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**GROWTH AND SURVIVAL OF THE POST LARVAE OF
Penaeus monodon UNDER DIFFERENT DIETARY CONDITIONS**

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Dissertation Submitted By

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IN PARTIAL FULFILMENT FOR THE DEGREE OF
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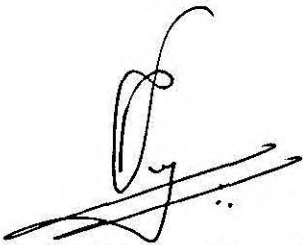
***DEDICATED TO MY LOVING PARENTS AND
BELOVED SISTER***

CONTENTS

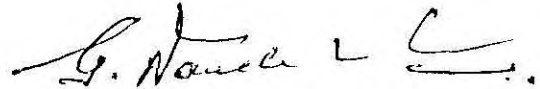
	PAGES
CERTIFICATE	I
DECLARATION	II
ABSTRACT IN HINDI	III
ACKNOWLEDGEMENT	IV
PREFACE	1 - 3
INTRODUCTION	4 - 20
MATERIALS AND METHODS	21 - 32
RESULTS	33 - 44
DISCUSSION	45 - 54
SUMMARY	55 - 57
REFERENCES	58 - 73

CERTIFICATE

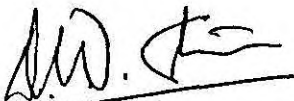
Certified that the dissertation entitled "**Growth and Survival of the Post larvae of *Penaeus monodon* under different dietary conditions**" is a bonafide record of the work done by **Kum. Roshni Gopal. V.** under our guidance at the Central Marine Fisheries Research Institute during the tenure of her M.Fsc (Mariculture) programme of 1995-97 and that it has not previously formed the basis for the award of any other degree, diploma or other similar titles or for any publication.



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I hereby declare that this thesis entitled "**Growth and Survival of the post larvae of *Penaeus monodon* under different dietary conditions**" is based on my own research work and has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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Roshni Gopal V.

सारांश

पेनिअस मोनोडोन के पश्चर्डिभकों {पी एल 22} को आइसोनाइड्रोजीनस {47% सी पी} और आइसोकलोरिक {4.5 कि.क/ग्रा} के आमिश्रित खाद्य में विटामिन और खनिज के पूरक मिलाकर और बिना मिलाकर दिया और पालन किया. खाद्य के सोल श्रोत के रूप में और बनाए गए खाद्य के साथ आमिश्रित करके जीवंत खाद्य {पादपप्लवक: कीटोसिरस कैल्सिट्रन्स और प्राणिप्लवक: आर्टीमिआ नोप्ली} भी दिया गया. नियंत्रित वातावरण में 21 दिन के पालन की अवधि के दौरान बढ़ती में कोई महत्वपूर्ण विभिन्नता नहीं {पी > 0.05} दिखाई पड़ी. खाद्य वस्तुओं में होने वाले विटामिन एवं खनिज पीएल 43 स्थिति के झींगों की बढ़ती के लिए पर्याप्त देखा गया. इससे यह व्यक्त होता है कि आमिश्रित खाद्य में विटामिन और खनिज और भी जोड़ने की आवश्यकता नहीं है. पादपप्लवक या प्राणिप्लवक खाद्य के सोल श्रोत के रूप में पर्याप्त नहीं है फिर भी आर्टीमिआ नोप्ली को आमिश्रित खाद्य में मिलाने पर अत्यधिक बढ़ती रिकार्ड की गई. परीक्षण किए गए अन्य संयुक्त खाद्यों की तुलना में कृत्रिम खाद्य देने पर झींगों की अतिजीवितता के प्रतिशत में उल्लेखनीय विभिन्नता देखी गई. जीवंत खाद्य अतिजीवितता और बढ़ती में ना कि वृद्धि लाते या पूरक खाद्यों को पोषण के तौर पर कोम्प्लिमेन्ट नहीं करते.

.....

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1. PREFACE

Globally during the last decade the production of marine finfish and shellfish has reached the peak and aquaculture - "the fish husbandry method to augment finfish and shell fish production," is the only hope for a quantum jump in fish production. Aquaculture emerged and established as an alternative to meet the requirement of seafood consumers. The important factors in the success of any culture operation are seed and feed coupled with water management. Of these factors feed is the largest single item in the running expenditure in aquaculture. It accounts for about 50-60% of the total variable cost. Feeds and feeding are intricate processes that can increase both production and profit. So, it is necessary to have adequate knowledge on the feeding habits and preferences of the cultured organisms and their nutritional requirements.

In nature crustacean, finfish and other larvae and juveniles feed by predation, foraging and scavenging. Typically, the larval habitat is a rich, dense and diverse biotope with a broad array of microorganisms including zooplankton, phytoplankton, diatoms, copepods, ciliates, bacteria and others. Thus the natural nursery functions efficiently and is a supermarket of microscopic life forms. Where as, aquatic husbandry violates the organisms natural predisposition as programmed within its genetic code.

When aquatic organisms are bred in captivity, their nutrition is totally dependent on the aquaculturist. Larval nutrition is one of the most crucial aspects in running a commercial hatchery. The current technology allows for the inhouse culture of several livefeeds including monoculture of live microalgae (eg.:- *skeletonema*, *isochrysis*, *chaetoceros* and *chlorella*), culture of *artemia nauplii* and rotifers. Other livefeed organisms include diverse species such as copepods, *daphnia* and mollusc larvae. The majority of hatcheries rely upon live culture of algae, rotifers and other microorganisms along with the feeding of *artemia nauplii*. These live feeds are good as they do not pollute the environment. But, maintenance of these livefeed cultures require specialised technical input, labour and time. Further more the livefeed cultured in the hatchery may in fact, be nutritionally deficient due to limitations on physical and chemical conditions of livefeed culture methods. The other method is to provide artificial or balanced compounded feed of required particle size so that the larvae can feed on them. Different compounded feeds have been tried in larviculture with varied success in different parts of the world.

Limitations in the knowledge of nutrition particularly that of marine shrimp under score the need to continually develop, test and apply new nutrition concepts. This is particularly true for rapidly expanding shrimp feed

industry where feed formulations are presently based largely on intuitions and unknown growth factors rather than nutritional science.

The present study aims at determining the food preferences and growth performance of the Postlarvae of *Penaeus monodon* fed livefeeds (*Chaetoceros* and *Artemia nauplii*) and artificial diets in different permutations and combinations.

2. INTRODUCTION

The technological developments on the large scale culture of shrimps have been significant all over the world since the successful rearing of *Penaeus japonicus* by Hudinaga (Fujinaga) in 1942. In the initial phase the efforts were mainly directed to develop appropriate breeding technologies for cultivable species under controlled conditions. Of late, however there is a great awareness in the importance of the knowledge on the nutritional requirement of these species and the development of an appropriate feed technology for their successful culture operation. There is also a thinking that the triumph of future aquaculture of these animals shall largely depend on a sound understanding of all the aspects of the nutritional environment of the shrimp. However, information on shrimps nutrition is relatively less, compared to those available for finfish.

2.1 Live food organisms in the larval and postlarval rearing of penaeid shrimp.

Inadequacy of knowledge on the nature of food consumed by the larvae in the wild formed a constraint for a long time in the successful rearing of shrimps from egg through different larval stages. The development of pure

cultures of *Skeletonema costatum* by Hudinaga (1942) for feeding the protozoa of *Penaeus japonicus* paved the 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 postlarval stages. In 1962, Hudinaga and Miyamaru found that *Chaetoceros ridigus* is also suitable for the culture of early larvae of *P. japonicus*. *Chaetoceros* spp. was found to be superior over *Skeletonema* as they could be cultured in high temperatures. Moreover, it was also found to be easily digestible by the larvae. It is also reported that the shrimp larvae can be successfully reared through different stages by feeding entirely on a monoculture of the diatom, *Chaetoceros* at a higher concentration of 30,000 - 1,00,000 lakhs cells/ml (New, 1979).

Fuginaga and Kittaka (1967) and Fuginaga (1969) used mixed cultures of diatoms in outdoor tanks with appreciable survival rates of the larvae of *P. japonicus*. Since then, several diatoms of either a pure culture or in combinations were developed and used to feed the larvae by different workers. Mock (1972), Brown (1972) and Furukawa (1973) prescribed the use of preserved algae and yeast as supplemental feed, when live algae were scarce. Villeges et al., (1980) reported high survival of *P. japonicus* larvae fed with a mixture of *chaetoceros* and baker's yeast.

The mysis and the postlarval stages of penaeid shrimps are normally fed with *artemia nauplii*. Though it is a nutritious and a convenient food, its availability and large scale use are restricted due to high cost and variations in quality and food value of different strains. In an attempt to find 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, 1974) and cladocerans like *Moina* spp. It was found that *brachionous* and *moina* offered in frozen form serve as effective food for advanced larval stages.

Postlarvae of *Penaeus merguensis* weighing 34-30 mg were reared on mysids at 35% for 30 days, and their food intake, growth and conversion efficiency was determined (Reddy et al., 1980). In 30 days, 1 Kg. of initially stocked postlarvae in the weight range of 34-30 mg requires 22.25 Kg. of mysids to produce 10.85 Kg. of new juvenile flesh.

Algal diets were used to rear the larvae of *Metapenaeus ensis*. It was found that the larvae could be reared from protozoa to PL1 on *Chaetoceros gracilis*, *Isochrysis galbana* or *Platymonas* spp., but the survival of the larvae fed on *platymonas* spp. was considerably lower.

In addition *C. gracilis* could substitute for artemia nauplii as a diet for postlarvae during first few days of development. There were no difference in the size of PL fed on *C. gracilis* and *artemia nauplii* (Chu and Lui 1988).

P. monodon larvae reared to PL-1 stage were fed with diatom, *Biddulphia longicuris*. A density of 5000 - 10000 cells/ml of *B. longicuris* was maintained in the larval rearing tank. The mean survival rate from Z1-Mysis larval stage was 88.7% and mysis to postlarvae (PL 1) stage was 91.6% (Samarasinge et al., 1993)

2.2 Larval and postlarval rearing of fresh water prawn with live feeds

Sandifer et al., (1976) reported that palaemonid prawns like *Macrobrachium rosenbergii* can be reared successfully on mixed algal cultures which were predominantly composed of *chlorella* at concentrations ranging from 5×10^5 - 2×10^6 cells/ml. Sick and Beaty (1974) showed that phytoplankton was not an essential requirement for the culture of *M. rosenbergii*.

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. Subrahmanyam (1969) successfully reared the *M. rosenbergii* through all the larval stages using different particle sizes of tubifex.

