MICROENCAPSULATION AND ITS EFFECTS ON QUALITY AND SHELF LIFE OF PRAWN LARVAL FEED

BY

REMA M.K

THESIS

Submitted in partial fulfilment of the requirement for the degree

MASTER OF FISHERIES SCIENCE

FACULTY OF FISHERIES

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF PROCESSING TECHNOLOGY

COLLEGE OF FISHERIES

PANANGAD - KOCHI

1998

To,

My Parents, Brother Raju

&

Sister Radha

DECLARATION

I hereby declare that this thesis entitled **"MICROENCAPSULATION AND ITS EFFECTS ON QUALITY AND SHELF LIFE OF PRAWN LARVAL FEED"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Panangad.
11-9-98

REMA M.K

CERTIFICATE

Certified that this thesis entitled **"MICROENCAPSULATION AND ITS EFFECTS ON QUALITY AND SHELF LIFE OF PRAWN LARVAL FEED"** is a record of research work done independently by Kum. Rema.M.K under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Dr. Lizy Behanan

Lizy Behanan

(Chair person, Advisory Board)
Asst. Professor (Seln.Grade)
College of Fisheries,
Panangad
Kochi

NAME AND DESIGNATION OF THE MEMBERS OF THE ADVISORY COMMITTEE/EXAMINATION COMMITTEE

Signature

CHAIR PERSON

Dr. Lizy Behanan
Asst. Professor (Seln. Grade)
Department of Processing Technology
College of Fisheries, Panangad, Kochi

MEMBERS

Dr. D.D. Nambudiri
Associate Professor and Head,
Department of Processing Technology
College of Fisheries, Panangad, Kochi

Sri. S. Krishna Kumar
Asst. Professor (Senior Scale)
Department of Processing Technology
College of Fisheries, Panangad, Kochi

Smt. Alphi Korath
Asst. Professor
Department of Management Studies
College of Fisheries, Panangad, Kochi

EXTERNAL EXAMINER:

CIFT, Kochi 682029

ACKNOWLEDGEMENT

I am immensely grateful to ***Dr. Lizy Behanan***, Asst. Professor (Seln. Grade), Dept. of Processing Technology, College of Fisheries, for her valuable guidance, timely advice and constant encouragement throughout the course of my study. Her patience, meticulous attention and valuable suggestions helped me a lot in the preparation of this thesis. She has been the primary source of inspiration and motivation during the development and progress of research.

I owe a great deal to ***Dr. D.M. Thampi***, Dean I/C, Professor and Head, Department of Aquaculture for granting permission and for providing necessary facilities for the successful conduct of research work.

I am greatly indebted to ***Sri. S. Krishna Kumar***, Asst. Professor (Senior Scale), Department of Processing Technology, for the timely advice and valuable help and support during the course of study. I thank him for critically going through the draft and making all the necessary corrections and modifications to prepare the thesis in the present form.

I wish to express my gratitude to ***Dr. D.D. Nambudiri***, Professor and Head, Department of Processing Technology for his valuable suggestions and timely advice.

I am also thankful to ***Smt. Alphi Korath***, Asst. Professor, Mgt. Studies for the statistical experiment planning which has made the interpretation of results very meaningful.

I wish to extend my gratitude to ***Dr. C. Mohana Kumaran Nair***, Associate Professor, Dept. of Aquaculture for providing hatchery facilities for the successful conduct of research .

My special thanks also due to my friends ***Anjana,V., Sunitha, P.S., Sheeela Bai, Bindhu, Sreeja Menon, Neeraja,T.*** and ***Smitha Gopal*** for the kind assistance rendered during the research work.

The assistance rendered by the library staff of College of Fisheries, Central Marine Fisheries Research Institute, Central Institute of Fisheries Technology and Cochin University of Science and Technology are gratefuly acknowledged.

I wish to place on record my sincere thanks to ***Smt. Tessy Thomas***, Jr. Programmer, College of Fisheries and ***Anish Paul Behanan*** and ***Anooj Kurian Behanan***, sons of my Major Advisor for their nice piece of work in printing of the thesis.

I also thank **The Kerala Agricultural University** for providing me with the Junior Fellowship, during the tenure of my study.

CONTENTS

1.	INTRODUCTION	1
2.	REVIEW OF LITERATURE	
2.1.	Larval rearing	7
2.2.	Nutritional requirements of *Macrobrachium rosenbergii* larvae	11
2.2.1.	Protein and amino acid	12
2.2.2.	Energy	13
2.2.3.	Carbohydrate	13
2.2.4.	Lipid	14
2.2.5.	Minerals	16
2.2.6.	Vitamins	17
2.3.	Larval feeds	19
2.3.1.	Microcoated diets	20
2.3.2.	Microbound diets	20
2.3.3.	Microencapsulated diets	
2.3.3.1.	Microencapsulated diet for crustaceans	23
2.3.3.2.	Microencapsulated diet for bivalves	32
2.3.3.3.	Microencapsulated diet for fish larvae	37
2.3.3.4.	Microencapsulated diet for zooplankton	40

2.4.	Gelatin and Collagen	42
2.5.	Storage stability of feed	44
3.	MATERIALS AND METHODS	
3.1.	Preparation of feed encapsulating material	46
3.1.1.	Extraction of collagen	46
3.1.2.	Extraction of gelatin	47
3.2.	Preparation of the feed	
3.2.1.	Feed Ingredients	47
3.2.2.	Processing of the protein source	47
3.2.3.	Proximate composition of the feed ingredients	48
3.2.4.	Formulation and processing of experimental diet	48
3.2.5.	Proximate composition of the experimental diet	50
3.3.	Acceptability study of the experimental diet	
3.3.1.	Source of larvae	50
3.3.2.	Conditioning of the larvae	51
3.3.3.	Monitoring the acceptability of the feed by the larvae	51
3.4.	Storage study of the experimental diet	52
3.4.1.	Enumeration of Total Plate Count	53
3.4.2.	Determination of Peroxide Value	53
3.4.3.	Determination of Thio Barbituric Acid Value	53

3.5.	Water stability	54
3.6.	Statistical analysis of the data	54
4.	RESULTS	
4.1.	Proximate composition of feed ingredients and formulated feeds	
4.1.1.	Proximate composition of feed ingredients	56
4.1.2.	Proximate composition of formulated encapsulated feeds	56
4.2.	Water stability	57
4.3.	Evaluation of microbiological quality and shelf life	
4.3.1.	Enumeration of Total Plate Count (TPC)	57
4.3.2.	Evaluation of shelf life	
4.3.2.1.	Evaluation of Peroxide Value (PV)	59
4.3.2.2.	Evaluation of Thio Barbituric Acid (TBA) value	61
4.4.	Evaluation of acceptability and survival rate	63
5.	DISCUSSION	65
6.	SUMMARY	77
7.	REFERENCES	79
	ABSTRACT	

LIST OF TABLES

1.	Proportion of ingredients used for the preparation of basal diet	49
2.	Type of encapsulated feed used in each bowl	51
3.	Storage conditions of the prepared diets	52
4.	Various encapsulated feeds	55
5.	Proximate composition of feed ingredients	56
6.	Proximate composition of formulated encapsulated feeds	56
7.	Water stability of encapsulated diet in percentage	57
8.	No. of colonies/g of each diet at different storage conditions	58
9.	PV in milli equivalents/kg fat at different storage conditions	59
10.	TBA values (mg malonaldehyde/kg) of different feeds at different storage conditions	61
11.	Survival values of the prawn larvae in percentage	63

ANOVA TABLES

1.	Analysis of variance of the data on Total Plate Count of different feeds stored at different storage conditions	58
2.	Analysis of variance of the data on peroxide value of different feeds stored at different storage conditions	60
3.	Analysis of variance of the data on thio barbituric acid value of different feeds stored at different storage conditons	62
4.	Analysis of variance of the data on the survival rate of larvae fed different feeds	63

LIST OF FIGURES

I. PV in milliequivalents/kg fat at different storage conditions

2. TBA values (mg malonaldehyde/kg) of different feeds at different storage conditons

3. Percentage survival values of prawn larvae

1. INTRODUCTION

The commercial production of freshwater prawn *Macrobrachium rosenbergii* is gaining a great momentum all over the IndoPacific region. In India, the promotion and culture of this species on scientific lines is being taken up as a priority sector development in aquaculture for ponds and tanks (Das,1993). The giant prawn is widely cultured in Asian countries such as Thailand, Taiwan, Malaysia, Vietnam, India, Indonesia and in Hawai and South Carolina states of USA. The latest global production of this species is 34,000 mt. Asia alone contributes to about 29,000t (92%) to the global production. Fresh water prawn production from India rose from mere 200 t in 1991 to 8000 t in 1994. If the same trend continues India may overtake Thailand, Taiwan, and Vietnam in the near future (Murthy,1996).

Eventhough it has been estimated that the total world production of compounded aquaculture feed constituted 16.8 mmt in 1995(Gill,1996), no official statistics are currently collected by FAO concerning compounded aquafeed production within member countries. Similarly no information was provided as to the quantities of aquafeeds produced for different major cultivated species within individual countries or regions (Gill, 1997) and for larval feed production.

The various types of artificial diets that have been considered for weaning larvae of fish and crustaceans are minced diets, wet microparticulate diets, dry microparticulate diets, spray dried diets, microbound diets, micro-coated diets and micro encapsulated diets . Each one of these has its own advantages and disadvantages.

Larviculture nutrition, particularly first feeding by the early larval stages, appears to be the major bottle neck for the industrial upscaling of the aquaculture of fish and shell fish. The cultivation and management of live feeds to support hatchery production of bivalves, crustaceans and fish remains costly, unpredictable in many instances, and often provides sub-optimum nutrition.The replacement of live foods by artificial diets in larval culture is necessary because many systems are still dependent upon one or even two live food-chain links to provide adequate nutrition of larval molluscs, crustaceans and fish.

One of the major constraints in the culture of *Macrobrachium rosenbergii* is the lack of cost effective and efficient feeds for its culture . Similar problem exists in its seed production also. *Macrobrachium* larviculture, like most other aquaculture operations, relies heavily on the use of the brine shrimp, *Artemia salina*, as an overnight live food for the prawn larvae. Brine shrimp eggs are not only difficult to obtain but are also expensive. The dependence of *Macrobrachium* hatcheries on the expensive and occasionally unobtainable cysts of *Artemia salina* is perhaps the primary

problem facing hatchery operators. The supply of *Artemia* cysts appears to be critically low and may be a major bottle neck constricting development of commercial hatcheries. Elimination of this heavy reliance on brine shrimp would be a major step in cutting down the cost of seed production. The limiting factor can be overcome if other natural and formulated feeds are developed.

Food and environment have a direct impact on the growth and development of all organisms. In prawn culture, feed assumes greater importance in view of its day to day need, its influence on the survival, growth and total production and its share in the total cost. The most important variables affecting the overall growth and feed utilization are stages of growth, water quality, water temperature, stability of the feed, palatability, mode of presentation of feed, bioavailability of protein and other nutrients and the manner in which food is distributed in a pond or tank. In this direction, microencapsulation seems to be a promising technique.

The technique of microencapsulation, currently used successfully for a variety of commercial purposes, is expected to find other new and exciting applications. Microcapsules are composed of a polymeric skin or wall (shell) enclosing a core. The capsule wall is a liner to the substance it contains, is strong enough to permit normal handling without rupture, and is relatively thin so as to permit a high core to wall ratio. The contents of the capsule are contained within the shell until released by means

that serve to break, crush, melt, dissolve or rupture the capsule wall, or until the internal phase is caused to diffuse through the capsule wall.

Microcapsules are used advantageously in many fields. The encapsulation of volatile aromas and flavours protects them from physical oxidation and from thermal decomposition. For medicinals, encapsulation serves to mask unpleasant odour and taste, and to protect against oxidation and spoilage. In addition, encapsulation can be such that the medicament will be selectively absorbed in the intestine rather than the stomach, or released gradually to provide action over a long period of time.

Recently it has been demonstrated that one, and in some cases, both live food chain links in larval aquaculture may be successfully replaced by microencapsulated diets. A major advantage of encapsulated diet is that there is minimal loss of nutrients within the aqueous environment thus minimising organic load in the system and alterations in oxygen and pH levels. Microencapsulated diets are specially prepared diets which are comparable to plankton in size and performance; the liquid and particulate dietary components are enclosed within a carefully engineered wall and are released under specific environmental conditons reducing the leaching of essential nutrients. Release of internal nutrients at active sites within the target system is the crux of the capsulation technology.

The main objectives of the present study were to formulate and prepare a microencapsulated feed for prawn larvae using cheap sources of fish waste materials to reduce the cost of feed and to study the acceptance of the feed by the larvae and also to study the effect of encapsulation on its quality and shelf life.

