus plicatilis	59.07	8.44	24.05	8.44	Charles John Bhaskar (1982)
Moina sp.	56.69	13.47	23.73	6.11	
Tubifex	65.0	15.0	14 0	6.0	Bardach *et al.* (1972)

* Grams per 100 gram Organic matter

cholesterol (0.5%), mineral mix (8.6%) vitamin mix (2.7%)' cellulose powder (9.3%) and water 130-135 ml. Agar was used as a binder. The powdered diet, passed through a sieve of mesh size 10-50 μm, was offered to the larvae from protozoea stage at a feeding rate of 0 16 mg/larva/day. The result of this dietary experiment showed that the larvae metamorphosed into mysis stage in 8 days with a survival rate of 53.2%.

In 1974 Sick and Beaty formulated 6 types of diets with varying composition of fish meal, soybean meal, shrimp meal, with or without albumin and *Artemia*, vitamin and mineral mix, alginate, linseed oil, Menhaden oil and cellulose in three forms, namely, freeze-dried, gel and dry flake, and experimented with *M. rosenbergii* larvae. The results of the experiment indicated that the larvae fed with the diet in freeze dried form with *Artemia* meat grew through all the stages, and the heighest rate of ingestion was observed by the 7-8 stage larvae with freeze-dried diet with *Artemia*.

At the NPCL of CMFRI, the larvae of *P. indicus* are being successfully reared from protozoea to postlarvae on a micro-particulate compounded feed prepared from mantis shrimp, prawn waste, ground nut cake, fish meal and tapioca with a survival rate of over 35%. Besides this, several feeds compounded with indigenously available raw material having different levels of protein, carbohydrate, lipid and other micro nutrients and different particulate size are experimented with the larvae and postlarvae of *P. indicus* with encouraging results (Ahamed Ali, MS).

Following the report on the use of the technique of micro-encapsulation for supplying artificial diet to filter feeding larvae by Jones *et al.* (1974) and Jones *et al.* (1976), Moller *et al.* (1979) experimented with the mysis larvae of *P. merguiensis* on a microencapsulated diet and successfully reared them to postlarva II. Subsequently, Jones *et al.* (1979) formulated different micro-encapsulated diets (particle size of 20 μm to 100 μm) with chicken egg, short-necked clam *(Tapes phillippinarum)*, soy cake and the purified diet (Diet B) earlier developed by Kanazawa *et al.* (1977) to rear *P. japonicus* from protozoea to postlarva. They found that optimum particle size of diet for protozoea I larvae was 10 μm and as the larva advanced to Mysis II and III stages it selected larger size particles of about 28 μm. The postlarva was seen to feed only on food particles larger than 28 μm size. They further observed that micro-encapsulated diet of *Tapes* and egg produced 50% survival rate and it would be possible to achieve still higher survival rate if the right particulate size diet is given in appropriate quantity and with proper management of water quality. Further studies on the large scale production and economic use of microencapsulated diets for larval rearing and to find out the nutritional requirement of the different larval stages are progressing.

Feeding Rate and Frequency

It is well known that, to achieve significant growth and survival, the food supplied should be properly ingested and assimilated by the prawn. In this context, information on the amount of food that is actually consumed by the

animal and frequency of feeding is essential. Such data are also essential for designing and evaluating the diets. In nature, it is observed that prawns eat frequently and rapidly. As their digestive tract is short, there is only a little time for absorption of food. Further, in caridean prawns the gastric mill is not present in the anterior chamber of proventriculous as in the penaeid prawns, and hence the dry food is observed to congest the proventriculous and hamper proper enzymatic mixing with the food (New, 1979).

The feeding rates in prawns have been reported to range from 3 to 20% of the total biomass of the animal per day (Subrahmanyam and Oppenheimer, 1969; Kanazawa *et al*. 1970 and Brown, 1972) and the ingestion rates are inversely related to the animal size.