The foregoing reports, and a few more which were not discussed, on the use of diatoms in shrimp larviculture and postlarval rearing are summarised in table 1.

Table 1

Important diatoms used in the rearing of Penaeid shrimps

Diatoms	Species of prawns	References
<i>Skeletonema costatum</i>	<i>P. japonicus</i>	Hudinaga 1942
<i>Chlamydomonas</i> spp. <i>Dunaliella</i> sp.	<i>P. duorarum</i>	Dobkin (1961)
<i>S. costatum</i> <i>Thalassiosira</i> spp. <i>Gymnodium splendens</i> <i>Isochrysis galbana</i> <i>Dunaliella</i> <i>Cyclotella nana</i> <i>Exuviella</i>	<i>Penaeus</i> spp.	Cook and Murphy (1969)
<i>S. costatum</i>	<i>P. japonicus</i>	Liao and Huang (1973)
<i>Coccinodiscus grandi</i> <i>C. centralis</i>	<i>P. kerachurue</i>	FAO (1974)
<i>Synechosystis</i> <i>Tetraselmis gracilis</i>	<i>Metapenaeus affinis</i> <i>M. dobsoni</i>	Thomas et al., (1976a,b)
<i>Cylindrotheca</i> <i>Tetraselmis</i>	<i>P. merguiensis</i> <i>P. japonicus</i> <i>P. aztacus</i> <i>P. semisulcatus</i> <i>M. ensis</i>	Aquacop (1978)
<i>Tetraselmis</i>	<i>P. monodon</i>	Platon (1978)
<i>Chaetoceros gracilis</i>	<i>P. stylirostris</i> <i>P. japonicus</i>	Simon (1978) Villeges and Kanazawa (1980)
<i>Skeletonema</i> <i>Thalassiosira</i> <i>Nitzzhia</i> <i>Tetraselmes</i>	<i>Penaeid</i> sp.	New (1979)
<i>Chaetoceros</i> spp.	<i>P. japonicus</i> <i>P. indicus</i> <i>P. monodon</i>	Kurata and Shigueno (1979) Muthu (1980)

2.3 Non-conventional feed

In the recent years several nonliving and nonconventional feeds were found useful in rearing of the larvae of shrimps and prawns. The protozoa stage of *P. japonicus* were successfully reared using soyacake powdered to a particle size of less than 100 microns (Hirata *et al.*, 1962). Later experiments showed that a combination of soyacake and diatoms produced best results. Some of the nonconventional feeds that are used for rearing the larval and postlarval stages include the following.

Non conventional Feed	Reference
1. Powdered fat free rice bran	Ishida (1967)
2. Activated sludge	Imamaru and Sugita (1972)
3. Marine yeast	Furukawa (1973)
4. Washings of filamentous algae	Anon (1976)
5. Sargassum juice	Anon (1976)
6. Fermented extracts of vegetable refuse	Anon (1977)
7. Egg yolk	
8. Estuarine detritus	Qasim and Easterson (1974)
9. Decomposed mangrove leaves	Sumitra and Ramadhas (1980)

The above non conventional feeds have been experimented with varying results.

Hameed Ali (1980) and Hameed Ali et al., (1982) reported successful rearing of the larvae of *Penaeus monodon*, *Penaeus indicus*, *Penaeus merguensis*, *Penaeus semisulcatus*, *Metapenaeus monoceros*, *Metapenaeus affinis*, *Metapenaeus dobsoni*, *Metapenaeus brevicornis* and *Parapenopsis stylifera* from proptozoea to postlarval stages by feeding exclusively a diet of crustaceans such as *Acetes indicus*. Alikunhi et al. (1980, 1982) achieved success in the large scale rearing of the larvae of penaeid shrimps fed with tissue suspension prepared with juveniles of *Metapenaeus dobsoni* and stomatopod crustaceans.

Subramanyan (1969) reared *Macrobrachium rosenbergii* by offering plant products such as soyabeans, maize, sorghum, coconut oil cake and cotton seed cake as supplementary feeds along with rotifers, brine shrimp nauplii and tubifex worms.

2.4 Artificial diets

Commercialisation of shrimp culture has led to an increased demand for suitable feeds for different stages in their life cycle. Large scale production of microalgae requires great amount of manual labour, large quantities of culture medium, vessels and equipments, adding considerably

to the running cost. Further more, their production is found to be widely fluctuating and often contaminated by unwanted species. In an attempt to overcome these constraints several attempts have been made to rear shrimps on artificial diets.

Shigueno (1975) successfully reared the larvae of *Penaeus japonicus* using a compounded diet with squidmeal, squid extract, petroleum yeast, activated gluten, alpha-starch, vitamin and mineral mixture.

Postlarvae of *P. monodon* (PL.10 - PL.40) were reared successfully using formulated feed in the Brackish Water Aquaculture Development Center, Jepara, Indonesia (1980). The formulated feed contained squid head meal 10%, fishmeal 20%, Soyabean cake 34%, shrimp meal 24%, Wheat flour 8%, Kel coalginate 2% and Aqua Mix 2%. Villeges and Kanazawa (1980) prepared an artificial diet which was composed of glucose (5.5% in dry weight), sucrose (10%), starch (4%), glucosamine (.8%), lipid and vitamin free ^{casein} ~~caesin~~ (50%), sodium succinate (.3%), pollack residual oil (8.1%), cholesterol (.5%), mineral mix (8.6%), vitamin mix (2.7%), cellulose Powder (9.3%) and water 130-135 ml. Agar was used as the binder. The feeding rate was .16 mg/larvae/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%.

At the NPCL of CMFRI, the larvae of *P. indicus* were reared successfully from the protozoa to postlarval stages on a microparticulate compounded feed prepared from mantis shrimp, prawn waste, ground nut cake, fish meal and tapioca with a survival rate of 35%. (Silas et al., 1985)

Growth and survival of *P. monodon* postlarvae given different feeding regimen were determined in earthen brackishwater nursery ponds by Tabbu (1985). The natural food consisting of lab-lab and natural food and an artificial diet were the two feeding regimes. It was observed that shrimps given artificial feed obtained higher mean weight gain, survival, metamorphosis and growth. In another experimental study the postlarvae (PL.20) of *Penaeus monodon* were reared in a nursery pond at Portonovo (Sriraman et al., (1988). They were reared to a stockable size of 30-48 mm from an initial size of 8-12 mm. The main feed used was clam meat (*Meretrix* spp.) and were fed at 100% of the body weight. A survival rate of 98% was reported.

In an attempt to ensure all time availability of stocking materials of *P. monodon* experiments were undertaken on a large scale in the lower Sunderban areas of West Bengal for growing the wild caught postlarvae (10-12mm) of the species to an advanced juvenile (35-45mm) stage under semi-controlled conditions. Besides encouraging the growth of natural food, the postlarvae were fed with well balanced

artificial diet having protein from both animal and plant origin. Encouraging results with high survival (upto 73%) and faster growth rates were observed in some of the trial runs (Hardial Singh (1988)).

Natural food in combination with either SEAFDC formulated or other commercial larval diets were tested for large scale production of *P. monodon* postlarvae. The shrimps were reared from nauplii to PL and dietary treatments included (a) Natural food alone (b) Natural food and Commercial phytoplankton substitute (c) Natural food and SEAFDC diet. Larval survival was significant in treatments containing SEAFDC diets than in treatments receiving natural food alone or natural food in combination Bautista *et al.*, (1991)

Godfred (1991) conducted a study to formulate a pellet feed for penaeid shrimps using different ingredients. An evaluation was made of the growth parameters of *P. monodon*, *P. indicus*, *P. semisulcatus* and *M. monoceros*. Shrimp head meal was the chief ingredient and fish meal, bajra flour, rice bran, GNOC, millet flour, alpha meal and dried cane molasses were added. The proximate composition of the feed was 39% protein, 23% carbohydrate, 6.2% lipid and 16.4% ash. Good growth results were obtained indicating that this pellet feed is suitable for all species tested.

Postlarvae and subadults of *Penaeus monodon* were reared in plastic bowls and tanks fed on different natural diets such as mussels, chopped shrimp and beef liver and compounded dry and moist diets in order to investigate the relationship of moulting to growth. Moulting frequency varied with diets and smaller shrimps had greater frequency of moults than larger ones (Kibria 1993).

Levay (1993) reared *P. japonicus* larvae using live food and artificial diet or artificial diet plus *C. gracilis*. Larvae from all the treatments were equivalent in weight and length at third protozoal stage but mysis and postlarvae from both the live food and artificial diet plus *C. gracilis* treatments were significantly larger and reached metamorphosis earlier than those fed with artificial diet alone. There were significant increase in percentage nitrogen and carbon contents during the development in larvae fed the live food or artificial diet plus *C. gracilis* but not of larvae fed an artificial diet alone. It is suggested that the algal complement contributes either extra digestible nutrients or some other specific growth enhancing factors.

2.5 Microdiets

The pioneering work of Jones *et al.*, 1974 led to the development of microcapsules as artificial food particles for aquatic filter feeders.

Microarticulated feeds have been well accepted in many penaeid shrimp hatcheries (Kurumaly et al., 1989, Liao and Liv 1989). Kuo (1986) reported a doubled survival rate (40% Vs. 20%) of *P. monodon* larvae fed micro particulated diet than those fed natural foods (*Skeletonema costatum*) and *Artemia nauplii*. A Kappa - Carrageenan Micro bound diet alone or in combination with natural food in yielding high survival of the larval *P. monodon* was as good as the all natural food group (Bautista et al., 1989) and was suggested to be a potential diet for partial or complete replacement of traditional algal food.

Kumulu and Jones (1995) conducted larval culture of *Penaeus indicus* using microencapsulated diet. The study was conducted for partial or total replacement of live feeds. Slower growth and lower survival rate of the larvae fed experimental MED were significantly improved by a supplement of 15 cells/ml frozen mixed algae (1-2 *Tetraselmis* and *Skeletonema*) during PZ stages (PZ1 - PZ3). The low level of algal supplement to MED resulted in high rate of survival (85-92%). These significant improvement in larval growth and survival are due to higher larval enzyme activities and hence more efficient digestion of artificial diet.

Sudersanan and Sukumaran (1996) conducted experiments using chicken microencapsulated diet fortified with vitamin premix and was fed to the postlarvae of *Penaeus monodon*.

The diet was highly acceptable to the prawn larvae resulting in high rate of survival (91%) and growth (2.1 mg/day) in a 45 day feeding trial in water recirculating system.