Feed storage and maintenance of feed quality are important economic considerations. Changes in amino acid, fatty acid, and protein profiles could result in reduced feed quality, thereby adversely affecting growth of the cultivated species and contributing to economic losses. The development of aflatoxin, proliferation of pathogenic bacteria and rancidity are the major problems in stored feed stuffs. Eventhough a variety of microencapsulated feed has been prepared for various species, little information is available on the formulation of a microencapsulated feed for fresh water prawn larvae and the effect of encapsulation on its quality and shelf life . Fish skin,offal and bones are rich source of collagen and gelatin. Fish procesing wastes are abundant and could be a valuable source of collagen and gelatin. In the present study collagen and gelatin extracted from fish processing waste and commercially available egg albumin were used as the encapsulating material.

The present study highlights the significance of the production of a low cost feed and the importance of the storage study of the prepared feed. Here, microencapsulation seems to be a promising technique in the

production of *Macrobrachium* larval feed which can replace live *Artemia* nauplii, that constitutes 60% of the cost of hatchery operations.

2. REVIEW

2.1. Larval rearing

There are a number of microscopically distinct stages during the laval life of freshwater prawns (New,1982) and eleven larval stages (zoea stages) have been recognised for *Macrobrachium rosenbergii* which last for about 25-40 or 45 days depending on the temperature, water quality and food (Uno and Soo,1969). Larvae eat continously and in nature their diet is principally zooplankton (mainly minute crustaceans), very small worms and the larval stages of other aquatic invertebrates (New, 1982).

After the first successful attempt in rearing the larvae of *Macrobrachium rosenbergii* (de Man) through metamorphosis by Ling and Merican (1961) and Ling (1962) great interest was aroused in many countries to investigate the aquaculture of this species of freshwater prawn. Over a short span of years, the techniques of larval rearing underwent considerable refinement and improvement. Fujimura (1966) was the first to standardise the method of juvenile production by using "green water" consisting of unicellular algae. This method is still used by many hatcheries in South East Asia. Apart from green water, some workers have experimented on static clear water, closed recirculating water and recirculating synthetic sea-water, with varying degrees of succes(Dugan *et al*, 1975; Smith *et al*,1976 b).

Attempts to rear *Macrobrachium rosenbergii* (de Man) larvae were first initiated in Philippines in January 1976 with 6000 larvae from two

berried females (De jarme *et al*, 1982). The larvae were reared in three 60 litre capacity glass aquaria and attained stage VIII of development in 22 days but increasing mortalities followed thereafter. Two months later some 800 larvae which were hatched from eggs spawned by a spent female and fertilized by a male in the laboratory were reared similarly. Within ten days most of the larvae metamorphosed to stage IV. In later stages, however, the number gradually decreased.

A major problem in *Macrobrachium* seed production is that posed by feed. *Macrobrachium* larviculture, like most other aquaculture operations, relies heavily on the use of the brine shrimp, *Artemia salina*, as an overnight live food for the prawn larvae. Brine shrimp eggs are not only difficult to obtain but are also expensive (Sorgeloos,1976). Sandifer *et al* (1976) reared *Macrobrachium* larvae using bonito meat and frozen *Artemia* along with brine shrimp nauplii.

Adisukresno *et al* (1982) got quite impressive results when the larval culture was conducted using locally formulated feed with the aim of reducing the cost of production and simplyfying the operation within the means of the artisanal fish farmers. Aniello *et al* (1982) carried out experiments to determine the feasibility of using the cladoceran (*Moina* sp) as a substitute for *Artemia* as an overnight feed for prawn larvae. Chineah (1982) reported improvements made in the larval rearing technique for the giant prawn since 1975. For feeding the prawn larvae conventionally filleted

frozen skipjack or tuna is subjected to a jet of water under pressure to break the flesh into small particles which are passed through a series of BSI standards superimposed stainless steel sieves of 20, 40 and 60μ to sort out particles of varying size.

By 1989 few hatcheries except in Taiwan, still used modifications of the pioneering 'Fujimura' green water rearing systems. The green water system is very difficult to manage consistently and remains out of favour with hatchery operators generally, except in Malaysia (Brown, 1991) and Mauritius (Thompson *et al*,1992). Rearing *M. rosenbergii* larvae on *Artemia* alone in a closed recirculating system, Devresse *et al*(1990) found that pronounced improvements were obtained in growth, survival , metamorphosis and stress resistance when *Artemia* were enriched with 200 mg/g and 400 mg/g n-3 HUFA concentrations.Cut pieces of *Tubifex* sp have been used to feed cultured larvae of *Macrobrachium malcolmsonii* (Rao, 1991). The use of both *Moina macrura* and *Artemia* nauplii was described by Reddy *et al* (1991). Daniels *et al* (1992) suggested a supplemental diet formula for use from 10th day onwards. This consists of 85% cleaned squid, 10% chicken eggs, 3% beef liver powder and 2% cod liver oil bound with an alginate hardened by calcium chloride and sieved. Following the research of Jahangir (1992) *Moina macrura* was investigated as an alternative to *Artemia* sp for *M.rosenbergii* larvae (Alam *et al*, 1993a,1993b). As backyard hatcheries become larger there is a tendency for them to convert from the use of farm made larval feeds for *M rosenbergii*

to the use of commercial larval diets because of the labour requirements of the former (Boonyaratpalin and New, 1993). Higher postlarval production was obtained through the use of a 50:50 *Artemia:Moina* mixture than with *Artemia* alone if the *Moina* were cultured on poultry manure, which contained an enhanced level of n-3 HUFA. Nair and Hameed (1992) used a coagulated suspension of 1 kg of 'thelly' (*Metapenaeus dobsonii*) meat plus 10 hen's eggs to feed *M.rosenbergii* larvae stocked at 50 ± 5/litre. Later, Alam *et al* (1993 c) found that weaning larvae on to *Moina* at stage X resulted in a higher yield of post larvae. Egg custard was also used in all these experiments (Alam *et al*, 1993 a, 1993 b, 1993c).

Artificial diets have not been found to be so successful for *M.rosenbergii* larval rearing as with marine shrimp. Exclusive replacemnt with microencapsulated diets throughout the larval rearing period has not been successful, although Deru (1990) found that fresh water prawns could survive on microencapsulated diet alone from Z stage 6-7 to metamorphosis. Jones *et al* (1993) demonstrated that *M.rosenbergii* trypsin levels increase dramatically from Z5 and peak at Z6, which explains the findings of Deru(1990). Fresh water prawn also have very fast gastroevacuation times. Although prawn larvae fed with microencapsulated diets for more than 24 hr. show substantially increased trypsin enzyme activities, survival is no better than in animals that are starved. Bell (1994) concludes that, although the early larval stages have sufficient trypsin activity (or exogenous trypsin from the live feed) to digest *Artemia* nauplii they cannot cope with

microencapsulated diet until stage Z6, when the relative hepatopancreas size has increased. To achieve full replacement of live diets throughout the larval rearing period requires diets of increased digestibility or enzyme incorporation to be developed.

2.2. Nutritional requirements of *Macrobrachium rosenbergii* larvae

Adequate nutrition is a pre requisite for the survival, normal developemnt and proliferation of any animal species (Biddle, 1976). In the natural environment, the giant prawn, *Macrobrachium rosenbergii* is able to satisfy its particular nutrient requirements from a variety of sources which include aquatic worms, insects, small molluscs, crustaceans, plankton and organic detritus (Ling,1969 a). However, under the restrictive and rigidly controlled conditons of intensive culture,it will become necessary to provide the prawn with appropriate levels of nutritionally balanced feeds. The selected food should be physically available close to the larvae, easly tackled, digestible, metabolizable and meet the nutritional requirements of the larvae. It is only since the past two decades the nutrition attracted the attention as a ways and means of solving the problems of larval diet . Besides the requirement of suitable particle size of the food, the artificial larval diet should be palatable, its nutrient must not be leached out and it should not pollute the water and it should easily available to the delicate larvae. Most of the studies relates to the nutritional requirements of juveniles and adults,the nutritional requirements of larvae being very scarce (Sherief,1990).

2.2.1. Protein and amino acid

Protein is indispensable for growth and maintenance of life of all animals. It is one of the most expensive single ingredient in animal feeds and therefore more time, resource and effort have been devoted in the investigations of protein requirements of prawns than any other dietary component. New (1976 b) has discussed numerous studies reporting on growth rates, feed efficiencies etc. of various crustacean feed, different levels of dietary protein. The study suggested that the crude protein level of *M.rosenbergii* larvae as 10-20 % and optimum levels suggested as 15-20% (Sick,1975). From their studies there is still considerable uncertainty as to prawn's quantitative requirement for dietary protein (Sherief,1990). According to New (1976a) a consensus of opinions indicates that the optimum level of dietary protein for different species of prawns is between 27 and 35% . In the case of young juvenile *M.rosenbergii* the requirement may be somewhat higher. The overall role of dietary protein is to provide sufficient quantities of those amino acids which are essential to the normal health and survival of an animal. Experiments conducted in *M.rosenbergii* have revealed that it has quantitative requirements for the amino acids such as Arginine, Histidine, Leucine, Isoleucine, Methionine, Phenyl alanine, Threonine, Tryptophan and Valine (Watanabe,1975). The non essentiality of lysine in the giant prawn *Macrobrachium* has been suggested by Watanabe (1975).

2.2.2. Energy

Dietary energy has been shown to exert a significant influence on the growth rates and nutrient assimilation efficiencies of both domestic animals and fish. Of the various factors which affect the overall energy requirement of the cultured crustacean, the most important one is the stage of the life cycle, the form in which the diet is presented, and the specific energy sources in the diet. Based on data on respiration, Stephenson and Simmons (1976) have reported the calorific requirement of *M. rosenbergii* larvae as seven times higher than that for juvenile prawns. According to Stephenson and Simmons, the higher energy expenditure of the larval stages may reflect their perpetual swiming habit and activity associated with food gathering as well as their extremely rapid development. Comparison of calorific values were made to assess larval growth of *M. rosenbergii* fed on different forms of various experimental diets (Sick and Beaty, 1974). They prepared six artificial diets in three forms (freeeze dried, flakes, gel) and fed to stage 7-8 *M. rosenbergii* larvae under laboratory conditions. Results showed higher energy assimilations when diets in the freeze-dried form were fed.

2.2.3 Carbohydrates

Unlike fish, crustaceans show limited ability to tolerate monosaccharides like glucose in their diet (Andrews *et al*,1972). They demonstrated that disaccharides such as sucrose and maltose and polysaccharides such as wheat starch, corn starch and potato starch can be utilized more efficiently than simple sugars. Incorporation of

polysaccharides at levels between 30 and 40 % of the dry diet is most common in crustacean diets (Sherief, 1990). However, extensive research is required to define the carbohydrate requirement of *Macrobrachium rosenbergii* at various stages of growth and development.

2.2.4. Lipid

In the case of dietary fat fish are apparently capable of using levels as high as 20-30% of their diet (Halver, 1976). This unique ability to efficiently metabolize high levels of dietary fat has a concomitant sparing effect on the protein required for maximum growth (Biddle, 1976). Unlike fish, there is rather convincing evidence that crustaceans cannot tolerate high level of dietary fat (Andrews *et al,* 1972). It has been demonstrated that the inclusion of a lipid supplemented at levels of 10% or more in the diet of *Penaeus setiferus* adversely affects growth and survival. Significant depression in growth was observed in *Penaeus serratus* fed diets containing 15% or more lipid (Forster and Beard,1973).Studies on essential fatty acid requirements (Sick and Andrews,1973) for crustaceans have suggested that the nutritive value of lipids for prawn is probably related to the types and contents of essential fatty acids. Marine crustaceans have higher levels of series (**ω3**) fatty acids and higher amounts of C20 and C22 PUFA than freshwater crustaceans. This indicates that essential fatty acids of linolenic series have greater value to marine crustacean, while the fresh water crustacean require more linoleic series or a mixture of both. A dietary requirement of 1-2 % linolenic acid is indicated in the diet of prawns

(Shewbart & Mies , 1973). Devresse et al (1990) found that pronounced improvements were obtained in growth, survival,metamorphosis and stress resistance when *Artemia* were enriched with 200 mg/g and 400 mg/g n-3 HUFA concentrations. The n-3 HUFA requirements of larval prawns had not previously been thought to be critical, because *M. rosenbergii* was known to spend most of its life cycle in fresh water (Sorgeloos & Leger,1992). However, the work of Devresse *et al* (1990) has shown that significant improvements of commercial value to farmers can be achieved when n-3 HUFA enriched *Artemia* are fed. Romdhane *et al* (1994) have shown that the value of n-3 HUFA enriched *Artemia* is enhanced, the earlier it is fed to prawn larvae.

The quantitative and qualitative requirements for an exogenous source of sterols in artificial diets for marine crustaceans have been studied since the early 1970's. Utilization of dietary cholesterol as well as other sterols by crustaceans are discussed by New (1976). Reported levels required by larval and juvenile *Penaeus japonicus* range from 0.1 to 0.2 %(Shudo *et al*, 1971) and 0.5% (Kanazawa *et al*, 1970,1971; Teshima,1982; Teshima *et al*; 1983) to 1.4% (Deshimaru and Kuroki; 1974). Such studies are very limited in the fresh water prawn, *M. rosenbergii*. The only report available in this aspect is that of Briggs *et al* (1988) who have shown that supplementary dietary cholesterol at levels in excess of 0.12 % is not essential for juvenile *M. rosenbergii*. Later Sherief *et al* (1992) suggested that supplemental cholesterol is not required in the semi-purified diet

(containing 0.12 % endogenous cholesterol) developed for *M.rosenbergii* when reared under the experimental conditions.