In the rearing of penaeid prawns. *Artemia* nauplii are usually supplied at densities varying from 0.3-3 nauplii/ml, while the postlarva is found to consume 50-90 *Artemia* nauplii per day. In the culture of *Macrobrachium* larvae with *Artemia* as food, a density of 5 to 10 nauplii/ml is generally provided. Sick and Beaty (1974) studying the relative rate of ingestion in *M. rosenbergii* with 6 formula diets, found that stage 7 and 8 larvae consumed higher amount of the diet containg *Artemia* meat. Similarly, the diets having a balanced starch albumin ratio and 15% egg albumin were taken at a relatively higher rates in freeze dried form than any other diets prepared in flake or gel forms. Villegas *et al*. (1980) recorded the highest survival (76 8%) of *P. monodon* larvae fed on a mixture of *Chaetoceros* and baker's yeast at feeding levels of $10 - 50 \times 10^3$ cells/ml and 1g / ton / day respectively. When the larvae were fed on *Chaetoceros* sp. alone, the optimum feeding level was found at a density of $10 - 50 \times 10^3$ cells/ml.

Venkataramaiah *et. al* (1975b) observed that pellets of 1-2 mm diameter were taken by the postlarvae of 9 mm size and above. If the pellets offered were of larger size, the postlarvae (9-15 mm long) were seen sitting on them and nibbling. Sometimes a single pellet was carried by more than one postlarva. They also found that the feeding ratio in *P. aztecus* during the process of growth from postlarvae (9.5 mm) to sub-adults (100 mm) bear roughly inverse relationship to the body weight, the feeding level being 100% of body weight in the early postlarvae, decreasing gradually to 5% of the body weight in the sub-adults Food consumption in *P. aztecus* was found to depend on water temperature and to a lesser extent on salinity concentration of medium. At a temperature of 31°C, *P. aztecus* was very active feeding at more than 11% rate while at a low temperature (21°C) it was sluggish and failed to consume the food offered at 8% feeding rate. However, increase in food consumption with temperature failed to yield the proportionate growth. The optimal feeding levels for the species was found at 6.2, 8.1 and 11% at 21, 26, and 31°C respectively (Venkataramiah *et al*. 1975 b)

Experimenting with microencapsulated diet Jones *et al*. (1979) recorded that *P. japonicus* larvae died within 10 days at Mysis 1 stage when fed at a concentration of 100 capsules/ml, but a

feeding concentration of 500–1000 capsules/ml, the larvae metamorphosed successfully from protozoea to postlarva. At the 1000 capsules/ml level, the larval mortality was found to be high, particularly in the protozoea stage. The feeding level of 500 capsule/ml was found to give better results. With the microparticulate diet, Kanazawa *et al.* (1977) found better growth rates at a feeding rate of 0.16 mg/of diet per larva per day. At a higher concentration of 1.6 mg/larva/day of feeding rate, mass mortality occured due to pollution of the rearing medium by the diet. Hirata *et al.* (1962) reported a survival rate of 85.9% in the rearing of P. japonicus larvae at the end of 6th day feeding soy cake particles at a rate of 0.16 mg/larva/day.

Several factors such as age, behaviour and physiological conditions of the species, light, substratum, biomass composition and physical and chemical characteristics of food influence the rate of ingestion of food. Feeding 3-4 times per day is found to be better than feeding once in a day. Sick *et al.* (1973) found that the ingestion rate decreased significantly in *P. setiferus* fed on a pelleted diet after 6 hours of exposure to the same food, probably due to changes in the physical and chemical characteristic of the food

The chemosensory properties of diets are found to attract shrimps for feeding. Substances such as betaine (trimethylamonium hydroxide), morin (a fragrant aromatic compound), egg-white protein and non-essential amino acids such as glutamic acid, glycine, and taurine are found to stimulate the prawns into feeding activity. It is reported that one private company in the USA has patented a food using a mixture of mono-sodium glutamate with sodium or potassium aspartate, which induces hunting and feeding reactions in prawns.