2.6 Bioenrichment

During the last decade much research has been devoted to a better understanding of the role and importance of lipids, particularly, the fatty acids. The most important essential fatty acids for brackishwater marine crustaceans, fish and molluscs are the ^ωW3 - highly unsaturated eicosapentaenoic acid (20:5W3) and docosahexaenoic acid (22:6W3). These are essential compounds required for membrane formation, osmoregulation, synthesis of prostaglandins and also play an activating role in the immune system. Since marine fish and shrimp larvae cannot synthesise these W3 - HUFA's to fulfil their physiological requirements these vital components should be acquired through the diet. Analysis of essential fatty acids in the live food organisms used in shrimp larviculture revealed that 20:5 W3 is low in all, except *chaetoceros*, and that in no case is 22:6 W3 present in significant amounts. It has also been demonstrated that the W3 HUFA content for both algae and *artemia* vary much within the same species or strain depending on their culture condition. (Leger and Sorgeloos, 1992).

The variation in W₃ HUFA content in the traditional live feeds used in shrimp larviculture has led to the development of W₃ HUFA enriched algal substitutes/supplements. (eg: microcapsules, W₃ - enriched yeast products, microparticulate diets). The W₃ HUFA enriched products for zooplanktonic organisms include microcapsules, emulsions, microparticulate diets etc. Significant improvement in the growth rates and survival has been demonstrated when W₃ HUFA supplementation is applied in Zoea Stages. Further improvements in the mysis and postlarval stages were noted when extra W₃ HUFA enrichment was provided. Hatchery operators feeding W₃ HUFA fortified diets observed more consistent results and production of more robust PL. (Sorgeloos 1988; Chamberlain 1988).

PL-5 Comparable to PL-10 in terms of size was reported by Sorgeloos (1988) and Chamberlain (1988) by feeding W:3 HUFA enriched *artemia*.

From the foregoing account of relevant literature collected, it is evident that exhaustive efforts have been made the world over in establishing the food preferences of the different larval stages in shrimp. This was obviously due to the necessity of upscaling the hatchery technology. Natural predisposition of the larvae very drastically shifts from a phytoplankton feeding protozoa to a zooplanktivorous mysis. However, artificial plankton, hatchery encapsulon,

microparticulate diets and microcoated diets are available today off-the-shelf to tide over crisis situations in hatcheries. Further more, bioencapsulation and bioenrichment is a very nascent area of research in establishing certain macronutrient requirements of shrimps in their larval as well as postlarval phases.

Postlarval rearing of shrimps appears to be a neglected area of research. Except in extensive and super intensive systems of culture as well as in hatcheries with a nursery rearing facility, feeding does not appear to be a cause of concern. Mortality and cannibalism at this stage is very high and an appropriate dietary environment may be a right path which needs to be investigated. It was after the scanning of peripheral and primary literature, ^{the} the present study was undertaken with the following objectives:

- (1) To study the growth and survival of *Penaeus monodon* postlarvae (PL22) with a particulate compounded feed, with and without vitamin and mineral supplements.
- (2) To find out the growth and survival of the same animals with phytoplankton (*Chaetoceros calcitrans*) and zooplankton (*Artemia nauplii*) as sole source of feed.

- (3) To find out the growth and survival of postlarval *Penaeus monodon* fed with compounded feeds and live feeds in combination.
- (4) To compare and contrast the dietary regimen provided and to arrive at the appropriate feeding regimen.

3. MATERIALS AND METHODS

In this chapter, the preparation of diets (artificial diets and live diets), collection and rearing of *Penaeus monodon* Postlarvae, their feeding regimes and other experimental details followed for determining the gain in weight and survival are presented sequentially.

3.1 Selection of feeds

Artificial and live diets were selected for conducting the experiment. The artificial diets selected for the experiment were,

1. Compounded diet including vitamins and minerals.
2. Compounded diet without vitamins.
3. Compounded diet without minerals.
4. Compounded diet without vitamins and minerals.

The live feeds selected were

1. *Chaetoceros calcitrans*
2. *Artemia nauplii*

3.2 Preparation of Artificial diet

For the preparation of the artificial diet the ingredients selected were 1) Fishmeal 2) Prawn meal 3) Clam meal 4) Ground nut oil cake and 5) Tapioca flour. These ingredients were purchased from the local market.

Prior to the preparation of the feeds their proximate chemical composition was analysed and ascertained. (Table 4)

3.3 Procedure for chemical evaluation of feed ingredients and feeds.

The chemical evaluation of the feed and feed ingredients were performed by the following method (AOAC, 1984).

3.3.1 Dry Matter

Dry matter content present in the feeds were determined gravimetrically by oven drying the samples at 80°C till concurrent dry weights were obtained.

$$\text{Moisture \%} = \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

3.3.2 Crude protein

Total nitrogen in the feed samples and feed ingredients were determined by kjeldhal method. This was multiplied by a conversion factor of 6.25 to arrive at the crude protein content.

3.3.3 Crude fat

Lipid content of the feed samples as well as the feed ingredients were determined by soxhlet extraction method using petroleum ether as the solvent.

3.3.4 Crude fiber

Crude fiber was determined by successive refluxing of feed samples with 1.25% H_2SO_4 and 1.25% NaOH under carefully controlled conditions.

3.3.5 Ash content

Ash content of the feeds were determined by incinering preweighed feed samples in a muffle furnace at 600 degree celsius for 8 hours.

After analysing the proximate composition of the feed stuffs, four feeds were formulated containing approximately 47% protein and 450 Kcal/100 grams gross energy (Isocalorific and Isonitrogenous).

Table - 2

Percentage ingredient composition of the experimental feeds

Ingredients	Level of incorporation(grams/100gms)			
	Feed 1	Feed 2	Feed 3	Feed 4
Fish meal	20	20	20	20
Prawn meal	20	20	20	20
Clam meal	20	20	20	20
GNOC	20	20	20	20
Tapioca flour	8	10	11	13
Oil	6	6	6	6
*Vitamin mix	3	-	3	-
**Mineral mix	2	2	-	-
Cholesterol	0.5	0.5	0.5	0.5
Lecithin	0.5	0.5	0.5	0.5

* Contains Vitamin B₁ - 10mg Calcium pantothenate- 10mg
 Vitamin B₂ - 10mg Folic acid - 1500mcg
 Vitamin B₆ - 3mg Vitamin B₁₂ - 15mcg
 Nicotinamide-100mg Vitamin C - 150mg

** USP Salt mixture XIV from M/s.Sisco Research Laboratories

Wherever the vitamin mixture and mineral mixture was deleted in the formulation tapioca flour levels were adjusted to make up the percentage composition.

3.4 Feed Preparation

All the dry ingredients except tapioca flour were thoroughly mixed. Tapioca flour was gelatinised, cooled and added to the dry mixture to make a dough of uniform consistency. The dough so made was extruded through a kitchen noodle maker with a die of 2 mm pore size. The feed strands were oven dried, crumbled and sieved through 200 microns mesh and stored in air tight containers and used (Rani and Ali, 1991). The proximate composition of the pelleted feeds were also determined. (Table 5)

3.5 Live feeds

3.5.1 Zooplankton diet

Artemia nauplii

In the present study, *Artemia* cyst belonging to the strain Great Salt lake (utah) was used.

Procedure for cyst hatching

About 0.5 gm of the cysts to be hatched were taken in a 2 litre measuring cylinder. About 1 litre of clean filtered seawater was added. Vigorous aeration was provided from the bottom of the measuring cylinder. Artificial light

was also provided by placing a bulb of 100 W. The breaking of the cyst could be observed after 12-24 hrs and all the nauplii hatched out within 48-72 hours. The hatched nauplii were separated from the unhatched cysts using a siphoning tube. The count of the hatched artemia nauplii were determined by counting the number of nauplii/100 ml and were used for feeding the experimental animals.

3.5.2 Phytoplankton diet

Chaetoceros calcitrans

Chaetoceros calcitrans was selected to feed the experimental animals. In this study, the algal cultures were maintained in three 5 liter tanks.

3.5.3 Maintenance of algal cultures

To fresh filtered seawater Walne's medium (Walne, 1974) of the following composition was added.

Composition of Walne's medium

A.	Potassium nitrate	100 gm
	Sodium orthophosphate	20 gm
	EDTA (Na)	45 gm
	Boric acid	33.4 gm
	Ferric Chloride	1.3 gm
	Manganese chloride	36 gm
	Distilled water	1 liter

B.	Zinc chloride	4.2 gm
	Cobalt chloride	4.0 gm
	Copper sulphate	4.0 gm
	Ammonium molybdate	1.8 gm
	Distilled water	1 liter
C.	Vitamin B ₁ (Thiamin)	200 mg in 100 ml distilled water
	Vitamin B ₁₂ (Cyanocobalamin)	100 mg in 100 ml distilled water
D.	Sodium silicate	1.0 gm (only for diatom
	Distilled water	1 liter culture)

A, B, C and D were prepared separately. 1 ml of A, 0.5 ml of B, 0.1 ml of C and 1 ml of D were added to 1 litre of filtered and sterilised seawater. To the seawater fertilised with Walne's medium, *Chaetoceros innoculum* was added. Sufficient aeration and artificial light was provided. The blooms developed within 2-3 days. The algal counts were determined using a haemocytometer. The culture was maintained at a cell concentration of 2 million cells/ml.

3.5.4 Determination of algal cell densities

Regular counts of the algal cells were taken in order to schedule inoculation of the culture tanks, to monitor the growth of the algal cultures and to determine the quantity of algae to be fed to the experimental animals.

Since most of the nanoplankters measure less than 10 microns a haemocytometer was used for counting the cells. One drop of a well stirred sample was taken with a sterilised pipette. A coverslip was placed on the haemocytometer and the pipette was brought to the edge of the haemocytometer to touch it. The sample runs inside the coverslip and thus a thin film of the culture is obtained and the cells are equally distributed. Since the haemocytometer has got nine chambers and four sides having sixteen divisions, it is restricted the counting for atleast four chambers. The average number of cells in 1 ml is calculated as

Average count per chamber $\times 10^4$ = Total Number of cells/ml

3.6 Collection of Experimental Animals

The postlarvae of *Penaeus monodon* (PL 22) was procured from the MPEDA Hatchery Vallarpadam. For transportation to the lab the postlarvae were packed in 5 litre capacity oxygen packed polythene bags containing 2 litres of seawater. In the hatchery the animals were maintained at a salinity of 25 ± 2 ppt and a temperature of 28°C . In the laboratory the animals were transferred to a 30 litre fibreglass tank containing seawater at a salinity of 27 ± 2 ppt and temperature of 28°C for acclimatisation. The acclimatisation was done by floating the bags containing

the postlarvae in the tank and adding small volumes of water periodically until both temperature and salinity within the bag and outside became same. After acclimatisation the next day the animals were segregated according to their size and were transferred to 2 litre plastic jars.