Crustaceans have long been known to have a limited ability to synthesise phospholipids *invivo.* This biosynthesis is not rapid enough to satisfy the metabolic requirements of young crustacean (Sherief, 1990). Thus dietary phospholipids such as soya lecithin at levels between 0.5 and 8% of the diet have proved essential in terms of growth and survival in casein purified diets. Lecithin at levels betwen 0 and 10 % of the diet has however proved unnecessary in purified diets containing 1 % supplemental cholesterol when fed to post larval *M.rosenbergii.* It was shown that there is no advantage confirmed by supplementary cholesterol and or lecithin to the basal diet (containing 0.12 % endogenous cholesterol and 0.048% total phospholipids) formulated for *M. rosenbergii.* Mahesh (1996) suggested that supplementation of lecithin at a level of 2.5% in the diet can accelerate growth and improve FCR during the early post larval phase of *M. rosenbergii* (i.e upto four weeks after larval settlement); beyond this, supplementation of lecithin is not needed in the diet.

2.2.5. Minerals

Dietary requirements of minerals have virtually been ignored in research investigations with *Macrobrachium* species (Biddle, 1976). Many micronutrient additions to formula feeds used for *Macrobrachium* sp. by virtue of supplementation may actually be deleterious for maximum

rates of growth (Sick and Milliken, 1983). Saju (1996) reported the optimum requirement of 1.8% dietary phosphorous for the giant fresh water prawn juveniles, the deficiency and excess of which leads to adverse effects.

2.2.6. Vitamins

Knowledge of the vitamin requirements of crustaceans in general is very scanty (Walker, 1975), particularly if one is referring to those candidate species identified for commercial culture. This situation is in contrast to that of several valuable fishes such as salmon, trout, catfish, carp etc.for which rather specific requirements have been established for a number of the water and fat soluble vitamins (Ketola,1976). The available data indicated that most of the studies on vitamin requirements were carried out in penaeid prawns. Researches has been reviewed (New, 1976 b) in which the importance of β- carotene and ascorbic acid in penaeid nutrition was investigated. Dey and Raghuvaran (1988) reported that the requirement of vitamin C vary depending upon the environment. They also reported that the ascorbic acid content was less in fresh water prawn *Macrobrachium* compared to brackish water shrimp *Metapenaeus* sp. Survival rate was the most important parameter affected by ascorbic acid deficiency on *M.rosenbergii* post larvae (Heinen, 1988). He reported the deficiency symptoms of vitamin C exhibited by *M.rosenbergii* as higher incidence of small cuticular black or dark brown lesions, moulting of only the abdomen (or only the posterior part of it) or prawns dying while trying to moult and presence of subcuticular blotches in the rostrum and other parts of non-

moulting animals, these blotches usually being white in living animals and brown in dead ones. Ascorbyl palmitate, a stable and lypophilic source of vitamin C, was used in a 20% (w/w) enrichment medium for brine shrimp nauplii to increase the vitamin content upto 2-3.4 mg/g DW, the level of which was maintained after 24 hr. storage following a 24 hr. enrichment (Merchie *et al* 1993). Although no significant differences were found in growth or survival of *M.rosenbergii* larvae fed with *Artemia* nauplii enriched with 0,10 and 20% ascorbyl palmitate, a significantly positive effect was noted on the physiological condition of the post larvae as measured by a salinity stress test. A noticeable drop in the vitamin C level of post larvae, compared to larvae, indicated a need for vitamin C during metamorphosis, a stress sensitive period. He also reports that under stress situations high vitamin C supplementation might enhance production characteristics. The requirement of vitamin C by *Macrobrachium rosenbergii* as reported by D'Abramo *et al* (1994) as 104.3 mg L-ascorbyl-2-monophosphate, L-ascorbyl-6-palmitate and ascorbyl palmitate AAE/kg dry diet. Phosphate derivatives were utilized effectively in *M.rosenbergii* (D'Abramo et al 1994). The requirement of ascorbic acid by the larvae of *M.rosenbergii* is reported by New(1995). He had suggested a method of enriching *Artemia* nauplii with ascorbyl palmitate. Although there was no significant differences in growth and survival of larvae, a positive effect was noticed in the physiological condition of the post larvae which indicated vitamin C requirement during metamorphosis. According to him a high supplementation of vitamin C might enhance production characteristics

under stress situations. Gijo (1996) reported that the growth of *M.rosenbergii* juveniles was found to be significantly influenced by different dietary levels of vitamin C. She reported that the maximum growth was obtained with a dietary level of 135 mg AAE/kg dry weight.

There are some reports on the importance of β-carotene in prawn nutrition. As yet no quantitative requirements have been defined. Infact, the role of β-carotene has been considered more from its involvement in pigment formation than as a precursor of vitamin A. This has been proved in the case of *Penaeus japonicus* (Kitayama *et al*, 1972). The qualitative and quantitative vitamin requirement of *M.rosenbergii* are yet to be studied. Based on the preceding review it is evident that existing knowledge on the nutritional requirement of *M.rosenbergii* larvae is very limited. A major research task is hence needed in several aspects of larval nutrition, the important areas being total nutritional requirements of different larval stages of *M.rosenbergii* , development of inexpensive but balanced diet of suitable particle size with locally available ingredients, nutritional diseases of larvae and the development of viable feed technology to produce enormous quantities of larval feed required in large scale hatchery production of *M.rosenbergii* seed.

2.3. Larval feeds

The various types of artificial diets that have been considered for weaning larvae of fish and crustaceans are minced diets, wet

microparticulate diets, dry microparticulate diets, spray dried diets, microbound diets, micro-coated diets, microencapsulated diets (Paulraj, 1993). Kanazawa (1986) categorised micro-particulate diets into three groups as micro-coated diets (MCD), micro bound diets (MBD) and micro encapsulated diets (MED).

2.3.1. Microcoated diets

Kanazawa (1982) obtained good results when zein microcoated diets were fed to prawn larvae. Kanazawa *et al* (1983) studied the survival rate of larval crab *Portunus trituberculatus* with micro coated diets along with microbound and microencapsulated diet and cholesterol-lecithin microcoated diets. Methods for the production of microcoated particles has been given (Gatesoupe,1986). A method for the formulation and evaluation of a particulated feed for *M.rosenbergii* post larvae has been given . Abdel-Rahman *et al.*(1993) reared shrimp larvae using a combination of live food and microparticulated diet and obtained good survival rate. Microcoated diets are prepared by coating microbound diets with some materials such as zein and cholesterol-lecithin (Paulraj,1993).

2.3.2. Microbound diets

After Andrew and Mac Leod (1970) described the application and control of algin-calcium reaction to food products, these hydrocolloids were adopted as binding agents in various rations (Forster, 1971;1976). Alginate and gum products have been used as binders for fish and shrimp

feeds by Meyers and associates (Meyers and Zein-Eldin, 1972; Zein-Eldin and Meyers, 1973; Meyers and Brand, 1975). Although these binders were judged to be adequate, no quantitative evaluation of their improved binding was performed (Farman - farmain *et al* ,1982). Digestibility of microbound diets could be improved by using soft processing methods to preserve the native properties of protein source (Gabaudan *et al*, 1980). Farman - farmain *et al* (1982) observed the pellet stability of repelletised Kelco HV algin bound Purina Marine Ration M20 at different levels of binders. They found that RM20-2.0% bound pellet gave the best results in both physical stability and efficient biological utilization. Kanazawa *et al* (1982b) demonstrated that 10 day old larval red sea bream and Ayu, *Plecoglossus altivelis* grew and survived reasonably well when fed microbound diet containing a mixture of chicken egg , egg yolk powder, squid meal, clam extract, krill and milk casein as major protein sources. However, the growth of larvae fed microbound diet solely was inferior to that of larvae receiving both rotifers and microbound diet. Teshima *et al* (1982, 1983) demonstrated that larval *Penaeus japonicus* fed microbound diet which include kappa-carrageenan as a binder, grew and survived well from zoeal to post larval stages. Kanazawa *et al* (1983) conducted experiments to rear the larval crab, *Portunus trituberculatus*, with carrageenan microbound diet along with other artificial microparticulate diets. The carrageenan microbound diet supported growth of crab larvae from zoea1 to juvenile stages when supplied as a sole diet, but the survival rate with the carrageenan microbound diet was lower than that with the live feeds. Microbound diets have been employed

for the study of larval fish nutrition . The results of growth and survival of larval ayu *Plecoglossus altivelis* fed with microbound diets containing 50% rotifer and live food alone for 90 days were reported (Kanazawa,1986).The survival of the microbound diet group at the end of the feeding trial was 88.5 %. He observed similar results for the total length and body weight for two groups of feeds. The procedure for the preparation of carrageenan microbound diets and agar gelatin microbound diet for feeding prawn and bivalve larvae were given by Kandasamy (1987). The performance of kappa-carrageenan microbound diet was assessed on *Penaeus monodon* larvae (Bautista *et al*, 1989). They reported that feeding with carrageenan microbound diet in combination with natural food resulted in the high percentage of survival rate. Kanazawa *et al* (1989) reported that the microbound diet in which the amino acid pattern approximated to that of larval whole body protein together with small amount of live food could sustain the growth and survival of larval red sea bream and Japanese flounder. Koshio *et al* (1989) reported kappa-carageenan as an effective binder for a microparticulate diets. Cofeeding live and microbound diets could produce growth and survival in early milk fish larvae superior to that achieved with either live feeds or artificial diets alone (Marte and Duray, 1991). Ruyet *et al* (1993) reported that due to relative unreliability in growth results and in juvenile quality (the level of skeleton abnormality was unacceptable) alginate microbound diets made from fresh materials were considered as unrealistic foods for larval feeding and obviously for nutrition studies. Further, they reported that microbound diet using zein as a binder

(Teshima *et al*, 1982) was relatively easy to prepare compared to alginate microbound diet. According to them zein microbound diet could be considered as a suitable basal diet to start the study of the nutritional requirement and digestive physiology of seabass and other marine fish larvae.

2.3.3. Microencapsulated diet.

2.3.3.1. Microencapsulated diet for crustaceans

The preparation of microencapsulated diet for rearing the larvae of *Penaeus japonicus* was described by Jones *et al* (1979 a). They reported that a survival rate of 50% to post larvae were possible. Comparative experiments using artificial diets in the form of free particulates indicate that survival rate may be even higher. They also reported that microencapsulated semi-defined diets may be used to determine the nutritional requirements of *Penaeus* larvae.

Fatty acid biosynthesis in the larval stages of *Penaeus japonicus* Bate was examined by feeding microencapsulated diet containing (1-14 C) palmitic acid, and fat free diets supplemented with defined fatty acids (Jones *et al* ,1979b).

For improving the stability of commercial feed pellets for the giant shrimp *Macrobrachium rosenbergii*, Farman - farmain *et al* (1982) encapsulated Purina Marine Ration M 20 with 0.5% Kelco HV algin and they

found that the physical stability of the pellets can be improved by algin encapsulation. They observed best results when the ration was repelletized with Kelco HV algin binder at 2.0% level. Zein microencapsulated and Nylon microencapsulated diets were prepared and their dietary values for the larvae of *Penaeus japonicus* were evaluated by Kanazawa *et al*(1982a). They found that zein microencapsulated diet and nylon microencapsulated diet supported the growth of prawn larvae from zoea- 1 to post larva-1 in terms of both survival and growth rates. However, they observed better results when powdered diets with zein as a coating (zein MCD). The dietary values of the zein MCD containing a defined diet was almost comparable to those of live feeds, *Chaetoceros gracilis* and *Artemia salina*. The ingredients and procedure involved in the preparation of a microencapsulated diet are presented (Kanazawa,1982a).

Attempts to rear the larval crab, *Portunus trituberculatis* with artificial microparticulate diets, nylon-protein- microencapsulated diets, gelatin-arabic gum-microencapsulated diet, cholesterol-lecithin microcoated diet and carrageenan microbound diet were made (Kanazawa *et al*,1983). They found that every artificial diet sustained the growth of the larval crab from zoea1 to juvenile stages, although the survival rates of the crab receiving the artificial diets were lower than that of the crab receiving the live feeds (control group).

Levine *et al* (1983) described the method of producing calcium alginate microcapsules. Food particles or droplets were dispersed in an alginate-gelatin matrix which was precipitated as the calcium salt. They obtained a stable microparticle of irregular shape. They reported improved growth and survival of larvae when the rotifer diet was supplemented with microencapsulated nutrients. Maugle *et al* (1983) used microencapsulated digestive enzymes to study the effect of digestive enzyme additives on growth, feed efficiency, hepatopancreatic glycogen and digestive enzyme activities of shrimp.