Nutritional Requirement

In recent years several studies have been carried out to understand the nutritional requirements of prawns and shrimps with diets of different protein, carbohydrate, lipid, mineral and vitamin composition. While the results of these studies have considerably contributed to the knowledge of the nutritional demands of these animals, there is wide difference in the observation of the various workers on the requirements of the optimum dietary level of both macro and micronutrients which provide the optimum growth and the highest survival rate in a given species of prawn. Further, most of these studies relate to the nutritional requirements of juveniles and sub-adults, the information on dietary requirements of larvae being utterly scarce.

It is observed that the nutritional requirements of larvae may be different from those of juveniles and adults consequent upon the behavioural and dietary changes occuring during metamorphosis through different stages. The pattern of life and mode of feeding also changes as the larvae grow to advanced stages. An attempt is made here to summarise the existing knowledge on the dietary requirements of prawns as it would facilitate investigations on the nutritional requirement of larvae which forms an area of immediate concern in the development of suitable feed in the hatchery production of seed.

Determination of optimum dietary levels of protein has been the subject of several studies (Kanazawa *et al.*, 1970; Lee, 1970; Kitabayashi *et al.* 1971 a, b, c and Deshimaru and Shigueno, 1972; Andrews *et al.* 1972; Balazs *et al.* 1973; Forster and Beard, 1973; Colvin, 1976; Deshimaru and Kuroki, 1975; Venkataramiah et al., 1975a; New, 1976 and Ahmed Ali, 1982a). Although the protein level as investigated in these studies ranges from 15 to 80% in the diet, it is generally opined that a protein level of 27-35% is the optimum requirement for the juvenile penaeids. It is also observed that with better understanding of the aminoacid profile of the prawn, source of protein and synergetic effects of the dietary component, still lower levels of protein may be adequate to obtain satisfactory growth. Working with the larvae of *Palaemon serratus* Van wormhoudt (1973) found that protease activity in the larvae is related to dietary changes occuring during metamorphosis. Recently, Charles John Bhasker (1982) conducted dietary experiments with purified (casein, starch, lipid (fish oil and ground nut oil), vitamins, minerals and cellulose) and semi-purified (casein, tapioca powder, ground nut oil, vitamins and minerals) diets with the post-larvae of *P. indicus* and found that the protein requirement in the diet decreased with increased in size of the post-larva and the optimum protein requirement was between 30 and 40% when adequate levels of carbohydrate (35-40%) and lipid (10%) were used.

Requirements of specific amino acids were studied by Cowey and Forster (1971), Kittabayashi *et al.* (1971 a, c, d,) Deshimaru and Shigueno (1972) and Torres (1973) in penaeid prawns and Miyajima *et al.* (1975) and Watanabe (1975) in *Macrobrachium ohione* and *M. rosenbergii* respectively. About 10 amino acids such as arginine, histidine, leucine, isoleucine, lysine, metheonine, phenylalanine, threonine, tryptophan and valine are found to be essential for penaeid prawns. In *M. rosenbergii*, however it has been shown that the species could synthesize most of the essential amino acids in adequate level (Watanabe, 1975; Stahl and Ahearn, 1978). The amino acid profile is found to differ in different parts of body of the prawns (Shewbart *et al;* 1972) and from species to species.

The nutritional importance of carbohydrates in prawns was studied by Tyagi and Prakash (1967), Cowey and Forster (1971), Forster and Gabbott (1971), Kitabayashi *et al.* (1971,d), Andrews *et al.* (1972), Sick and Andrews (1973) and Ahamed Ali (1982). Herbivorous shrimps showed strong carbohydrase activity. Generally, glucose is found to be poorly assimilated than corn or wheat starch and oyster glycogen (Forster and Gabbott, 1971). However, a suitable dietary carbohydrate is found to be necessary to spare or preclude the use of carbon chain from amino acids for chitin synthesis (Cowey and Forster, 1971). Van wormhoudt (1973) reported that in the larvae of *Palaemon serratus* amylase activity varied in different larval stages reaching the high level in the 2nd stage. Ahamed Ali (1982) studying the effect of carbohydrate in purified diets observed that the growth of *P. indicus* juveniles enhanced with the increase in carbohydrate dietary level upto about 40%. Highest growth was recorded at

5% lipid at each carbohydrate level. This observation indicates that the performance of the diet could be improved by increasing the dietary carbohydrate level due to its protein sparing action. Studies have also been carried out on carbohydrate metabolism (Dall, 1964) and on the absorption rate of specific monosaccharides (Sick ank Andrews, 1973).