3.7 Experimental Set up

The experiment consisted of 12 treatments and was carried out in 2 litre plastic jars. Duplicates were maintained for each treatment. The stocking density was 15 animals/jar. The animals were maintained in 27 ± 2 ppt seawater and the seawater temperature was 28°C . The dissolved oxygen concentration of the seawater was maintained at 4.5 ± 0.5 mg/litre with constant aeration provided through one air stone in each jar. (Plate - 1)

Prior to starting the feeding experiment the wet weight of 10 samples of animals; each sample containing 15 animals were taken. After taking the wet weight they were kept in the oven for drying overnight. The dry weight was taken the next day.

The animals were given 12 different types of diets.

Table - 3

Treatments No.	Feeding regimes
T ₁	Compounded feed containing vitamins and minerals.
T ₂	Compounded feed without vitamins.
T ₃	Compounded feed without minerals.
T ₄	Compounded feed without vitamins and minerals.
T ₅	Only phytoplankton (<i>Chaetoceros calcitrans</i>)
T ₆	Only zooplankton (<i>Artemia nauplii</i>)
T ₇	Phytoplankton and zooplankton
T ₈	Compounded feed containing vitamins and minerals + phytoplankton + zooplankton.
T ₉	Compounded feed without vitamins + phytoplankton.
T ₁₀	Compounded feed without minerals + phytoplankton
T ₁₁	Compounded feed without vitamins + zooplankton.
T ₁₂	Compounded feed without minerals + zooplankton.

The artificial diets were fed to the animals at the rate of 15% of the body weight. The algal feed was provided at a concentration of 25000 cells/animal/day. The artemia nauplii were added at a density of 8-12 nauplii/day/animal. All the feeds were provided once a day in the morning. Daily survival was noted and feeding was regulated based on it. 50% water was exchanged daily. The feeding was conducted for a period of 21 days. After the experimental period of 21 days the wet weight of animals in each treatment was noted. Their dry weights were also recorded after oven drying for 12 hours to a constant weight.

The data thus obtained were subjected to statistical analysis. The parameters studied during the experimental period were,

$$3.7.1 \text{ Survival percentage} = \frac{N_0 - N_1}{N_0} \times 100$$

Where,

N_0 =Initial number of animals and N_1 =Final Number of animals.

$$3.7.2 \text{ Relative growth rate} = \frac{W_t - W_i}{W_t} \times 100$$

Where,

W_t = Final wet weight/animal and w_i = Initial wet weight/animal.

$$3.7.3 \text{ Specific growth rate (SGR)} = \frac{[l_n (W_t - l_n W_i)]}{t} \times 100$$

Where,

l_n = Natural logarithm t = experimental duration

The afore mentioned parameters were choosen from Hopkins (1992).

3.8 One way analysis of variance was carried out for wet weight gain, dry weight gain, survival %, RGR and SGR. When treatment F is found significant the treatments were grouped into homogenous groups by applying SNK test (Snedcor and Cochran 1967). The statistical analysis was done with the SPSS/PC program at the computer center of C.M.F.R.I.

EXPERIMENTAL SET-UP



PLATE - 1

4. RESULTS

The composition of the artificial diets are presented in Table-2. The live feeds selected were *Chaetoceros calcitrans* and *Artemia nauplii* (Treatments T5 and T6 respectively).

The compounded feeds had a moisture content of 1.8% (T1), 2.6%(T2), 2.6%(T3) and 6.70(T4) respectively. The crude protein in the four diets were estimated to be between 45.5% and 47.25% i.e., The crude protein content of T1, T2, T3 and T4 were 45.5%, 47.25%, 45.5% and 46.43% respectively. The crude fat in the experimental feeds were 8%, 10.5%, 10.5% and 9.02% and the crude fiber content was found to be 1.8%, 1.94%, 1.75% and 1.89%. The total ash content of the feeds were estimated to be 15, 13.8, 13.4 and 21.41% respectively. The nitrogen free extract (soluble carbohydrates) was calculated by difference and was found to be 27.9% for T1, 23.9% for T2, 26.25% for T3 and 21.41% for T4. The proximate chemical composition of the four feeds are presented in Table-5.

The nutritive value of *Chaetoceros calcitrans* was estimated by Kaladharan et al., (1996). It had a protein content of 56.37 ± 5.7 , lipid content of 16.7 ± 3.3 and its soluble sugar content was 33.62 ± 5.2 as percentage of dry

matter. The proximate chemical composition of artemia nauplii is reported to be 60.12% protein, 20.20% total lipids, 8.06% carbohydrates and 9.81% total ash. (Uday Ram Jothy, 1983).

The feeding regimen chosen for conducting the experiment is presented in Table-3. Throughout the experimental period the dissolved oxygen content of the water was 4.5 ± 0.5 mg/liter. The salinity of the water was maintained at 27 ± 2 ppt and the temperature of the water was 28°C .

After feeding and maintaining the animals for a period of 21 days the following parameters were determined

1. Wet weight/animal/treatment.
2. Dry weight/animal/treatment.
3. Survival Percentage.
4. Relative Growth Rate (RGR).
5. Specific Growth Rate (RSGR).

The results obtained indicate that variations in final wet weight, final dry weight, relative growth rate and specific growth rate are not statistically significant ($P > 0.05$), Tables 6, 7, 8, 10, and 11. Survival percentage was found to be statistically significant ($P < 0.05$) Tables 6 and 9. Those treatments with similar survival percentage

and differing significantly from other treatments were classified into three groups according to Student-Newman-Keuls procedure and means with significant differences were assigned different superscripts (Table 6).

The average survival percentage was found to be fluctuating in different treatments. The average wet weight/animal/treatments was also compared. It was observed that in the treatments provided with artificial diet (T1-T4), the average survival percentage was found to be highest for T4 (83.3%) and the lowest was observed for T1 and T3 (70%). The average survival percentage observed in T2 was 73.35%. Among the treatments provided with artificial diets (T1-T4) the final wet weight/animal/treatment was found to be highest in T3 (0.03515g) and the lowest average weight was observed in T4 (0.03075g). The average wet weight/animal/treatment observed for T1 and T2 are 0.0349g and 0.03175g respectively.

In the treatments provided with live diets (Phytoplankton and Zooplankton) Solely and in combination (T5, T6 and T7) the highest average survival percentage was observed in T7 (36.65%). 6.7% and 26.65% are the average survival percentages obtained for T5 and T6. 0.0224g was the highest average wet weight/animal/treatment. This was observed in T7. Lowest weight gain was observed for T6

(0.0145g). The weight gain observed for T5 was 0.0205g.

The survival percentage in treatments fed with live and artificial diets in combinations (T8-T12) were also observed. The highest survival percentage was observed for T12 (63.3%) and lowest was for T11 (20%). 46.65%, 53.35% and 56.65% were the survival percentages observed for T8, T9 and T10. T11 showed a highest average wet weight of 0.0496g. The lowest average wet weight was observed for T9 0.263g. The average wet weight observed for T8, T10 and T12 were 0.0269, 0.0297 and 0.0275g respectively.

From the wet weight's observed the relative growth rate and specific growth rates were calculated (Table 6).

In treatments provided with artificial diets (T1-T4) relative growth rate was found to be highest in T3(266.1) and the lowest RGR was observed in T4 (220.3). Relative growth rates observed in T1 and T2 are 263.5 and 230.72. The highest specific growth rate was observed in T3 (6.17) and the lowest was observed in T4 (5.54). 6.14 and 5.68 are the specific growth rates observed in T1 and T2.

It was observed that in the treatments provided with live diets (solely and in combination) highest relative growth rate was observed in T7. The relative growth rates observed in T5 and T6 are 113.5 and 112.5 respectively. The highest specific growth rate observed was 3.89. This was

observed in T6. The specific growth rates observed in T5 and T7 were 3.6 and 3.54.

In the treatments provided with artificial and live diets in different combinations the highest specific growth rate was observed in T11 and the lowest was observed in T8 (4.66). 4.79, 5.17 and 4.95 were the specific growth rates observed in T9, T10 and T12 respectively. 416.65 was the highest relative growth rate observed. This was observed in T11. The lowest relative growth rate 165.65 was observed in T9. 179.69, 209.4 and 185.95 were the relative growth rates in T8, T10 and T12.

The overall picture evident from table 6 and figures 1 and 2 are that in terms of nutritional performance T11 which is a feeding regimen consisting of an artificial feed devoid of vitamins along with a live zooplankton viz. *Artemia nauplii* performed the best. The next best dietary regimens were T3 which is an artificial diet with vitamins and devoid of minerals followed by T1 which is an artificial diet fortified with vitamins and minerals.

Table - 4

Proximate Chemical Composition of feed ingredients
(% on dry matter basis)

Ingredients	Moisture %	Dry matter	Crude protein	Ether extract	Nitrogen free extract	crude fiber	Ash
Fishmeal	5.40	94.60	61.25	3.20	2.95	2.00	25.20
Shrimp Meal	13.40	86.60	64.75	4.00	1.30	2.95	16.20
Clam Meal	5.40	94.60	57.75	10.8	17.15	3.7	5.20
Gnocmeal	4.00	96.00	47.25	10.50	30.92	.83	6.60
Tapioca flour	8.20	91.80	1.75	3.20	84.26	.84	2.00

Table - 5

Proximate Chemical Composition of Experimental Feeds
(% on dry matter basis)

Proximate composition	Feed - 1	Feed - 2	Feed - 3	Feed - 4
Moisture	1.8	2.6	2.6	6.70
Dry Matter	98.2	97.4	97.4	92.82
Crude Protein	45.5	47.25	45.5	46.43
Ether Extract	8	10.5	10.5	9.02
Crude Fiber	1.8	1.94	1.75	1.89
Nitrogen free extract*	27.9	23.9	26.25	14.07
Ash	15	13.8	13.4	21.41
Calculated Gross Energy (Kcal/100g)**	437.44	453.42	453.43	425.33

* NFE = 100 - Moisture + CP + EE + CF + Ash

** Calculated assuming that 4.1, 5.5 and 9.1 Kcal/g for Carbohydrate, Protein and fat respectively. (ADCP, 1983)

Table 6

Effect of different dietary treatments on wet weight, dry weight, survival %, RGR and SGR in the PL of *Penaeus monodon*

Diet	Initial wet weight	Initial dry weight	Final wet weight (average)	Final dry weight (average)	Survival % (average)	Relative growth rate (% wt.gain in 21 days)	Specific growth (% wt gain per day)
T1	.0096	.0025	.0349	.0092	70.11 ^{ae}	263.5	6.13
T2	.0096	.0025	.0318	.0085	73.35 ^{ae}	230.72	5.68
T3	.0096	.0025	.0352	.0095	70 ^{ae}	266.1	6.17
T4	.0096	.0025	.0308	.0083	83.3 ^{ae}	220.3	5.54
T5	.0096	.0025	.0205	.0040	6.7 ^{be}	113.5	3.6
T6	.0096	.0025	.0145	.0044	26.65 ^{acd}	112.5	3.89
T7	.0096	.0025	.0224	.0052	36.65 ^{acd}	133.0	3.54
T8	.0096	.0025	.0269	.0072	46.65 ^{acd}	179.69	4.66
T9	.0096	.0025	.0264	.0061	53.35 ^{acd}	165.65	4.79
T10	.0096	.0025	.0297	.0072	56.65 ^{acd}	209.4	5.17
T11	.0096	.0025	.0496	.0123	20 ^{bdc}	416.65	5.78
T12	.0096	.0025	.0275	.0071	63.31 ^{ad}	185.95	4.95
			N.S.	N.S.	Means with common superscript do not differ significantly in (P < 0.05)	N.S.	N.S.