Jones *et al* (1984) reported that live foods used for penaeid culture have been successfully replaced by microencapsulated diets, both in the laboratory and at the hatchery level. He also reported that the technology has reached the level at which dietary requirements of individual species can be met by the incorporation of specific nutrients. Microencapsulated diet was tried to study the nutritional significance of long chain polyunsaturated fatty acids to the zoeal development of the brachyuran crab, *Eurypanopeus depressus* (Levine & Sulkin, 1984 a). When the lipid fraction of brine shrimp was added in encapsulated form to the rotifer diet, brachyuran crab larvae showed enhanced survival and development rate to the megalopa as compared to rotifer-fed controls. They got enhanced larval survival and development rate when the rotifer diet was supplemented with microcapsules containing specific polyunsaturated fatty acids. Microencapsulated diets have been used to study feeding and assimilation of

polyunsaturated fatty acid (PUFA) by brachyuran crab larvae (Levin &Sulkin, 1984 b). Ingestion of microcapsules was demonstrated by feeding to newly hatched crab larvae, microcapsules which contained fluorescent particles. Gas liquid chromatographic analysis of megalopae obtained from zoea reared on diets enriched with specific microencapsulated PUFA demonstrated that assimilation of the respective fatty acids had occurred.

The details of the procedures for preparation of nylon- protein microencapsulated diets were described by Kanazawa (1986). Clarke(1987) used a microencapsulated diet containing palmitate in cod-liver oil to study the fate of dietary palmitic acid in the Antarctic krill, *Euphausia superba.* Newly hatched crangon zoea were raised separately using live foods and calcium alginate microencapsulated *Artemia* nauplii and calcium alginate microencapsulated *Daphnia magna* (Villamar & Brusca, 1987). They developed a dependable "atomizer" design for laboratory preparation of calcium alginate microencapsulated diet. They reported that zoeae that were fed *Chaetoceros* supplemented with calcium alginate micro encapsulated *Artemia* nauplii has significantly greater growth rates than siblings fed only *Chaetoceros.*

A microencapsulated particulate feed, developed to replace live foods conventionally used in penaeid larval culture, has been used in preliminary trials during 1984-85 in commercial hatcheries (Jones *et al* , 1987). This diet composed of a balanced crustacean diet microencapsulated

within a biodegradable capsule wall designed to protect the internal diet from bacterial degradation while allowing leaching of food attractants. The microcapsules were prepared by emulsifying the dietary components in aqueous solution in cyclohexane containing a natural surfactant. Active sites on protein molecules contained within the diets are then cross-linked by interfacial polymerisation using an acid chloride. They suggested that encapsulated feed may be used to replace part or all of the conventional live foods used in penaeid larval culture.

Mylvaganam (1988) has given details of the development of microencapsulated feed for hatchery reared shrimp. He reported that microcapsules seals the nutrients with a water stable protein membrane that only breaks down in the digestive tract of the shrimp larvae, thus releasing the nutrient at the point of absorption. Sumeru (1988) described egg microencapsulated diet together with other diets for shrimp larvae.

Amjad and Jones (1989b) and Amjad *et al* (1993) in comparative trials with six other artificial feeds demonstrated that microencapsulated feed was the only feed to remain stable for 24 hrs. Canavate & Marin (1989) have given details of an experiment conducted to investigate the effect of different types of containers on the growth and survival of *Penaeus japonicus* larval culture using microencapsulated diets. They reported that the culturing of *P. japonicus* larvae entirely on microencapsulated diets is feasible, but dependent on the development of a

proper system and management. Jones *et al* (1989) found that the addition of only 10 algal cells/ml of the culture water containing microcapasules during protozoeal stages was sufficient to produce growth similar to live feed controls.

Kurmaly *et al* (1989) studied the practicality of rearing *Penaeus monodon* larvae on microencapsulated diets. Results for growth and survival indicated that all the microencapsulated diets investigated (RDX10, X13D, RDX24, CAR005, CD435) adequately supplemented algal diets and RDX24 proved a reasonable algal replacement They suggested that CD435 could successfully replace *Artemia.*

Promising results were reported by Deru (1990) who achieved complete replacement of live *Artemia* in culturing *Macrobrachium rosenbergii* from stage Z-6 to Z-11 with over 80% survival using Frippak CD3 microencapsulated diet designed for penaeid larvae. The diet, however, could not sustain development of the early larvae further than stage Z4.

A wide range of microparticulate and encapsulated diets have been used in an attempt to replace live or natural diets for lobster *Homarus gammarus* larvae (Kurmaly *et al*, 1990). Despite the range of dietary materials presented, larvae did not moult beyond stage III on artificial diets and lack of success is attributed to poor diet digestibility together with long gut retention time.

Microencapsulated test diets supplemented with formalin-killed vibrio cells to increase the number of survivors and to improve the quality of larvae in an early developmental stage of the giant tiger prawn *Penaeus monodon* was used by Itami *et al* (1991).A discussion is presented on the use of microencapsulated feeds in shrimp culture by Kumar and Kesavanath (1991). According to them the crux of the encapsulation technology is the release of the encapsulated internal nutrients at active sites within the target biological system. An account of some of the encapsulation methods like the zein microencapsulated diet, nylon protein linked microencapsulated diet and X-linked protein microcapsules has been given. Ottogali (1991) reported the total replacement of cultured algae with microcapsules for the culture of several penaeid species in hatcheries in New Caledonia with similar growth and survival to controls on live foods.

Amjad and Jones (1992) evaluated the effectiveness of microencapsulated feed along with other eight brands of artificial larval diets manufactured by different process technologies. They observed that highest growth and survival of shrimp larvae were obtained using microencapsulated feed in conjunction with 10 cells/ μ l of microalgae.

Chen *et al* (1992a,1992b) and Chen (1993) developed microencapsulated amino acid (L-arginine) that can be assimilated by *Penaeus monodon.* Using this microcapsule they could quantify the arginine requirement of the species. Kumlu *et al* (1992) studied the growth, survival

and trypsin activity of *Penaeus monodon* larvae fed live diet, microencapsulted diet (Frippak) and a combination of both. They observed best survival and growth using live diets. Their results suggest that some substances in phytoplankton trigger digestive enzyme production during early larval stages of penaeids and therefore it might be possible to improve the effectiveness of microencapsules by incorporating those substances into them.

Villamar and Langdon (1993) developed a novel food particle type that retained low molecular weight, water soluble nutrients (vitamins and glucose) within lipid walled microcapsules embedded with dietary ingredients in particles of gelled alginate-gelatin. They prepared complex microcapsules consisting of dietary ingredients and lipid walled microcapsules embedded in particles of a gelled mixture of alginate and gelatin to obtain a single food particle type that could be used to provide suspension feeders with potentially all dietary essential nutrients.

Jones *et al* (1993) had summarised achievements in the substitution of artificial diets for conventional live feeds in crustacean larval culture. Samara Singhe *et al* (1993) used microencapsulated diet for culture of *Biddulphia longicuris* which forms a food for *Penaeus monodon* larvae. The resistance to bacterial decay of two of the microencapsulated diets was investigated under microbial conditions similar to those found in hatchery culture of larval penaeid prawn or shrimps (Muir & Sulton,1994). They

detected a 2-3 fold increase in total ammonia concentration and a 7-14 fold increase in total bacterial numbers with in 48 hrs. when microcapsules were added to larval culture water at a density recommended by the manufacturer for one day of supplementary feeding.

According to Kumlu and Jones (1995) microencapsulated diet can partially replace live *Artemia* for the larval rearing of *Macrobrachium rosenbergii*. They reported that microencapsulated diet sustained *M.rosenbergii* larvae from Z5-6 to PL1 with a 28% survival. Kumlu and Jones (1995) reported that partial and total replacement of live diets (microalgae and *Artemia* nauplii) with microencapsulated diets are possible for larval culture of *Penaeus indicus*. They also reported that the slower growth and lower survival rate of larvae fed on experimental microencapsulated diets were significantly improved by supplementary frozen mixed algae during protozoeal stage.

Preferences between microencapsulated particles and phytoplankton cell food particles by *Penaeus japonicus* larvae were studied by Marin-Margain and Canavate (1995). They reported that mysis feeding was based on a preferential ingestion of microencapsulated particles. Abdel-Rahman (1996)tested microencapsulated diet (commercial product) along with three other foods to determine their effects on the growth and survival of larvae of the penaeid shrimp *Penaeus japonicus* Bate. A microencapsulated diet using chicken egg fortified with vitamin premix was

prepared and fed to the post larvae of *Penaeus monodon* (Sudarsanam & Sukumaran; 1996). The diet was highly acceptable to the prawn larvae resulting in a high rate of survival.

2.3.3.2 Microencapsulated diets for bivalves

Gabbot *et al* (1976) tested microencapsulated diet with bivalve molluscs. Jones, Gabbot and coworkers used nylon protein walled capsules in their studies. Langdon and Waldock (1981) prepared gelatin-acacia capsules and fed to *Crassostrea gigas* spat. The capsules were prepared containing either triolein, docosa hexa enoic acid or oyster lipid extract, anti-oxidant and vitamin E. The stability of capsule suspension was shown to be satisfactory under the conditions used to grow the spat. They found that addition of encapsulated oyster lipid extract or 22:6 ω_3 to the diets increased growth of spat.

Microgel particles have been described by Langdon (1983), Langdon & Levin (1984) and Langdon & Siegfried (1984). Langdon & Siegfried have reported that *Crassostrea virginica* (Gmelin) could utilize a microparticulate diet for growth; However they found that the growth of oysters fed on the artificial diets varied among experiments. Microencapsulation techniques have been developed for artificial foods for bivalves (Gaither, 1984). Lipid walled vitamin capsules were prepared (Langdon & Bolton ,1984) by dissolving ascorbic acid and vitamin mix in 1 ml of 0.1 M sodium phosphate buffer. This capsule was used as a diet

component of bivalve mollusc *Crassostrea virginica.*

Stroemgren *et al* (1986) studied short term effects of microencapsulated hydrocarbons on shell growth of *Mytilis edulis*. Microencapsulated oils were fed to *M.edulis* in different concentrations and the growth in terms of shell length of the mussels was measured.

Chu *et al* (1987) reports the results of two feeding experiments in which oyster larvae (*Crassostrea virginica*) were grown to metamorphosis on microencapsulated diets and provides the first successful case of culturing oyster larvae to metamorphosis on an artificial diet. They used gelatin-acacia and lipid walled microcapsules to deliver nutrients to oyster larvae. Gelatin-acacia microcapsules were prepared as described by Chu *et al* (1982) and lipid walled capsules were prepared using a method modified from Langdon & Siegfried (1984). Microcapsules with capsule walls made of lipid mixture containing ethyl cellulose and stearic acid improved the retention of water soluble components. Capsules were more stable at low temperatures than at higher temperatures. Inclusion of lipid algal extract in gelatin-acacia capsules promoted better growth of larvae than those not containing the algal extract. They suggested that attractants or phagostimulants could play an important role in microencapsulated diet.

Kandasamy (1987) described the procedure for preparation of nylon-protein microencapsulated diet, gelatin-acacia encapsulated diets and

ethyl cellulose capsules. Edible oyster larvae and spat were fed with *Isochrysis galbana* supplemented with microencapsulated diet prepared from oyster, clam or fish oil extracts (Kandasamy & Muthiah; 1988). They reported that spat setting was higher in the larvae fed with algal diet supplemented with oyster oil extract encapsulated diet than those fed with algal diet. They observed better growth and more weight increase among the spat fed with algal diet supplemented with microencapsulated diet containing oyster oil and fish, compared to that in oyster spat fed with algal diets alone.

Commercially prepared diets encapsulated in protein walled capsules were successfully used in growth experiments with bivalve molluscs (Laing 1987). The growth reported was 54-64 % of that achieved on live algal diets. Addition of 15-40% algae (w/w)gave similar growth to control.

To supplement natural food *Isochrysis galbana* for the pearl oyster larvae, Dharmaraj & Kandasamy (1988) prepared microencapsulated diet using edible oyster oil, fish oil and soya lecithin. They observed that the control with *Isochrysis galbana* gave good results. Among the artificial diets tested encapsulated diet with edible oyster oil showed better results. Chu and Gibbons (1988) conducted an experiment in which juvenile oysters are grown on a commercial microencapsulated diet (Frippak diet). They observed increase in dry weight , ash weight and organic weight. They reported that when the microencapsulated diet was substituted with 25 % or

50% of the amount of algae fed to the algal controls, higher spat dry weight, ash weight and organic weight were obtained.

A modified calcium alginate microparticulate diet carrying a fluorescent dye was tested for sea scallop larvae (Kean-Howie *et al* , 1989). Their findings indicate appearance of normal particle ingestion and moderate growth of early veligers. Lane (1989) reported that feeding a 50:50 (w/w) diet of *Isochrysis* together with microcapsules high in highly unsaturated fatty acids to *Ostrea edulis* produced significantly more larvae than live algal fed controls. Langdon (1989) reported that protein-walled capsules prepared with 20% v/v 1,3,5, benzene tricarbonyl trichloride (BTC) and 80% v/v Sebacoyl chloride (SBC) as cross linking agents, lost less than 5% encapsulated 14C with an efficiency of 40.2 $\pm$ 5%.