Athough lipids form an important source of energy, it is found that prawns do not require high levels of dietary fat and lipase activity is limited (Andrews and Sick, 1972). It is reported that addition of lipid to the diet at different levels (7.5 to 20%) adversely affects the growth and survival of the prawn (Andrews *et al.* 1972; Forster and Beard, 1973). However, it has an attractant quality. Tissue lipid levels are found to vary from season to season and during the moulting cycle. The Important fatty acids in prawns are palmitic acid and W_3 polyunsaturated fatty acids. It is observed that W_3 fatty acids are retained in the tissue, while W_6 fatty acids are mobilised for energy. However, the low levels of the former and the high levels of the latter are found to have inhibitory effect. Recently, it has been shown that linoleic, linolenic and W_3 - long chain polyunsaturated acids are essential in the diet of the prawn (Kanazawa *et al.* 1979) and a requirement between 1 and 2% for linolenic acid has been indicated (Shewbart *et al.* 1973). Jones et al., (1979) examined the fatty acid biosynthesis in the larval stages of *P. japonicus* and found that the highest larval growth rates were achieved on diets containing *Tapes phillippinarum* lipid. This study also revealed that 16 : 1W 7, 18 : 0 and 18 : 1W 9 fatty acids may be synthesised from palmitic acid and the larvae possess the ability to elongate 18 : 3W3 to 20 : 5W3 and 22 : 2W6 and 18 : 2W6 to 20 : 4W6. However, as their conversion rate is found to be slow, it is reported that eicosapetaenoic acid (20 : 5W3) and decosahexaenoic acid (22 : 6W3) are essential in the diet.

Prawns, like other crustaceans, cannot convert acetate into sterols which are hence required in the diet (Teshima and Kanazawa, 1971). A cholesterol level of 0.5% in the diet is found to be better than 0.05 or 1% while a higher level of 5% seem to depress the growth. A basic diet containing 0.5% cholesterol supplemented with inokosterone, cyasterone or ecdysterone extracted from plants is found to increase the moulting frequency. It is reported that *P. japonicus* converts desmonsterol into cholesterol and C_{28} - and C_{29} - sterols for growth.

Information on nutritional requirements of mineral and vitamins of prawns is limited. Kitabayashi *et al.* (1971 a) recorded greater growth rates when phosphorus and calcium were added to the diet. However a calcium/phosphorus ratio of above 2.4 : 1 was found to depress the growth. It was reported that Mg and Fe are nonessential, but 2% P, 1% K and 0.2% of a trace element premix was useful (New, 1979)

Fisher (1960) reviewed the requirement of vitamins in crustaceans in general. Most of the B-group vitamins, vitamin C and E are found to be essential. Although vitamin A seem to be not essential, it is found in several species of penaeids. Kitabayashi *et al.* (1971 b) found accelerated growth of *P. japonicus* with vitamin C in the diet. Most of

the prawn diets include vitamin A or β-carotene and vitamin K. There is little information on the dietary level of vitamins, although 0.5-1% of vitamin C and 0.4% of inositol were found to be optimal requirement by some workers (New, 1979). It is cautioned that improper use of vitamins without understanding their role in the diet may produce toxic or antagonistic result.