Table - 7

One Way Analysis of Variance
(Wet Weight Gain)

Source	D.F	Sum of Squares	Mean Squares	F Ratio	F Prob
Between Groups	11	.0017	.0002	.5545	.8310
Within Groups	12	.0033	.0003	.5545	.8310
Total	23	.0050			

Table - 8

One Way Analysis of Variance
(Dry Weight Gain)

Source	D.F	Sum of Squares	Mean Squares	F Ratio	F Prob
Between Groups	11	.0001	.0000	.6263	.7766
Within Groups	12	.0002	.0000	.5545	.8310
Total	23	.0003			

Table - 9

One Way Analysis of Variance
(Survival Percentage)

Source	D.F	Sum of Squares	Mean Squares	F Ratio	F Prob
Between Groups	11	12384.7500	1125.8864	.5331	.0037
Within Groups	12	2533.3500	211.1125		
Total	23	14918.1000			

Homogenous Subsets (Subsets of groups, while highest and lowest means do not differ by more than shortest significant range for a subset of that size).

SUBSET 1					
Group Mean	Grp 5 6,7000	Grp 11 20,0000	Grp 6 26,6500	Grp 7 36,6500	Grp 8 46,6500
Group Mean	Grp 9 53,3500	Grp 10 56,6500			
SUBSET 2					
Group Mean	Grp 11 20,000	Grp 6 26,6500	Grp 7 36,6500	Grp 8 46,6500	Grp 9 53,3500
Group Mean	Grp 10 56,6500	Grp 12 63,3000	Grp 1 70,0000	Grp 3 70,0000	Grp 2 73,3500
SUBSET 2					
Group Mean	Grp 7 36,6500	Grp 8 46,6500	Grp 9 53,3500	Grp 10 56,6500	Grp 12 63,3000
Group Mean	Grp 1 70,0000	Grp 3 70,0000	Grp 2 73,3500	Grp 4 83,3000	

Table - 10

One Way Analysis of Variance
(Relative growth rate)

Source	D.F	Sum of Squares	Mean Squares	F Ratio	F Ratio
Between Groups	11	184937.4423	16812.4948	.5585	.8280
Within Groups	12	361212.9124	30101.0760	.5545	.8310
Total	23	546150.3548			

Table - 11

One Way Analysis of Variance
(Specific growth rate)

Source	D.F	Sum of Squares	Mean Squares	F Ratio	F Prob
Between Groups	11	37.7475	3.4316	.5986	.7980
Within Groups	12	68.7945	5.7329		
Total	23	106.5420			

Fig 2. Survival % and relative growth rate of *Penaeus monodon* under 12 dietary regimen

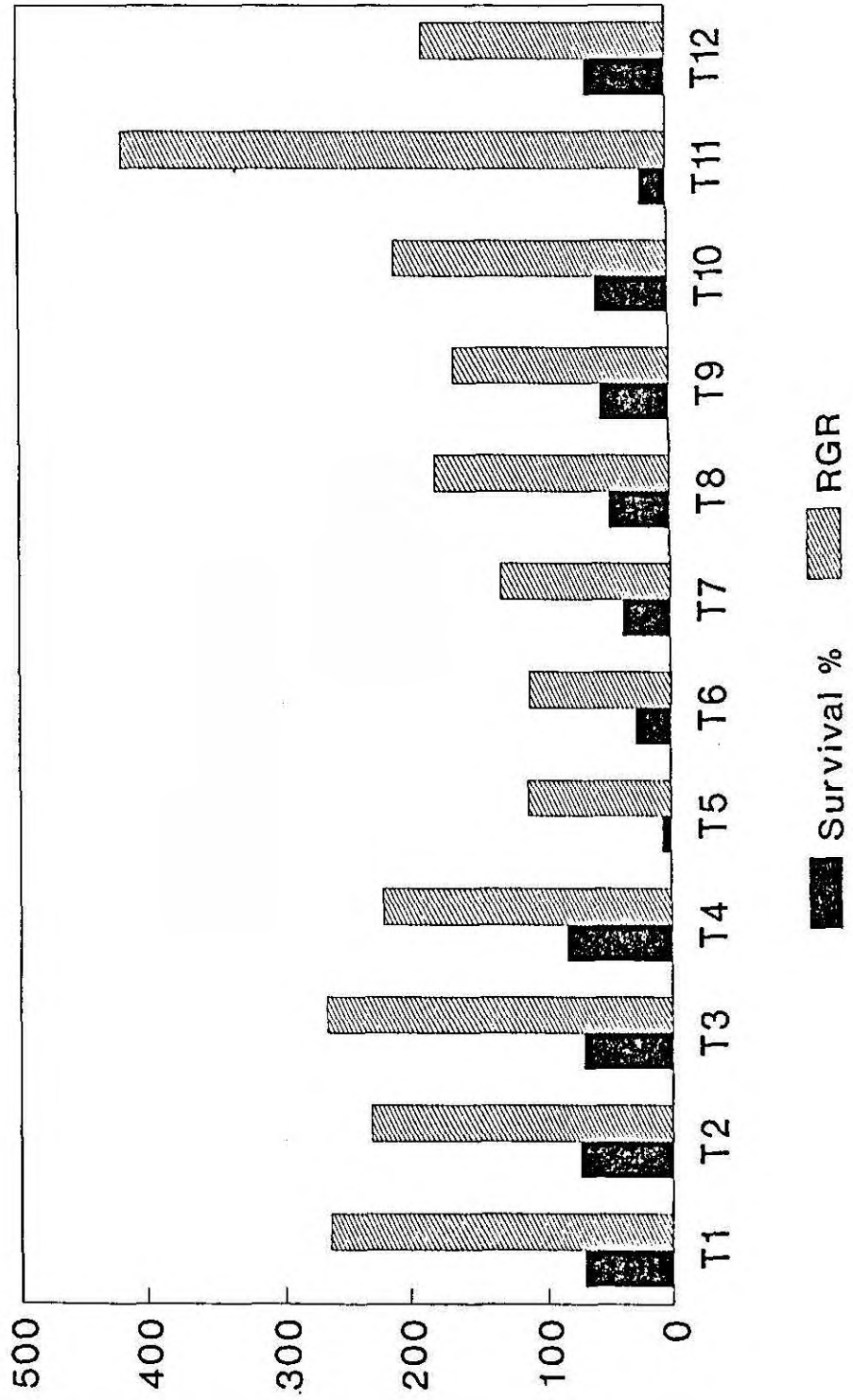
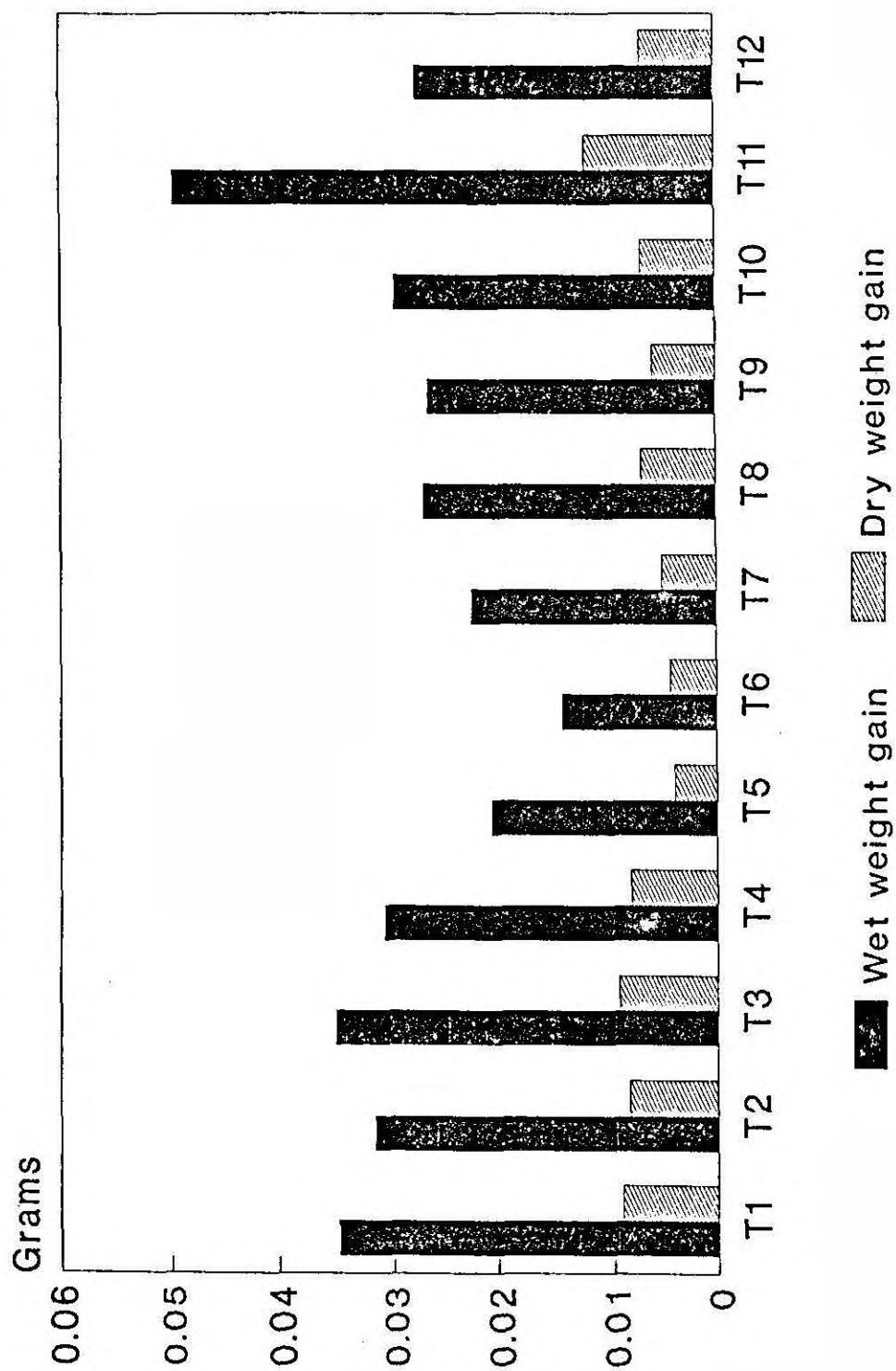


Fig 1. Wet weight gain & Dry weight gain
Penaeus monodon under 12 dietary regimens



5. DISCUSSION

In this section the results of the present investigation are discussed in the light of available reports.