In their study Langdon & De Bevoise (1990) used protein walled capsules, and glyceride coated nylon protein walled capsules and compared for the delivery of dietary protein to oysters. Protein walled capsules incubated at 25° C for 24 hr. in non-axenic seawater lost up to 38% 14 C-protein compared with losses of only 14% 14 C-protein from glyceride coated nylon-protein walled capsules. Both capsules were equally digested *invitro* by extracellular style enzymes of *Crassostrea gigas.* A study was conducted to assess the nutritional value of a new type of microcapsule produced by Frippak feeds (Vergara-Martin *et al* ,1990). He reported that microcapsules as a single diet did not support growth in the clam larvae.

Southgate *et al* (1992) reported that microencapsulation techniqueshave been investigated as a means of presenting artificial diets to bivalve larvae. They also reported that microcapsules have suitable physical characteristics for ingestion and digestion by larvae and can be engineered to have the correct nutritional composition and consistent quality. Nell (1993) recommended microencapsulated diets as a promising technique in the formulation and preparation of oyster diets.

Heras *et al* (1994) described a method for preparing lipid microsphere. They reported that these microspheres have shown potential as a diet alternative suitable for supplementing algae as source of essential fatty acids and other lipids in the culture of marine suspension feeders such as oyster. According to them the preparation technique was fast, not labour intensive and uses inexpensive raw materials. The method was by sonication of a mixture of fish oil, soy lecithin, vegetable oil and vitamin E in the ratio 50:20:29:1 (w/w/w/w). Microspheres were stable in recirculating sea water. The oxidation stability of stock emulsion was assessed over a storage period of eight days. To test the ingestion and digestion process under laboratory conditions, they encapsulated fluorescent beads into the microspheres and fed to adult oyster together with algal cultures. They observed that oysters were able to ingest and digest microspheres in a concentration of 50% of the total particles supplied as an algal-microsphere mixed diet.

For comparing the relative utilization of ingested carbohydrate, protein carbon and protein nitrogen in suspension feeding organisms, Kreeger (1996) used dual labelled microcapsules.

The efficiency with which lipid contained in gelatin-acacia microcapsules (GAM) was assimilated by Pacific oyster *Crassostrea gigas* spat was determined (Knauer and Southgate, 1997). They reported high assimilation efficiencies which indicates the potential of GAM as carrier of lipids in nutritional studies with bivalves.

2.3.3.3 Micro encapsulated diet for fish larvae

Kanazawa *et al* (1982b) fed cross-linked nylon-protein membrane microcapsules (Chang *et al* 1966) to first feeding larvae of red sea bream, *Chrysophrysis major* and the ayu, *Plecoglossus altivelis* and reported that the larvae could not digest the micro capsules.

A microencapsulated diet was formulated for wels (*Silurus glanis* L.) using egg as reference protein (Toth and Papp 1986). They found no significant difference between the relatively high survival rates of fish groups fed on the encapsulated diet and on natural food. They also found markedly higher relative weight gain on group fed on natural food. Verreth *et al* (1987) used microencapsulated egg diets to compare the nutritional value of several dry test diets for the larvae of the African cat fish, *Clarias gariepinus*. They reported high survival rates (varying between 64 and 43%)

but low and varying growth rates. No success has been reported for the use of all protein-membrane microcapsules as a feed for early larvae of marine fishes (Walford and Lam, 1987). They suggested that although microcapsules cannot be fed directly to fish larvae (at least during the first 10 days after hatching), they may be used as a substitute for algae as a secondary feed to improve the nutritional value of live foods for marine fish larvae.

Singh and Kumar (1989) used microencapsulated egg diet for rearing mrigal spawn. They reported that the mrigal spawn attained a length of 7.41 mm and average weight of 46 mg in 10 days. The diet was also reported to be well acceptable to rohu fingerlings.

Martinez-Tapia *et al* (1990) compared microencapsulated and live food for larval survival and growth in sea bream and reported that microencapsulated diet is an alternative to be used in aquaculture but mixed with live food.

Awaiss (1991) used microencapsulated dry feed diet for comparing and finding the suitability of rotifers for Gudgeon and perch larvae. Leibovitz *et al* (1991) developed a new microcapsule and evaluated for leaching, buoyancy, and ability to support survival and growth of *Menidia beryllina* larvae. Walford *et al* (1991) reported that cofeeding live and microencapsulated diets can produce growth and survival in early larvae of *Lates calcarifer* superior to that achieved with either live feeds or

artificial diets alone. They reported that microcapsules were broken down in the larval intestine when rotifers were present and that the protein membrane was absorbed.

Ashraf *et al* (1992) reported that microencapsulation even though it can enhance buoyancy and reduces leaching may be inappropriate for striped bass larval diets. Rimmer *et al* (1992) used brine shrimp supplemented with a microencapsulated enhancement diet for feeding barramundi in an attempt to determine whether a mortality syndrome identified in artificial reared fish could be attributed to diet. They found that the use of brine shrimp supplemented with a microencapsulated enhancement diet prevented stress symptoms and mortality.

Lopez Alvarado & Kanazawa (1993) carried out experiments to assess the utilization of crystalline amino acids included in microbound diets by fish larvae and found out that leaching of free amino acids is very fast. He recommended microencapsulation technique to reduce leaching. Petitjean & Csengeri (1993) has reported microencapsulation of hydrosoluble additives to artificial diets for cyprinid larvae to avoid leaching. He adopted the procedures by Chang *et al*(1966) with modification.

Garzon *et al* (1994) used microencapsulated lysine to study the influence of dietary lysine availability on the fractional rates of protein synthesis and degradation in liver and white muscle of rainbow trout.

Lopez-Alvarado *et al* (1994) tested the effect of coating and encapsulation of crystalline amino acids on leaching in larval feeds. They found that binding crystalline amino acids to alginate, carrageenan or zein microparticles gave poor results. Microencapsulation of crysalline amino acids within protein walled capsule gave better results, with capsules retaining up to 60% of some crystalline amino acids within lipid-walled capsules. Feeding studies with Olive flounder (*Paralicthys olivaceous*) indicated that larvae older than 20 days could break tripalmitin-walled capsules. They suggested lipid walled capsules show promising potential for use in nutritional studies with marine fish larvae.

Fernandez-Diaz and Yufera (1995) and Yufera *et al* (1995) determined the ingestion rates of larvae of gilthead sea bream *Sparus aurata* fed on microcapsules with different shell rigidities at the beginning of feeding, in an attempt to establish the feeding response and ingestion ability of microcapsules. They found that gilt head sea bream larvae could be transferred directly from live to microencapsulated food without appreciable variation in the incidence of feeding or in the rate of food consumption from first feeding onwards.

2.3.3.4. Microencapsulated diet for zooplankton

The technique of microencapsulation may be used to introduce semi-defined artificial diets as particulates to *Artemia* which successfully promote growth and survival of the nauplii (Jones *et al.*,1974, Jones and

Gabbot.,1976).

Holland and Jones (1981) used cod liver oil as a source of polyunsaturated fatty acid such as 22:6ω_3 and very small droplets are coated with gelatin to make a water stable microencapsulated oil feed for the newly hatched nauplii of *Artemia.* They found that the maximum number of capsules appeared in the gut of nauplii after 4 hrs. Analysis of the fatty acids present in these nauplii demonstrated that the 22:6 ω_3 is retained in the tissue of *Artemia* for up to six hours of starvation. From this they reported that if *Artemia* nauplii are fed on one feed of microcapsules containing cod liver oil, then they provide a live food supplemented with 22:6 ω_3 available for feeding to larval cultures for a period of up to six hours.

Teshima *et al* (1981) described the culture of rotifers *Brachionus plicatilis* with microencapsulated diet possessing a nylon-cross linked protein membrane. Two types of microencapsulated diet were prepared. The results of feeding trials showed that both supported the growth of rotifer for several days as successfully as the live diets examined.

The development of a partially refined artificial encapsulated diet for *Artemia* nauplii was described (Sakomoto *et al*, 1982). Analysis showed that fatty acids such as 20:5ω_3 and 22:6ω_3 supplied in the encapsulated diet appeared in the tissue of *Artemia* nauplii fed on these diets, thus improving the nutritional value of *Artemia* as a live food for marine fish

and invertebrate larvae which have a dietary requirement for long chain poly unsaturated fatty acids.

Rimmer and Reed (1989) reported that supplementation of rotifers and brine shrimp with a commercially available microencapsulated diet increased the levels of several polyunsaturated fatty acids in the food organism. The possibility of replacing green algae, the commonly used food for the cladoceran *Daphnia magna*, by a microencapsulated diet was tested by Elendt (1990). The microencapsulated diet "Frippak No.1 CAR" was compared with the Chlorophycean algae *Scenedesmus subspicatus*. He reported that docosa hexa enoic acid, present in the microcapsules, was not accumulated by the daphnids.

2.4.Gelatin and Collagen

Gelatin is formed when collagen (the proteins in connective tissues and bones) is heated with water. Collagen has a molecular weight ranging from 300,000 to 700,000, while gelatin has a molecular weight of about 100,000 (Swaminathan, 1979). He has also described the preparation of gelatin from skin, bone or tendon. Gelatin is soluble in hot water and the solution on cooling sets to a gel (Swaminathan, 1979) on account of hydrogen bonding (George,1998). Properties of gelatin has been given by Swaminathan(1979)

Information on fish gelatin is scarce (Norland1987, 1990; Osborne et al., 1990; Leuenberger, 1991).A technical difficulty in producing fish gelatin for human consumption has been elimination of an unpleasant fish odor from the product but Grossman and Bergman (1992) developed a method that made the gelatin odourless. Declaration of fish gelatin as GRAS (Anonymous,1994) is under consideration but it has been produced since 1960 with acid extraction and most of it has been for industrial uses (Norland, 1990). No published studies have concerned optimization of processing fish gelatin from skins(Gudmundsson and Hafsteinsson,1997). Fish skins are abundant and could be a valuable source of gelatin, especially in ethnic foods where pork may not be acceptable. Gelatin has been derived from fish skins and bones but has been much less studied than the conventional gelatin from animals (Gudmundsson and Hafsteinsson,1997)

Several preliminary nutrition studies were conducted using pelleted diets bound with collagen (Sick *et al.*,1971; Sick and Baptist, 1973; Sick and Beaty, 1975) and gelatin (Lobao *et al*,1993; Ali,1998). Gelatin-acacia microcapsules were prepared and described (Chu *et al*, 1982 ;Chu *et al*, 1987). Paulraj (1993) suggested collagen and gelatin as binders in fish feeds. Villamar and Langdon (1993) developed a novel food particle type that retained low molecular weight water soluble nutrients with in lipid walled microcapsules embedded with dietary ingredients in particles of gelled alginate-gelatin (complex microcapsules).

2.5. Storage stability of feed

Cockerel and Holiday(1975) has reviewed the harmful effects of the autoxidation products of the stored feeds. Methods for evaluation of protein quality, including the undesirable changes in proteins that occur during processing (Friedman, 1975) and processing and storage (Hurrel,1984) have been reviewed. Chow (1980a) reported oxidative rancidity as one of the major deteriorative changes in stored feed stuffs. De la Cruz *et al* (1989) have demonstrated the effects of storage temperature on diets designed for the shrimp *Penaeus monodon*. He also observed changes in the peroxide values of feeds stored above 10 C°and recommended that feeds should not be stored at ambient tropical temperatures for more than 15 days.Analytical methodology applicable to oilseeds, oils, and fats, both during and after processing and storage, was reviewed by Patterson (1989). A two-year comprehensive study of low moisture aquaculture feeds were conducted by five US government laboratories (Flower *et al*,1990). Fish feeds were stored for two years at -2°C and vitamin concentrations and thiobarbituric acid (TBA) values were determined during and after this period. Stansby(1990) suggested peroxide value and thio barbituric acid value as two indices of rancidity in fish meal, shrimp meal and fish oil. Bautista *et al* (1992) studied the effects of antioxidants on feed quality and growth of *Penaeus monodon* juveniles. Murthy and Devaraj (1992) studied the effects of storage on the quality of three artificial feeds used in carp culture. Djunaidah (1993) has reported the allowed limit of microbial contamination in larval feeds. Divakaran *et al* (1994) designed an experiment

to quantify temporal changes in two low-moisture shrimp feeds stored at various temperatures and reported that the two low-moisture shrimp feeds were highly stable during long-term storage under various temperature regimes. Hwang *et al* (1995) reported the importance of antioxidants in fish feeds and fish meal to improve the storage stability of the feed and meal. Bautista and Subosa (1997) studied the changes in shrimp feed quality over a period of 62 days and studied its effects on growth and survival of *Penaeus monodon* juveniles.

3. MATERIALS AND METHODS

The aim of the experimental study is to prepare a microencapsulated feed for prawn larvae and carry out studies on its quality and shelf life. The experiment was carried out during the period from 17-8-1997 to 12-11-1997.