General Remarks

The successful application of aquaculture technology for increased production of prawns depends on the development of a viable culture system with a feasible technical, economic, marketing and social approach. Sustained demand, large scale production and marketing of the farmed prawns in its turn greatly depends on the acceptance of the produce by the consumer who is primarily interested in the quality of the product, its appearance, odour, flavour and texture. In all these, the quality and quantity of the food consumed by the prawn, its assimilation and absorption play an important role.

In the mass rearing of prawn larvae that transforms into different stages within a short period, with structural modification of feeding apparatus and changing their food regime, the selection of a suitable food of appropriate size is important. The selected food should be physically available close to the larvae, easily tackled, digestable, metabolizable and meet the nutritional requirement of the larvae; it should not pollute the medium in which the larvae are reared. In recent years considerable progress has been made in the technology of large scale culture of valuable live food organisms, particularly the microalgae which form the major food source in the controlled rearing of prawns. However, in the uncontrolled extensive method of mass production of microalgae in outdoor tanks using the natural source of light, the major problem encountered is the fluctuation in production The algal production is influenced by factors such as nutrients, characteristics of the medium and its environmental parameters and availability of light. Blooms of unwanted and often toxic species which develop in the culture tanks also pose problems, as they lead to mass mortality of larvae. Although this system is less expensive, it is labour intensive.

The controlled production of monospecific microalgae for direct feeding of larvae has met with some success in experimental and small scale operations. But constraints are encountered when the defined algal culture is upscaled to feeding the larvae in the hatchery due to premature collapsing of the cultured species and contamination with other species. In this context, further studies on the growth requirements of microalgae in order to increase their life span and to find out reasons for premature collapsing when upscaled are essential. Information on the nutritional value of the microalgae used in the larval rearing is also an essential prerequisite to select the most suitable species from among the several used at present. Data on the relative efficiencies of the different systems of algal production along with the cost would help in developing suitable design to meet the requirement of huge quantity of food in hatchery production of prawn seed. The

development of a technology for proper preservation of microalgae for use in adverse or emergency situation would go a long way in the successful hatchery production of prawn seed.

In the field of development of formula, compounded diet suitable for intensive culture of prawns, there has been appreciable progress in recent years. Several diets formulated by using locally available ingredients, commercial meals and chemical ingredients of different protein, carbohydrate, lipid, mineral and vitamin mix represent the attempt made and contribute to the understanding of their use. By and large the studies carried out with the formula diet describe the levels of macro and micro nutrients in the diet, the relative growth and survival rates obtained in the experiments, physical characteristics of the diet and to some extent, the nutrirional requirement of the species. However, comparison of the relative merits of the diets is made difficult due to the varieties of dietary ingredients used and contradication in the results obtained and the observations made. Thus there is inconsistency as to the optimum dietary requirement of protein, carbohydrate and lipids. Studies on nutritional requirements of micronutrients have received relatively less attention.

It is only since the past decade the larval nutrition attracted the attention and ways and means of solving the physical problems of larval diet were suggested. Besides the requirement of suitable particle size of the food the artificial larval diet should be palatable, its nutrient must not leach out, it should not pollute the water and it should be easily available to the delicate larvae. As a result of the investigations carried out so far, a few diets for the larvae have been developed, the latest being the microencapsulated feeds of 15–100 μm size. However, these diets are yet to be used in large scale production of seed and commercialised.

Data on nutritional requirements of larvae are limited to a few recent investigations (Sick and Beaty 1974; Sick, 1976; Jones *et al*. 1979; Villegas and Kanazawa, 1980). There is little information on the nutritional requirements of cultivable species of penaeid and palaemonid prawns of India, although the recent technical advances made on the development of microencapsulated diets have greatly helped to study these aspects. A major research task is needed on several aspects of larval nutrition, the important areas being total nutritional requirements of different larval stages of cultivable species, development of inexpensive but balanced diet of suitable particale size with locally available ingredients, determination of caloric requirements of larvae, nutritional diseases of larvae and the development of viable feed technology to produce the enormous quantity of larval feed required in large scale production of seed of cultivable penaeid and palaemonid prawns.

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