Particulate compounded feed consisted of locally available ingredients like shrimp meal, fish meal (serving as animal protein sources as well as attractants), solvent extracted ground nut oil cake (a vegetable protein source), tapioca flour (an energy source and a binder) and vitamin and mineral supplements.

This formulation is different from the particulate feed reported by Silas *et al.*, (1985) in which mantis shrimp (*Oratosquilla nepa*) powder formed an ingredient. Moreover, the aforesaid authors reported only 36.8% protein, 10.1% lipids, 29.8% carbohydrates and 18.9% ash on an as fed basis.

Feeds formulated and used in this study contained 47% protein on an average, and a gross energy content of 425 Kcal/100g to 453 Kcal/100g. These high levels of protein and energy were chosen because, using similar feed ingredients it was observed by Vijayagopal (unpublished) that the dietary requirement of protein under controlled conditions of culture of the early juveniles (0.38 - 0.48g) of *Penaeus indicus* was 47%.

Cholesterol and lecithin were also incorporated in these feeds according to the findings of Teshima and Kanazawa (1971) and Peidad - Pascual (1986) indicating the dietary essentiality of cholesterol and phospholipids in penaeid nutrition.

Variability in the artificial diets prepared were brought about by deleting supplementary vitamin and mineral premixes. This was because there exists a lack of sound knowledge regarding their requirements for shrimps Trino and Sarroza (1995). Eventhough, establishing their requirements does not form a part of the present investigation this study aims at finding out whether costly micronutrient supplementation is required during postlarval and nursery phases of rearing of *Peneaus monodon*. Moreover, this study was also expected to indicate whether the presence of live feeds, both phytoplankton and zooplankton could make up the micronutrient deficiencies in the culture environment. One report on this aspect in *Penaeus monodon* is by Trino and Sarroza (1995) who opined that growth, survival, apparent food conversion ratio (AFCR), net production and net cost of production were not significantly different between the two diets i.e., a diet lacking in vitamin and mineral supplements and a diet incorporating vitamins and minerals, in a modified extensive culture system. However, the cost of production of a diet lacking vitamins and minerals was favourably lower. It is important to note in this context

that Pascual and Catacutan, (1990) and Trino et al., (1992) reported that vitamin and mineral supplements can account for 20 to 23% of the total cost of the feed.

It is with this over view the dietary treatments were designed, wherein, treatments T₁ - T₄ contained only compounded feeds and treatments T₅ - T₇ contained phytoplankton (*Chaetoceros*) and zooplankton (*Artemia nauplii*) solely and in combination. Treatments T₈ - T₁₂ were the different permutations and combinations of compounded feeds and live feeds.

5.1 Growth under different dietary conditions

Growth in terms of wet weight gain and dry weight gain were not significant statistically ($P > 0.05$). However, the trends visible in figure 1 are self-explanatory. The weight gain in the treatments with artificial feed with vitamins, minerals and without vitamins and minerals do not vary significantly. These results are similar to that of Trino (1995); eventhough, rearing of *P. monodon* was under controlled conditions in the present study. Whereas, Trino (1995) reported from experiments on-farm with a duration of 120 days. This lack of any statistical variability reflects the sufficiency of vitamins present in the feed stuffs themselves for smaller sizes of *Penaeus monodon*. Trino and Sarroza (1995) has also opined that natural food organisms

could have also contributed to the growth as well as micronutrient supplementation. In the present investigation, this possibility is checked with the use of filtered sea water. Thus, vitamin and mineral supplementation in the feeds meant for rearing of shrimps in postlarval and nursery phases under different systems of culture requires revalidation before advocating abstinence from vitamin and mineral premix incorporation in artificial diets.

Haiqui et al., (1992) while evaluating the dietary essentiality of fat soluble vitamins in the *Penaeus Vannamei* had reported that growth of shrimps was not affected by a vitamin K₃ (Menadione) deficient diet. However, they had reported that vitamins A, D and E are essential nutrients in shrimp diets. Such reports are relevant here because, vitamin requirements for shrimps being fully unknown supplementation based on intuition appears wasteful.

In the treatments where phytoplankton and zooplankton were fed the lowest growth was recorded in the zooplankton treatment, followed by a better growth with phytoplankton alone. When phytoplankton and zooplankton were fed in combination there was still better growth and when artificial feed was also included in the dietary regimen best growth was recorded. This phenomenon can be explained in the light of the fact that, highly unsaturated

fatty acids (HUFA's) have been demonstrated to be essential compounds required for an array of functions like membrane formation, osmoregulation and synthesis of prostaglandins etc., in most of the marine crustaceans. HUFA's also appear to have an activating role in the immune systems of invertebrates. Among the microalgae used as live food *Chactoceros* spp. in general is reported to contain the highest concentration of 20:5 W3 fatty acid viz., eicosapentaenoic acid (Leger and Sorgeloos, 1992). This could have been the reason for a comparatively better growth of shrimps receiving a diet of phytoplankton compared to zooplankton. Moreover, artemia nauplii used in the present investigation could have had an incompatible fatty acid profile in terms of HUFA. Further more, it is reported by Leger and Sorgeloos (1992) that dietary protein requirements are met by most live feeds used for *Penaeus* spp. But, in terms of amino acid composition major deficiencies have not been reported to retard growth. However, low sulphur amino acid levels do exist in all zooplankton prey species including artemia and rotifers. Such nutrient imbalances could be attributed to the results obtained in the present investigation, where, phytoplankton treatment was better compared to zooplankton and when artificial feed supplementation was done best growth resulted due to the complementary effect of an artificial feed in terms of amino acids.

In the treatments where the compounded feeds were fed in combination with phytoplankton and zooplankton no specific trends were observed. An artificial feed devoid of vitamin supplements and combined with artemia indicated highest growth. This was followed by the treatment with artificial feed without minerals plus phytoplankton. Presence of phytoplankton and zooplankton along with compounded feeds does not seem to favour growth of shrimps to a statistically significant level in general. However, artemia supplementation to an artificial feed did have its own beneficial effect. This was reflected in terms of highest overall growth recorded in that treatment (T₁₁). These results are similar to that of Stahl (1979) who from his work on *Macrobrachium rosenbergeii* opined that an applied feed to supplement the natural foods produced significant growth than that obtained by natural foods alone, although this increase was not dramatic.

5.2 Survival under different dietary conditions

Survival cannot be directly correlated to the feeding regimen, it indirectly indicates the fact that improper and imbalanced nutrition leads to autolysis of cells, eventually leading to death. Moreover, in studies with organisms in the larval, postlarval and nursery phases survival percentage is chosen as a key index of its nutritional status.

In the present investigation, survival percentage is the only parameter which showed statistically significant ($P < 0.05$) variations which confirms the aforementioned fact. Highest survival of 83.3% was obtained in the treatment receiving a compounded feed devoid of vitamins and mineral supplements and the lowest survival was recorded in the treatment receiving only phytoplankton. Eventhough the result of the present study is similar to the report of Trino and Sarroza (1995) where higher survival was observed by feeding a compounded diet devoid of vitamin and mineral premixes, definite conclusions could not be drawn based on survival percentage with reference to the performance of a dietary regimen in this study.

However, certain trends observed in figure 2 where survival percentage is juxtaposed with the relative growth rate are that a well prepared compounded feed can result in consistent survival percentages and relative growth rates, when fed in appropriate quantities, which was 15% of the body weight in this study. Inconsistencies do occur when the feeding regimen has live feeds where the rate of feeding can be low or surplus. In either case the cultured organism reveals it and calls for accurate counts and culturing skills.

It is also interesting to note that the compounded feed without vitamin and mineral supplements performed

equally well as of that of a feed with both vitamins and minerals incorporated (T_1 and T_2) in terms of survival percentage in this study. Moreover, a feed without vitamins and with minerals showed a slightly higher survival percentage eventhough not statistically significant ($P < 0.05$). These results do not corroborate with the findings of Catacutan and de la Cruz (1989) and Sedgwick (1984), where the former reported that exclusion of water soluble vitamins resulted in poor growth of *Penaeus monodon* and the latter reported high mortality in *Penaeus merguensis*. When fed a diet of freeze dried *Mytilus edulis* meal. This may be because these two studies were made with semi-purified diets where the vitamin and mineral complements derivable from the feed as such is less. The animals may be solely dependent upon the supplementary vitamins and minerals provided.

The results of this short experiment also shows that a diet of phytoplankton alone (T_5) or zooplankton alone (T_6) is insufficient. Exogenous nutrition through natural feed ingredients improves survival dramatically. However, higher concentrations of live feed organisms with varying stocking densities needs long terms experimentation in the light of the fact that Anderson et al., (1987) with pond reared *Penaeus Vannamei* stocked at 20 m^{-2} showed that the natural food organisms accounted for 53 to 77% of the growth of the cultured shrimp.

5.3 Relative growth rate (RGR) and specific growth rate (SGR)

Relative growth rate indicates the percentage growth in body weight over the initial body weight of shrimps within the experimental duration, which in this study was 21 days. The trends observed in figure 2 and table 6 are that artificial feeds with and without vitamin supplements recorded a uniform growth rate of > 200% whereas a combination, the compounded diet without minerals and in combination with artemia recorded a relative growth rate of > 400%. These results eventhough not significant statistically ($P > 0.05$) reveals the propensity of artemia nauplii in growth enhancement.