3.1. Preparation of feed encapsulating material

The materials used for encapsulation of the diet were gelatin, collagen, commercially available egg albumin (Qualigens Fine Chemicals, Glaxo India Ltd.) and commercially available food grade gelatin (Rex.Corn Products Co. (India) Ltd.). The collagen and gelatin were extracted from shark skin (*Scoliodon sorrokowah*) obtained as a processing waste from fish market (Kadavanthra fish market, Kochi).

3.1.1. Extraction of collagen

The method adopted was that of Sato *et al* (1986). Shark skin was homogenised with distilled water and centrifuged in a refrigerated centrifuge (5 °C). Residue was then homogenised with dilute alkali solution and then again centrifuged. From the residue hot water soluble fractions were collected and dried until the moisture content was reduced to less than 10% and stored in a refrigerator until use for diet preparation.

3.1.2. Extraction of gelatin

The method adopted was a slight modification of Gudmundson & Hafsteinsson (1997). Cleaned shark skin was treated with dilute sodium hydroxide solution followed by treatment with dilute sulphuric acid and then with citric acid. Finally the gelatin was extracted from the skin with distilled water at 45 °C and the extract was evaporated to dryness till moisture content was reduced to less than 10%. The dried gelatin was then stored in a refrigerator until further use.

3.2. Preparation of the feed

3.2.1. Feed Ingredients.

The protein source used for the preparation of the experimental diet was clam meal (Sherief, 1989; Nair and Sherief,1993; Anil Kumar, 1994). Besides the protein source, other ingredients such as salt mixture (Salt mixture USPIX,Sisco Research Laboratoris Pvt. Ltd., Bombay), Vitamin mixture (Supplevite-M, Sarabhai Chemicals, Bombay), Cholesterol (CDH, analytical reagent Bombay),glycine and encapsulating material (collagen/gelatin/eggalbumin) were used for the preparation of the experimental diets.

3.2.2. Processing of the protein source

The clam meat of *Villorita cyprinoides* purchased from local market was steamed in an autoclave at ambient pressure for 15 minutes and then dried in an electric dryer for 12 hrs. at 60 °C. The dried clam meat was

powdered in a pulverizer and passed through standard sieve and the fine powder (300 μ) was packed in air tight plastic bottles and kept in refrigerator until it was used for feed preparation.

3.2.3. Proximate composition of feed ingredients

Proximate composition of the clam meal and that of the encapsulating material were analysed prior to feed formulation. Boyd's (1979) method was used to estimate the moisture content. The sample was heated to 105 °C for 30 minutes and then dried at 65 °C till a constant weight was obtained. Crude protein content was estimated by Microkjeldahl's method (AOAC, 1983). The nitrogen content was multiplied by the factor 6.25 to get the protein content. To estimate the crude fat content, solvent extraction using petroleum ether (40-60° C) in a soxhlet extraction apparatus was carried out for 6 hrs. The crude fibre content was estimated by the method of Pearson (1976), and ash content was determined by burning the sample at 550 °C$\pm$ 10 °C for 6 hrs. in a muffle furnace . The carbohydrate content (Nitrogen free extract,NFE) was determined by Hastings (1976) difference method on dry weight basis.

NFE=100-(%crude protein + crude fat + % crude fibre + % ash)

3.2.4. Formulation and processing of experimental diet

Three types of diets were prepared using three different encapsulating material viz. collagen,gelatin and egg albumin (as control). The

proportion of ingredients used for the preparation of basal diets are given in Table 1.

Table 1. Proportion of ingredients used for the preparation of basal diet

Ingredients	Weight in gm
Clam meal	86.5
Mineral mix.	2.0
Vitamin mix.	2.0
Cholesterol	1.0
Glycine	1.0
Encapsulating material	7.5

The ingredients were weighed out accurately in an electronic balance and all the ingredients mentioned in the table except vitamin mix and encapsulating material were mixed well in a dry mortar. The dried mixture was made into a dough consistency by adding sufficient volume of distilled water (1:1.25 w/v) and mixed well in a mortar . The dough was transferred to glass bowls and steam cooked for 30 minutes in an autoclave at ambient pressure. The cooked dough was rapidly cooled down and mixed well in a mortar along with vitamin mix.

The well mixed dough was then spread on aluminium trays of an electric tray drier and dried at 60 °C for 4 hrs. The dried material was then crushed into small pieces and sieved through standard sieves. The fine powder (300 μ) was then collected and used for encapsulation.

7.5g each gelatin , collagen, egg albumin were dissolved separately in 300 ml distilled water taken in three beakers. To each of this beaker 100g of the basal processed feed was added. It was then mixed throughly for one minute using a homogeniser. The homogenised contents were then centrifuged at 5000 rpm for 20 minutes (Sudarsanam and Sukumaran,1996) in a refrigerated centrifuge. The supernatant was decanted and the microencapsulated particles were dried under sun (in the absence of a freeze drier). The dried diets were then packed in airtight plastic containers and labelled as CED,GED & EED for collagen encapsulated feed, gelatin encapsulated feed and egg albumin encapsulated feed) and used for studying its shelf life and acceptability by the prawn larvae.

3.2.5. Proximate composition of the experimental diet

Proximate composition of experimental diet was analysed to evaluate the nutrient status. Methodology employed was Boyd's (1979) method for moisture, Microkjeldahl's method (AOAC, 1983) for crude protein, solvent extraction using petroleum ether for fat and Pearson (1976) method for crude fibre.

3.3 Acceptability study of the experimental diet

3.3.1. Source of larvae

The larvae of *Macrobrachium rosenbergii* produced at the College of Fisheries Hatchery were used for the study. Three larval stages (III,IV,V & VIII) were used for the study.

3.3.2. Conditioning of the larvae

The larvae were transferred to a circular flat bottom fibre glass tank (83 litre capacity) filled with filtered saline water of 10±2 ppt upto half the capacity and provided with vigorous aeration. The larvae were not fed for one day until transferred to individual rearing tanks.

3.3.3. Monitoring the acceptability of the feed by the larvae.

Ten circular flat bottom glass bowls having wide mouth (2 litre capacity) were used for studying the acceptability of the diets by the larvae. These bowls were labelled as B1,B2,B3,B4 & B5 (in duplicates). The type of feed used in each bowl was noted as in Table 2.

Table. 2 Type of encapsulated feed used in each bowl

Name of bowl	Type of feed/live food
B1	Basal feed encapsulated with egg albumin
B2	Basal feed encapsulated with collagen
B3	Basal feed encapsulated with gelatin
B4	Basal feed encapsulated with food grade gelatin
B5	*Artemia* nauplii

These bowls were then filled to half their capacity with 10± 2 ppt saline water. 20 larvae were introduced into each vessel. Aeration was supplied to each vessel and complete change of saline water was made every 24 hrs. The larvae were provided with sufficient feed or *Artemia* (as the case may be) twice every day. Different larval stages (III,IV,V & VIII) were tried. They were reared for fifteen days.

3.4. Storage study of the experimental diet

For assessing the shelf life of the experimental diet, each of the prepared microencapsulated diet (CED,GED & EED) was divided into three lots and packed in different packaging materials and stored at different storage conditions. This is illustrated in the Table 3.

Table 3. Storage conditions of the prepared diets

Feed	Lot No.	Storage conditions and packaging materials
1 CED	1.CRTB	RT,PB
	2.CBAG	RT,PE bag
	3. CREF	REF,PE bag
2.GED	1.GRTB	RT,PB
	2.GBAG	RT,PE bag
	3.GREF	REF,PE bag
3.EED	1.ERTB	RT,PB
	2.EBAG	RT,PE bag
	3.EREF	REF,PE bag

RT= Room temperature PB= Polyethylene bottle

REF= Refrigerated temperature PE= Polyethylene

From each lot of the three feeds, samples were taken at intervals of 15 days and analysis carried out for Total Plate Count (TPC), fat oxidation tests like Peroxide Value (PV) and Thio Barbituric Acid Value

(TBA) up to 45 th day of storage. Analysis were carried out on initial day also.

3.4.1. Enumeration of Total Plate Count(TPC)

The media and reagents used were nutrient agar and buffer solution. Method adopted ICMSF(1978).

Serial dilutions of feed samples were prepared in sterile buffer solution and inoculum from each dilutions was poured into petri plates kept in sets of duplicates. Media was added to each plates and incubated at 37 ± 1 °C for 48 hrs. in the inverted position. All the steps were conducted in an asceptic manner. Plates which gave colonies betweeen 30-300 were counted and reported as aerobic plate count or TPC/g of material.

3.4.2. Determination of Peroxide Value (PV)

Peroxide value in milliequivalents per 1000 g fat (AOAC Method: 965.33) were determined.

3.4.3. Determination of Thio Barbituric Acid Value(TBA)

TBA value measures a secondary product of lipid oxidation, malonaldehyde (or malonaldehyde type of products) with TBA to yield a coloured end product.The method is based on the spectrophotometric determination of extracted malonaldehyde (Tarladgis et al, 1960). TBA value is defined as absorbancy at 538 nm against Beckman DV Spectrophotometer and standard size of one cm cuvette (Tarladgis et al , 1964). TBA values in

terms of mg malonaldehyde per kg feed were determined.

3.5. Water stability

Micro encapsulated diet stability in the distribution system was evaluated in the laboratory at the end of feeding trials by determining dry weight losses and the physical integrity of particles (Heinen, 1981).

3.6. Statistical analysis of the data

All the data were analysed according to two way classification. The bacterial counts expressed as CFU (Colony forming units/g) were transformed to log x (where x is the observation). Angular transformation was done for the per cent survival rates. These transformed values were used for the statistical analysis.

4.RESULTS

The result of the experiment carried out to prepare a microencapsulated feed for prawn larvae and studies on its quality and shelf life are detailed below. The various feeds tested are described in Table 4.

Table 4. Various encapsulated feeds

Feed	Encapsulating material	Storage conditon and packaging material
CRTB	Collagen	RT, PB
CBAG	Collagen	RT, PE bag
CREF	Collagen	REF,PE bag
GRTB	Gelatin	RT,PB
GBAG	Gelatin	RT,PE bag
GREF	Gelatin	REF,PE bag
ERTB	Egg albumin	RT,PB
EBAG	Egg albumin	RT,PE bag
EREF	Egg albumin	REF,PE bag

For explanation on feed codes refer:

PE bag: Polyethylene bag

RT : Room temperature

REF: Refrigerated temperature

PB : Poly ethylene bottle

4.1.1. Proximate composition of feed ingredients

The proximate composition of the ingredients used in the formulation of experimental diets is presented in Table 5.

Table .5. Proximate composition of the feed ingredients

Ingredients	Moisture (%)	Crude protein (%)	Crude fat (%)	CHO (%)	Ash (%)
Clam meal	9.12	53.22	9.4	19.47	8.79
Encapsulating material					
Collagen	7.00	87.50	4.56		
Gelatin	6.90	82.45	4.80		
Egg albumin	7.50	69.48	2.54		

CHO– Carbohydrate

4.1.2 Proximate composition of formulated encapsulated feed

Proximate composition of formulated encapsulated feeds are given in Table 6.

Table 6. Proximate composition of formulated encapsulated feeds

Feeds	Crude protein (%)	Moisture (%)	Crude fat (%)	Ash (%)	CHO (%)
CED	49.8	7.00	12.00	11.5	19.70
GED	49.5	6.94	11.66	12.0	19.90
EED	48.12	7.90	13.38	10.98	19.62

The highest protein content was for collagen encapsulated diet (CED) followed by gelatin encapsulated diet (GED) and least for egg albumin encapsulated diet (EED).

4.2. Water stability

The results of stability test for different prepared diets are presented in Table .7. The most stable diet at the end of 8 hrs.was gelatin encapsulated feed, followed by collagen and egg albumin encapsulated feed. There was not much difference observed in the average stability of the diets as the immersion time increased .

Table 7. Water stability of encapsulated diet in per cent

	Hours			
Feeds	**2**	**4**	**6**	**8**
CED	90	90	88	88
GED	90	89	89	89
EED	90	89	89	88

4.3. Evaluation of microbiological quality and shelf life

4.3.1. Enumeration of Total Plate count (TPC).

Enumeration of TPC is done to provide an estimate of the total number of aerobic organisms in the prepared diets. It reflects the microbiological quality of the feed and is useful for indicating the spoilage of feed.

The number of colonies per g. of each diet at different storage conditions are given in Table 8.

Table 8. Number of colonies per gm of each diet at diferent storage conditions

Feeds/Days	D0	D15	D30	D45
CRTB	$1.89x10^3$	$3.48x10^3$	$1.33x10^3$	$3.00x10^3$
CBAG	$1.89x10^3$	$1.00x10^3$	$1.00x10^3$	$3.33x10^3$
CREF	$1.89x10^3$	$1.67x10^3$	$1.67x10^3$	$1.93x10^3$
GRTB	$1.42x10^3$	$1.60x10^3$	$1.00x10^4$	$1.00x10^4$
GBAG	$1.42x10^3$	$2.60x10^3$	$1.00x10^4$	$1.00x10^4$
GREF	$1.42x10^3$	$6.00x10^3$	$1.67x10^4$	$2.67x10^4$
ERTB	$1.95x10^3$	$2.70x10^3$	$1.00x10^3$	$6.67x10^3$
EBAG	$1.95x10^3$	$1.78x10^3$	$2.00x10^3$	$2.50x10^3$
EREF	$1.95x10^3$	$1.64x10^3$	$1.67x10^3$	$1.70x10^3$

D0- Initial day of storage **D30-30th day of storage**

D15-15th day of storage **D45-45th day of storage**

The analysis of variance (ANOVA TABLE 1) showed that there is significant difference ($P<0.05$) between feeds and between days of storage.

ANOVA TABLE 1

Source of variation	Sum of squares	df	Mean sum of Squares	F
Between feeds	1.7147	8	0.2143	2.765*
Between days	0.9905	3	0.3301	4.259*
Error	1.8608	24	0.0775	
Total	4.566	35		

*Significantly different at $P<0.05$.

Critical difference at 5% level=0.3366

Retransformed means:

CBAG	EREF	CREF	EBAG	CRTB	ERTB	GRTB	GBAG	GREF
1798.84	1967.88	2027.68	2317	2570.40	2760.57	4395.41	4977.37	8892.01

Between days:

Critical difference at 5% level= 0.2244

Retransformed means:

D0	D15	D30	D45
1865.09	2353.42	3066.90	5208.34

4.3.2. Evaluation of shelf life

4.3.2.1. Evaluation of Peroxide Value (PV)

The PV of different feeds at different storage conditions are given in table 9. In all the feeds the PV initially showed an increasing trend and then showed a declining trend.

Table 9. PV in milliequivalents/kg fat at different storage conditions

Feeds/Days	0	15	30	45
CRTB	75.00	140.00	288.00	257.28
CBAG	74.20	134.29	375.00	286.66
CREF	74.30	117.14	375.00	313.33
GRTB	74.80	114.28	633.33	325.00
GBAG	75.00	146.66	586.66	340.00
GREF	72.00	108.57	506.66	366.66
ERTB	75.50	150.00	365.00	340.00
EBAG	74.00	133.33	505.00	368.31
EREF	75.00	134.29	506.66	433.33

The analysis of variance (ANOVA TABLE. 2) showed there is no significant difference (P> 0.05) between feeds. But it showed a significant difference (P< 0.05) between days of storage. Comparison of means of days based on critical difference showed there is no significant difference between initial day of storage and 15th day of storage.

ANOVA TABLE 2.

Source of variation	Sum of squares	df	Mean sum of squares	F
Between feed	42246.67	8	5280.83	1.478
Between days	870139.594	3	290046.53	81.20*
Error	85725.47	24	3571.89	
Total	998111.73	35		

* Significantly different at P<0.05

Critical difference at 5% level=48.17

Days mean values:

D0	D15	D45	D30
74.42	130.95	336.73	460.15

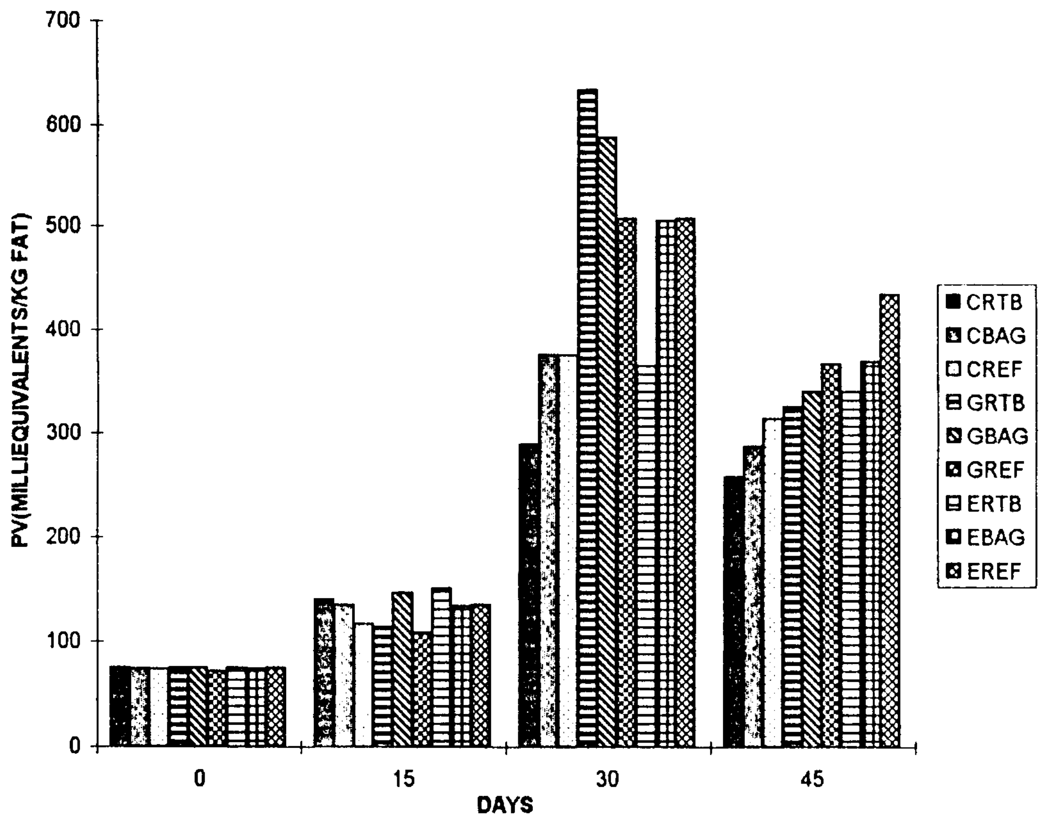
Fig. 1 PV IN MILLI EQUIVALENTS/KG FAT AT DIFFERENT STORAGE CONDITIONS
PV(MILLIEQUIVALENTS/KG FAT)
700
600
500
400
300
200
100
0
0
15
30
45
DAYS
CRTB
CBAG
CREF
GRTB
GBAG
GREF
ERTB
EBAG
EREF

4.3.2.2. Evaluation of TBA

TBA values of different feeds at different storage conditions are given in Table 10. The TBA value in terms of mg malonaldehyde /kg of feed showed an increasing trend with increasing days of storage.

Table 10. TBA Values (mg malonaldehyde /kg) of different feeds at different storage conditions

Feeds/Days	0	15	30	45
CRTB	0.058	3.283	29.52	31.02
CBAG	0.058	3.283	22.32	33.00
CREF	0.058	3.139	33.12	32.40
GRTB	0.058	2.995	27.0	32.00
GBAG	0.058	3.40	21.24	33.48
GREF	0.058	2.995	26.64	30.24
ERTB	0.058	3.13	23.04	29.52
EBAG	0.058	3.03	25.92	28.00
EREF	0.058	3.00	29.52	27.00

The analysis of variance (ANOVA TABLE 3) showed there is no significant difference ($P > 0.05$) between feeds. A significant difference ($P < 0.05$) was observed between days of storage.

ANOVA TABLE 3.

Source of variation	Sum of squares	df	Mean sum of squares	F
Between feed	31.50	8	3.9375	0.734
Between days	6690.87	3	2230.29	415.75*
Error	128.73	24	5.364	
Total	6851.10	35	195.75	

*Significantly dif ferent at $P<0.05$

Critical difference at 5% level= 0.0279

Days mean values:

D0	D15	D30	D45
0.058	3.139	26.48	30

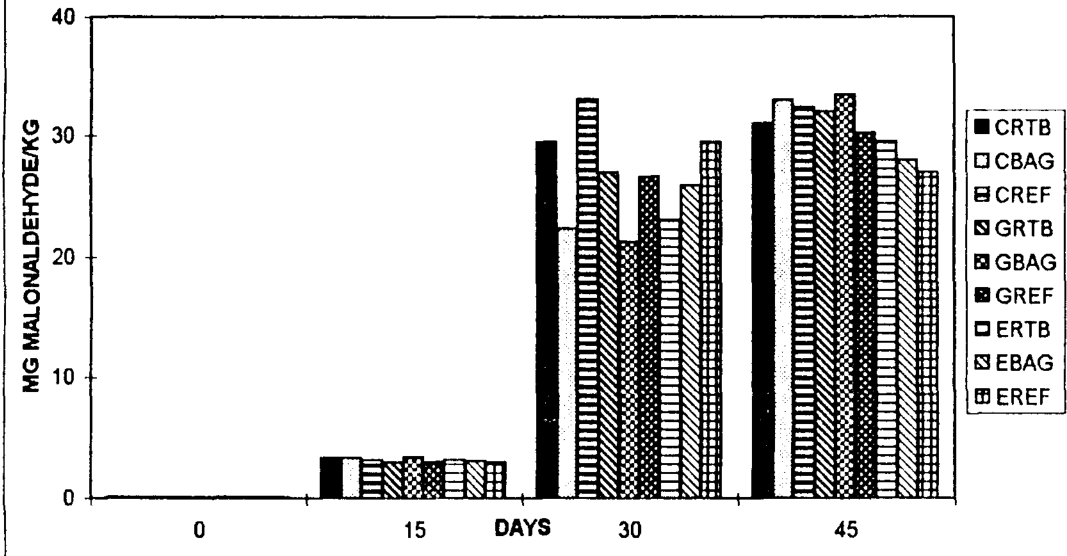
Fig. 2 TBA VALUES(MG MALONALDEHYDE/KG) OF DIFFERENT FEEDS AT DIFFERENT STORAGE CONDITIONS
MG MALONALDEHYDE/KG
40
30
20
10
0
0
15
DAYS
30
45
CRTB
CBAG
CREF
GRTB
GBAG
GREF
ERTB
EBAG
EREF

4.4. Evaluation of survival rate

The percentage survival values of the prawn larvae using various feeds are given in Table 11.

Table 11. Survival values of the prawn larvae in percentage

	Larval stages			
Feeds	**III**	**IV**	**V**	**VIII**
CED	20	44	45	80
GED	40	44	60	80
EED	16	38	90	90
FGED	40	22	60	80
Artemia	80	76	90	90

Maximum survival rate was observed for those larvae fed with freshly prepared *Artemia* nauplii.

ANOVA TABLE 4

Source of variation	Sum of squares	df	Mean sum of squares	F
Between feeds	1377.77	4	344.44	4.25*
Between larval Stages	2634.07	3	878.92	10.82*
Error	973.42	12	81.12	
Total	4985.26	19		

*Significantly different at $P<0.05$

Betweeen feeds:
critical difference at 5% level=11.33

Retransformed means:

CED	FGED	GED	EED	*Artemia*
47.24	50.61	56.53	60.74	84.49

Between larval stages:

Critical difference at 5% level= 10.14

Retransformed means:

III	IV	VI	VIII
38.59	44.70	70.90	84.34

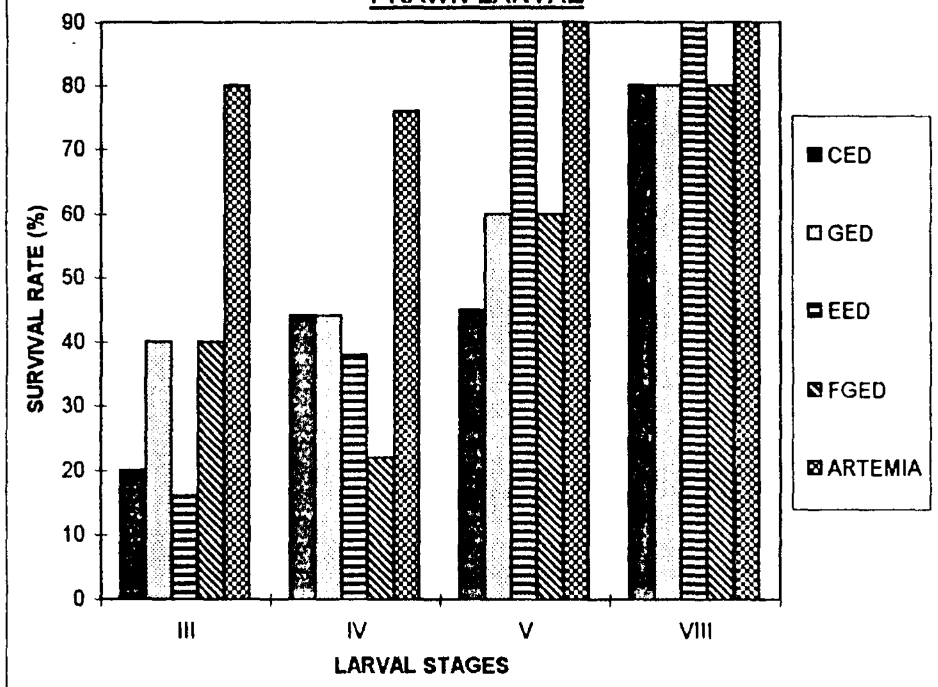

Fig. 3 PERCENTAGE SURVIVAL VALUES OF THE PRAWN LARVAE

5.DISCUSSION

The technique of microencapsulation, protecting diets from degradation and nutrient dissolution (Jones *et al*, 1974) provides a method of supplying artificial diets for larval culture. The results of the feeding trial (culture of rotifers with microencapsulated diets possessing a nylon-cross linked protein membrane) showed that both the microencapsulated diets prepared supported the growth of rotifers for several days as successfully as the live diets examined, when the freshly prepared diets were supplied to the rotifers (Teshima *et al*, 1981). Ingestion of microcapsules enriched with PUFA and containing fluorescent particles have shown that assimilation of the respective fatty acids had occurred in bracyuran crab larvae (Levine & Sulkin, 1984b). Inclusion of lipid algal extract in gelatin-acacia capsules promoted metamorphosis and growth of larvae than those not containing the algal extract which suggests the importance of attractants or phagostimulants in microencapsulated diets for *Crassostrea virginica* (Chu *et al* 1987). A microencapsulated feed used to replace part or all of the conventional live foods used in penaeid larval culture could proudce 1x 10^6 post larvae for less than 2.6 kg of encapsulated feed (Jones *et al* 1987). A high rate of survival (91%) and growth (2.1 mg/day) was obtained in a 45 days feeding trial with microencapsulated egg diet for post larvae of *Penaeus monodon* (Sudarsaam & Sukumaran, 1996).