Specific growth rate which indicates percentage weight gain per day also follows the pattern of relative growth rate. However transformation of the actual weights into natural logarithms lends clarity to the data presented. Compounded feed treatments (T_1 - T_4) were at par with that of combined feeding regimen (T_8 - T_{12}). Live feeds as sole sources of feed (T_5 - T_7) under controlled conditions of experimentation in this study performed the least in terms of daily body weight gain. However, these results were not significant statistically ($P > 0.05$). Thus, this short-term investigation, in general, shows that vitamin and mineral supplementation in compounded feeds for early stages of growth (PL 22 - 43) in *Penaeus monodon* based on intuition

can be avoided. Provided, the compounded feed contains protein and energy above 45% and 4.5 Kcal/g respectively derived from good quality feed ingredients like fish meal, shrimp meal and deoiled ground nut oil cake. These natural feed ingredients could have had sufficient quantities of vitamins and minerals required for the optimum growth of shrimps in their early phases of growth. Among the live feeds artemia nauplii establishes itself to be an apt zooplankton for the postlarval and nursery phases of shrimp rearing and phytoplankton alone as feed appears insufficient to shrimps. Survival percentage reflects the nutritional status with statistical significance in this short investigation also. Moreover, knowledge of HUFA and amino acid requirements appears imperative, but remains to be an unexplored area in shrimp nutrition.

6. SUMMARY

This study entitled "Growth and survival of the post larvae of *Penaeus monodon* under different dietary conditions" was carried out at CMFRI, Cochin during June to July 1997.

Hatchery bred postlarval shrimps of *Penaeus monodon* (PL-22) were procured and stocked in 2 liters of filtered seawater of 25 ppt salinity at the rate of 15 animals per treatment. The animals were given 12 different types of diets (artificial diets and live diets solely and in combination (Table 3). The feeding was conducted for a period of 21 days. After a period of 21 days the wet weight and dry weight of the animals were recorded. The parameters studied during the experimental period were 1) Survival percentage 2) Relative growth rate 3) Specific growth rate.

One way analysis of variance was carried out for wet weight gain, dry weight gain, survival percentage, RGR and SGR. The results obtained indicate that final wet weight, final dry weight, relative growth rate and specific growth rate are not statistically significant (Tables 6, 7, 8, 10 and 11). Where as, the survival percentage was found to be statistically significant ($P < 0.05$) (Table 6 and 9).

The salient findings of this study are

- 6.1 A well prepared compounded diet of the requisite particle size (200 microns) without vitamin and mineral supplements performed equally well in terms of growth and survival, when compared to the same compounded feed including supplementary vitamins and minerals.
- 6.2 Phytoplankton (*Chaetoceros calcitrans*) was found to be inadequate as a sole source of feed for the postlarvae of (PL 22-43) *Penaeus monodon* at a concentration of 25000 cells/animal/day. However feeding of phytoplankton at higher concentrations at different stocking densities requires long term investigation.
- 6.3 Phytoplankton as sole source of feed performed comparatively better than zooplankton (*Artemia nauplii*) as a sole source of feed in this study.
- 6.4 Neither phytoplankton nor zooplankton supplementation with compounded feeds devoid of either supplementary vitamins or minerals indicated any significant growth promotion.
- 6.5 However *Artemia nauplii* supplementation (T₁₁) resulted in a dramatic growth increment confirming the propensity of this live feed in the postlarval rearing of penaeids.

- 6.6 This short-term experiment confirms the validity of survival percentage as an index in postlarval rearing of shrimps with different diets, both natural as well as artificial.
- 6.7 From this study it appears that micronutrient deficiencies are not a cause of concern in designing the dietary regimen for post larval/nursery shrimp rearing. Hence, HUFA requirements and amino acid requirements can also be the areas requiring indepth investigations to enhance growth and survival during postlarval and nursery phases of shrimps.

7. REFERENCES

ADCP, (1983).

Fish feeds and feeding in developing countries,
FAO, Rome, ADCP/REP/83/18: 97 p.

AKIO KANAZAWA, (1984).

Nutrition of Penaeid prawns and shrimps.
Proceedings of the First International Conference on
the Culture of Penaeid prawns/shrimp, Iloilo City,
Philippines.

ALIKUNHI, K.H., MOHANKUMAR, G., RAVINDRAN NAIR, S., JOSEPH,
K.S., HAMEED ALI, K., PAVITHRAN, M.K., and SUKUMARAN,
P.K. (1980).

Observations on mass rearing of Penaeid and
Macrobrachium larvae at the Regional shrimp Hatchery,
Azhikode during 1979 and 1980.

Bulletin Department of Fisheries Kerala, 2 (I); 68 pp.

ALIKUNHI, K.H., RAVINDRAN NAIR, S., SUKUMARAN, P.K., and
PAVITHRAN, M.K., (1982).

Report on mass rearing of shrimp larvae at the Regional
Shrimp Hatchery, Azhikode during 1981.

ANDERSON, R.K., PARKER, P.L. and LAWRENCE. A.L., (1987).
A¹³C/¹²C tracer study of the utilisation of presented
feed by commercially important shrimp *Penaeus vannamei*
in a pond growout system.
Journal of World Aquaculture Society 18: 148-155 pp.

ANON., (1977).

Annual report 1976 - Aquaculture Department
Southeast Asian Fisheries Development Centre,
Philippines.

ANON., (1976).

Mass Production of the fry of Sugpo, *Penaeus monodon*
(Fabricius) and some notes on its biology.
Mindanao State University Institute of Fisheries
Research and Development, Annual Report, 1976
Contribution No. 75-1: 1-17 pp.

A.O.A.C., (1994)

Official methods of analysis.
Association of Official Analytical Chemists. 14th
Edition, Arlington VA, 1141 pp.

AQUACOP, (1978).

Study of nutritional requirement and growth of *Penaeus*
merguiensis in tanks by means of purified and
artificial diets.
Proceedings of World Mariculture Society 9: 225-232 pp.

BAUTISTA, M.N., MILLAMENA, O.M., and KANAZAWA, A., (1989).

Use of Kappa - Carrageenan microbound diet (C-MBD) as feed for *Penaeus monodon* larvae.

Marine Biology 103: 169-173 pp.

CATACUTAN, M. and de la CRUZ, M., (1989).

Growth and midgut cells profile of *Penaeus monodon* juveniles fed water soluble vitamin - deficient diets.

Aquaculture 81: 137-144 pp.

CHU, K.H. and LUI, L.K., (1988).

Evaluation of algal species as diet for larvae and post larvae of shrimp *Metapenaeus ensis*.

Third international symposium on Marine Biogeography and Evolution in the Pacific 26th June - 3rd July 1990, 255 p, Bulletin of Marine Science, Vol. 47 No.1.

CHAMBERLAIN, G., (1988).

Shrimp hatcheries.

Coastal Aquaculture 5: 1-9 pp.

COOK, H.L. and MURPHY, M.A., (1969).

The culture of larval Penaeid shrimp.

Transactions of American Fisheries Society 98 (4) 751-754 pp.

DOBKIN, S., (1971).

Early developmental stages of Pink shrimp, *Penaeus duorarum* from Florida waters.

Fisheries Bulletin. Fisheries and Wildlife Service
U.S., 61 (190), 321 - 341 pp.

FAO, (1974).

Feeding of larval shrimp.

FAO Aquaculture Bulletin 6(23), 3-4 pp.

FUJINAGA, M. and KITAKA, (1967).

The large scale production of young Kuruma prawn
Penaeus japonicus Bate.

Bulletin of Plankton Society Japan commemoration No: of
Dr. Matsue's 60th Birthday Dec. 1967: 33-46 pp.

FUJINAGA, M., (1969).

Kuruma Shrimp *Penaeus japonicus* cultivation in Japan.

FAO Fisheries Report, 57 (3) 811-832 pp.

FURUKAWA, A. (1973).

Cultivation of larvae of *Penaeus japonicus* (Bate) with
marine yeast.

In: Fisheries in Japan-prawns. Japan Marine Products
Photomaterials Assoc. Tokyo 1; (161-163) pp.

GODFRED, J., SRIRAMAN. K., RAMANATHAN, N., KANNUPANDI, T.,
(1991).

Feed formulation and its effects on the growth of 4 Sp.
of penaeid shrimp.

Aquacult. Eur. Vol. 16, No.1, 1991 26-28 pp.

HAIQI HE, ADDISON LAWRENCE and RUIYA LIU, (1992).

Evaluation of dietary essentiality of fat soluble
vitamins A, D, E and K for Penaeid Shrimp.

Aquaculture, 103: 177-185 pp.

HAMID ALI, (1980).

A new system for mass rearing of Penaeid shrimp
larvae.

Proceedings of first National Symposium on shrimp
farming, Bombay 16-18 August, 1978: 254- 262 pp.

HAMEED ALI, K., S.N. DWIVEDI and K.H. ALIKUNHI, (1982).

A new hatchery system for commercial rearing of Penaeid
Prawn larvae.

C.I.F.E Bulletin 2-3 pp.

HARDIAL SINGH, (1988).

Preliminary observation on the growth and survival of
Tiger prawn *Penaeus monodon* (Fabricius) postlarvae in
Pen nurseries.

C.M.F.R.I. Special Publication No.40 53 p.

HIRATA, H.Y. MORI and MIYAMARU, M., (1962).

Breeding of Kuruma prawn *Penaeus japonicus* Bate.

Journal of Oceanography Society Japan, 20th Anniversary
694-906 pp.

HOUNG - YUNG - CHEN, (1993).

Recent Advances in Nutrition of *Penaeus monodon*.

Journal of the world Aquaculture Society Vol. 24, No.2,
June 1993.

HUDINAGA, (1942).

Reproduction, Development and rearing of *P. japonicus*
Bate.

Japanese Journal of Zoology., 10 (2): 305-393 pp.

HUDINAGA and MIYAMARU, (1962).

Breeding of the kuruma prawn *P. japonicus*, Bate.

Journal of Oceanography Society, Japan. (20th
Anniversary) 694-706 pp.

IMMAMARU, S. and SUGITA, T., (1972).

Penaeus shrimp seedling mass production technology
development studies, Early rearing of larvae using
artificial organic suspended matters.

Culture Technology Research 1(2): 34-46 pp. (In
Japanese)

ISHIDA, M., (1967).

The culture of *Penaeus* Zoea on a diet of rice bran.
Fukuoka prefecture, Bizen Experimental Research
Station, Project Report, 1966, 87-88 pp.

JONES, D.A., MUNFORD, J.G. and GABBOTT, P.A., (1974).

Microcapsules as artificial food particles for aquatic
filter feeders.
Nature (London), 247 (233-235).

KEVIN, D. HOPKINS, (1992).

Reporting fish growth; A review of the basics.
Journal of World Aquaculture Society Vol. 23, No.3
September 1992.

KIBRIA, G., (1993).

Studies on the moulting frequency and growth of shrimp
(*P. monodon*) fed on natural and compounded diets.
Asian Fisheries Science Vol. 6, No. 2 , 1993 203-211
pp.

KURATA, H. and SHIGUENO, K., (1979).

Recent progress in the farming of kuruma shrimp
(*Penaeus japonicus*)
In: T.V.R. Pillai And Wn.A Dill (Eds). Advances in
Aquaculture, Fishing News Books Ltd., Surrey; 258-268
pp.

- KURMALY, K., JONES, D.A., YULE, A.B., EAST, J., (1989).
Comparitive analysis of the growth and survival of
Penaeus monodon (Fabricius) larvae from Protozoal to
postlarvae 1, on live feeds, artificial diets and on
combinations of both.
Aquaculture 81: 27-45 pp.
- KUMLU, M. and JONES, D.A., (1995).
Effect of live and artificial diet on growth, survival
and Trypsin activity in larvae of *P. indicus*.
Journal of World Aquaculture Society Vol. 26. No.4,
Dec. 1995.
- KUO, C.C., (1986).
Microparticulated diet for grass shrimp larvae 81-85
pp.
In: J.L. Chuang and S.Y. Shiau, editors. Research and
development of aquatic animal feed in Taiwan vol: 1.
F.S.T. monograph series No.5 Fisheries Society of
Taiwan, Taipei.
- LEGER, P. and SORGELOOS, P. (1992).
Optimised feeding regimes in shrimp hatcheries.
In Marine Shrimp Culture: Principles and Practices.
Editors, Fast, A. and Lester L.J., Elsevir. Amsterdam -
London - New York - Tokyo. 225-244 pp.

LEVAY, L., RODRIGUEZ, A., KAMARUDIN, M.S., and JONES, D.A.,
(1993).

Influence of live and artificial diet on the tissue composition and trypsin activity in *Penaeus japonicus* larvae.

Aquaculture Vol: 118, 1993 287-297 pp.

LIAO, I.C., and HUANG, T.L., (1973).

Experiments on propagation and culture of prawns in Taiwan.

In: T.V.R. Pillai (Ed.) Coastal Aquaculture in the Indopacific Region, FAO Fishing News (Books) Ltd., 282 - 312 pp.

LIAO, I.C. and F.G. LIU, (1989).

A brief review of nutritional studies for *Penaeus monodon*.

In: Advances in Tropical Aquaculture, Tahiti, Feb 20-March 4. Aquacop IFREMER Actes de colloque 9. 355-380 pp.

LING, S.W., (1969).

Methods of rearing and culturing *Macrobrachium rosenbergii* (de Man).

FAO Fisheries Report 57 (3), 607-619 pp.

MOCK, C.R., (1972).

Proceedings of the 3rd Ann. Workshop, World Aquaculture Society, 285 - 286 PP.

MUTHU, M.S., (1980).

Development and culture of *Penaeid* larvae: A Review:
First All-India Symposium on Invertebrate Reproduction
July 1980.

NEW, M.B., (1979).

The diet of prawn, Working paper based on lectures
given to the 2nd Inland Aquaculture course given at
National Inland Fisheries Institute, Bankhem Thailand
(June - August 1979) 11-19 pp.

PIEDAD - PASCUAL, F., (1986).

Effect of supplemental lecithin and lipid sources on
the growth and survival of *Penaeus monodon* juveniles.
In: J.L. Maclean L.B. Dizon and L.V. Hosillos
(Editors). The First Asian Fisheries forum, Asian
Fisheries society, Manila, Philipines, 615-618 pp.

PASCAUL, F.P. and CATA CUTAN, M., (1990).

Defatted soyabean meal and *Leucaena* leafmeal as protein
sources in the diets of *Penaeus monodon* juveniles.
In: R. Hirano and I. Hanyu (Editors) proceedings of the
second Asian Fisheries forum. 17-22 April 1989, Tokyo,
Japan, Asian Fisheries Society Manila, Philippines 345-
348 pp.

PLATON, R.R., (1978).

Design operation and economics of small scale hatchery for larval rearing of Sugpo, *Penaeus monodon* (Fab.). Aquaculture Extension Manual, No. 1, SEAFDC, Philippines.

QASIM, S.Z. and EASTERSON, D.C.V., (1974).

Energy conversion in the shrimp, *Metapenaeus monoceros* (Fabricius) fed on detritus.

Indian Journal of Marine Sciences, 1 (2); 131-134 pp.

RANI PALANISWAMY and AHMAD ALI. S. (1991).

The impact of particle size of ingredients in compounded diets on pellet stability and performance in *P. indicus* H. Miline Edwards.

Journal of Aquaculture in Tropics, 6: 119-127 pp.

REDDY, S.R., AHMED, M.Q., JOSHI, P.K., DWIVEDI, S.N., (1981).

Short term training programme in Brackish water prawn and Fish culture Sept. 8.30, 1980, at Brackish water Fish Farm, Beach Road, Kakinada - 1 (A.P); (1981) 120 P.

SAMARASINGE, R.P., DESILVA, FERNANDO, D.Y., (1993).

The culture of *Biddulphia longicuris* and its use as food for *Penaeus monodon* larvae.

Asian Fisheries Science Vol. 6 No. 2, 223-227 pp.

SAMOCHA, B.R. and LEWEINSOHN, C., (1977).

A preliminary report on rearing penaeid shrimp in Israel.

Aquaculture, 10: (3) 291-292 pp.

SANDIFER, P.A., HOPKINS, J.S. and SMITH, T.I.J., (1976).

Production of juveniles; status of *Macrobrachium* hatcheries, 14 p.

SEDGWICK, R.W., (1984).

The requirements of *penaeus merguensis* for vitamin and mineral supplements in diets based on freeze dried *Mytilus edulis* meal.

Aquaculture, 19: 127-137 pp.

SICK, L.V. and BEATY, H., (1994).

Culture techniques and nutrition studies for larval stages of the Gaint prawn *Macrobrachium rosenbergii*.

Georgia Marine Science Contributions.

Technical Report Series 74-5; 1-30 pp.

SILAS, E.G., MOHAMED, K.H, MATHU, M.S., PILLAI, N.N., LAXMI NARAYANA, A., PANDIAN, S.K., THIRUNAVAKKARASU, A.R. and SYED AHAMED ALI (1985).

Hatchary production of Penaeid Prawn Seed *Penaeus indicus*.

C.M.F.R.I. special publication No. 23-41 pp.

SIMON, C.M, (1978).

The culture of the diatom *chaetoceros gracilis* and its use as food for penaeid protozoa larvae.

Aquaculture 14 (2); 105-113 pp.

SNEDECOR, G.W. and COCHRAN, W.G. (1967).

Statistical methods. 6th Edition. The Iowa state coll, press. Ames, Iowa.

SORGELOOS, P., (1988).

Feeding HUFA enriched artemia to *Penaeus monodon* postlarvae.

Artemia Newsletter 8: 30-31 pp.

STAHL, M.S. (1979).

The role of natural productivity and applied feeds in the growth of *Macrobrachium nosenbergii*.

Proceedings of the World Mariculture Society 10: 92-109 pp.

SUBRAHMANYAM, C.B. and OPPENHEIMER, C.H., (1969).

Food preferences and growth of grooved penaeid shrimp.

In: H.L. Youngken Jr. (Ed). Proceedings Good-drugs from the sea marine technol. Soc. Washington D.S. 65-76 pp.

SUDERSANAM, K., SUKUMARAN. N; (1996).

A simple micro encapsulated egg diet for postlarvae of *Penaeus monodon*.

Seafood Export Journal Vol. 27 No:3 1996 5-9 pp.

SUMITRA, V. and RAMADHAS. V., (1980).

Conversion efficiency in the shrimp *Metapenaeus monoceros* (Fabricius) fed decomposed mangroove leaves.

Indian Journal of Marine Science: 9 (2): 123-125 pp.

TESHIMA, S. and KANAZAWA, A., (1971).

Biosynthesis of sterols in the lobster, *Panalirus japonicus*, and prawn *Penaeus japonicus*, and the crab, *Portunus trituberculatus*.

Comparitive Biochemistry and physiology 38.B: 597 p.

THOMAS, M.M., KATHIRVEL, M. and PILLAI, N.N. (1976 a)

Spawing and rearing of penaeid prawn *Metapenaeus affinis* (H.M. Edu) in the laboratory.

Indian Journal of Fisheries 21 (2): 543-556 pp.

THOMAS, M.M., KATHIRVEL. M. and PILLAI, N.N., (1976 b).

Observation on the spawning and rearing of *Metapenaeus dobsoni* under laboratory conditions (575-579) pp.

TRINO, A.T., PENAFLOIDA, V.D, and BOLIVAR, E.C., (1992).

Growth and survival of *P. monodon* juveniles feed a diet lacking vitamin supplements in a modified extensive culture system.

Aquaculture 101; 25-32 pp.

TRINO, A.T. and SARROZA, T.C., (1995).

Effect of diet lacking in vit and mineral supplement on growth and survival of *P. monodon* juveniles in modified extensive system.

Aquaculture 136 (3,4): 323-330 pp.

UDAY RAM JOTHY, (1983).

Food Value of Rotifer, Brine Shrimp and *Moina* to postlarvae of *P. indicus* (H. Milne Edwards) reared in laboratory.

M.Sc. (Mariculture), Dissertation. Cochin university of Science & Technology.

VEDAVYASA RAO, P., (1983).

Larval nutrition - Technical session Seed production and hatchery management. Jan. 21 to 22nd (1983).

VIJAYAGOPAL, P., MANPAL SRIDHAR, and PAUL RAJ, R.,
(unpublished) Project No. PNP/35 - Development of feeds for culturable marine crustaceans.

VILLEGES, C.T., TEON-LOON, KANAZAWA, A., (1980).

The effect of feeds and feeding levels on the survival of a prawn *Peneaus monodon* larvae.

Memoirs Kagoshima University Research Center, South Pacific (1); 51-55 pp.

VILLEGES, C.T., KANAZAWA, A.

Rearing of larval stages of Prawn *Penaeus japonicus* (Bate) using artificial diet.

Memoirs Kagoshima University Research Center South Pacific., (1); 43-49 pp.

WALNE, P.R., (1974)

Culture of Bivalve Mollusc.

50 years experience at Conway. Fishing News (Books Ltd.): 1-173 pp.