From practical point of view, microencapsulated diet digestibility could be improved by using good feed stuffs and binders and

applying adequate food processing (Ruyet *et al* ,1993). The digestibility of the microcapsules depends on the nature of wall membranes (Leibovitz *et al*, 1991). In the present study clam meal is used as the protein source(Sherief, 1989; Nair and Sherief,1993; Anil Kumar, 1994) and collagen and gelatin as the encapsulating material. Protein walled microcapsules with diameters ranging betweeen 400-600μ were used as food in the feeding experiment. According to New (1976b), a consensus of opinions indicates that the optimum level of dietary protein for different species of prawns is between 27 and 35%. Djunaidah(1993) reported the crude protein content of *Macrobrachium* larval feed in Malaysia as 48.9% and 55.6% in ACAC and SUTIMAL (feed brand name) respectively. There is till considerable uncertainity as to the prawns's quantitative requirement for dietary protein. Proximate analysis of the diets used in the present study revealed that they contained protein in the range of 48.12 to 49.8% which agrees with the protein content (40-57%) of the various microencapsulated diets prepared for *Penaeus monodon* larvae by Kurmaly *et al* (1989) and Djunaidah (1993).

The diets used in the present experiment contained carbohydrate in the range of 19.62% to 19.9% which is comparable with the carbohydrate content (14-27%) of the commercial microencapsulated diets (Kurmaly *et al* , 1989) for prawn larvae.

The lipid content of the diets used in the present study is in the range of 11.66 to 13.38 % which correlates with the lipid content of the

commercial encapsulated diets for *Penaeus monodon* larvae (Kurmaly *et al*, 1989).

Fair and Fortner (1981) reported that physical structure of feed played a greater role during the ingestion of feed. Stability of larval feed is of utmost importance in an aquaculture system, as the efficiency of the larval feeds depend on the retention of nutritive compounds in the larval feeds for longer time without leaching or disintegration. Also this makes the larval feed more economical. A primary function of microencapsulated diet is to prevent rapid nutrient leaching which may otherwise stimulate bacterial blooms and fowling of larval cultures. However, feeding attractants and gustatory stimulants incorporated into diets must be released. The problem of leakage of nutrients from encapsulated diets has been discussed by other investigators (Langdon and Levine, 1984). The current methodology for preparing lipid-walled capsules has improved the stability of the capsule wall. Leakage of water soluble nutrients from this type of capsule is not a serious problem (Langdon and Siegfried, 1984). Capsules with walls made of a lipid mixture containing ethyl cellulose and stearic acid improved the retention of water soluble components. The leakage of phenol red and by inference water-soluble nutrients from the lipid walled capsules was a gradual process. The stability of gelatin-acacia capsule suspension was shown to be satisfactory under the conditions used to grow the spat(Langdon & Waldolck, 1981). Leaching rates are greatly reduced in X-linked protein microcapsules during the first 24-36 hours (Jones *et al*, 1987). The protein

capsule efficiently retained protein, with losses of less than 5% protein when suspended in sea water for 24 hrs. (Langdon, 1989). After the complex microcapsules (consisting of dietary ingredients and lipid walled microcapsules embedded in particles of a gelled mixture of alginate and gelatin) had been suspended for 24 hrs. in sea water, 84.82 ± 6.66% of the C14 thiamine and 53.0 ±3.89 % of the riboflavin initially present within the capsules was retained(Villamar & Langdon, 1993). Encapsulating the crystalline amino acids with protein cross-linked walls significantly improved retention of amino acids compared with that of mocrobound diets(Lopez-Alvarado *et al*, 1994). Leaching losses of amino acids from cross-linked protein walled capsules were reduced to 59.4%(2 min.after the diet was suspended in water). Losses were further reduced to 39.4% when protein walled microcapsules were coated with 10% (w/w) triolein. By encapsulating crystalline amino acids within a lipid-wall , overall leaching losses after 2 min. in water were reduced to 47.4% when lipid -walled capsules were prepared with 70% tripalmitin and 30% triolein, and to only 3.7% when 100% tripalmitin walls were used (Lopez-Alvarado et al, 1994). By including 2% (w/w) span 85 in the tripalmitin wall, overall leaching in 2 min. was further reduced to 1.4% of the crystalline amino acids. Also lipid microspheres were stable in recirculating sea water (Heras *et al*, 1994). The larval feeds should remain stable (90%) for atleast 2 hrs. so that they become available for aquatic organisms (Djunaidah, 1993). In the present study, to make the various diets to remain water stable, different encapsulating materials were tried. Here, the inclusion of 7.5% of all

encapsulating material in diet gave 10% loss at 2 hrs. There was not much significant improvement in loss even after 8 hrs.in water. In the present study Gelatin encapsulated diet (GED) was the most water stable diet followed by Collagen encapsulated diet (CED) and Egg albumin encapsulated diet (EED).

Feed storage and maintenance of feed quality are important economic considerations. Changes in amino acid, fatty acid, and protein profiles could result in reduced feed quality, thereby adversely affecting growth of the cultivated species and contributing to economic losses. The development of aflatoxin, and proliferation of pathogenic bacteria if any contamination had occurred prior to storage, could also have deleterious effects. Rancidity is another major problem in stored feed stuffs. Eventhough a variety of microencapsulated feed has been prepared for various species, little information is available concerning the effect of encapsulation on quality and shelf life of the encapsulated feed.

The allowed limit of microbial contamination in larval feeds is reported as 7.5×10^3 col/g (Djunaidah,1993). After 45 days of storage all the diets except GED, under different storage conditions recorded colonies much less than the limit. After 15 days of storage the number of colonies for GED was also within the limit. Among the different diets, it was observed that the GED was highly infested with bacteria . However, no visible spoilage or fungal growth was observed in any of the tested diets. Qualitative evaluation

of the flora has not been done. There is scope for further studies in this field.

Prawn feeds always contain fat, either from a natural or supplemental source. These fats are extremely susceptible to oxidation (rancidity), especially when they contain high levels of unsaturated fatty acids. When these fats are added to feeds, they are readily absorbed to the surface of the feed particles, resulting in increased surface area exposed to air and establishing an environment for accelerated rates of oxidation. Furthermore, exposure of the stored feeds to sunlight elevates temperature, thereby creating an ideal environment for rapid oxidation of fats (Bautista and Subosa, 1997). During the process of fat oxidation, chemical degradation products such as free radicals, peroxides, hydroperoxides, aldehydes and ketones are formed. These products, in turn, react with other dietary ingredients (vitamins,proteins and other lipids) and reduce their biological value and availability during digestion (Cockerel and Holiday,1975; Azad,1996). At present, oxidative rancidity is believed to be one of the major deteriorative changes which occur in stored feed stuffs (Chow,1980a). Feeding any animal species with feeds containing oxidised fat may result in increased mortality, growth depression, poor feed conversion and can therefore lead to economic loss. The use of oxidised feed can also result in increased disease outbreaks including one known as 'lipoid liver degeneration' (Robert,1978 ; Tereza-Ventura *et al* ,1987). Feedstuffs such as shrimp meal, fish meal, fish oil etc. are commonly used in feeds for prawn. They are very rich in poly unsaturated acids which are

highly susceptible to oxidation. Measurement of the state of oxidation of lipid in shrimp feeds is necessary to determine what level of rancidity can be tolerated by the animal. There are no chemical tests at present for which a given value will invariably correspond to a definite degree of rancidity (Stansby,1990). Neverthless, there are several tests that are used and, when their limits are kept in mind, can be of value. Two most frequently used such tests are peroxide number or peroxide value (PV)and thio barbituric acid (TBA) value. PV is a measure of the formation of peroxide or hydroperoxide groups that are initial products of lipid oxidation; and TBA measures a secondary product of lipid oxidation, malonaldehyde (or malonaldehyde type products) with TBA and tetra ethoxy propane to yield a coloured end product. The sample may be reacted directly with TBA, but is often distilled to eliminate interfereing substances, then the distillate is reacted with TBA. Sinnhuber and Yu (1958) suggested the use of various concentrations of standard 1,1,3,3, tetra ethoxy propane (TEP) solution to establish a standard curve for the quantitative measurement of malonaldehyde in rancid foods.

In the present study, the different diets were analysed at 15 days interval for PV (AOAC Method 965.33) and TBA value (modifications of Tarladgis *et al*, 1964 and Sinnhuber and Yu, 1958). Initially each diet showed a comparatively lower value for PV then showed an increasing trend and finally a declining trend(Fig.1). The initial increasing trend was due to the accumulation of products of oxidation (peroxides and hydroperoxides).

In due course, these products further undergoes breaking down (aldehydes and ketones) and shows a comparatively low value. However, the TBA value initially showed the lowest value and then shows an inreasing trend (Fig.2). This is because TBA value measures secondary products of lipid oxidation which at the initial stages of oxidation are comparatively less. Kaneda *et al*(1955), and Common *et al* (1957) found toxocity in autoxidation products. Kaneda *et al* (1955) reported that peroxides were the most toxic of the auto oxidation products. The lethal dose of peroxides was about 278 mg total peroxide per kg of fat. These and other workers have shown that high levels of peroxides are damaging to animals and that the level of peroxide rather than the source of oil, is the cause of toxicity. Ethyl esters with a peroxide value of 240 mg percent were toxic to rats (Matsuo; 1954a, 1954b). Rats grew normally when the peroxide value was less than 24 and decreased growth occurred when the PV was greater than 54 (Groot & Kleinobbok,1953). When menhaden oil was oxidised to PV 125 and above toxic symptoms began to appear in rats (Rasheed *et al*,1963). Herring oil with a PV of 142 to chicks at a level of 6 % in the diet found no depresion in weight gain or in feed consumption (Carpenter *et al*, 1963). The PV of a fish feed and fish meal treated with antioxidant was 0.2-60.8 meq/kg and 5.6-85.6 meq/kg and the TBA values for the same were 6.4-45.6 mg/kg and 4.6-88.6mg/kg respectively (Hwang *et al*, 1995). Even mild oxidation of fish oil has been shown to depress salmon growth (PV 14 meq/kg oil) where vitamin E was not supplemented (Koshio *et al* ,1994).According to Bautista and Subosa(1997), *Penaeus monodon* juveniles can tolerate levels of fat

oxidation up to 828 mg malonaldehyde/kg fat. In shrimp(*Penaeus vannemi*) diets containing fish oil with peroxide value of 100 m eq/kg depressed growth , oils with lower values (50 and 60 m eq/kg) did not (Pike,1998). In all these studies the tolerable level of rancidity in feeds vary among species and a definite tolerable level of feeding oxidised feed to prawn is not known. Hence, to find the definite level of toxicity of oxidised feed much work has to be done in this field. In the present study peroxide value of the different diets studied varied from 72 to 75.5 meq/kg on the first day of storage itself. This comparatively high value of peroxide can be probably due to the drying method adopted for the feed. The feeds were sundried in the absence of a freeze drier. This sundrying might have accelerated oxidative rancidity. If the feeds were freeze dried the amount of PV and also other quality deteriorating parameters could have been lower. Even with this level of PV the larvae could be reared successfully with appreciable survival rate. During larval rearing the larvae had undergone moulting which also indicates that the diet was acceptable and non toxic at this level of PV. This high level of PV in the diet could be reduced by incorporating vitamin E or other antioxidants within the limit in the diet.. None of the feed contained any antioxidants or antibacterial agents . However, the PV of each diet under different storage conditions, up to 15 days of storage, were well below the lethal dose suggested by Kaneda *et al* (1955).

The quality of each diet was also assessed by conducting physical evaluation on samples every 15 days. The sense of touch, sight and

smell were used for physical evaluation.It was observed that all the feeds were free of any fungal infestation or physical spoilage. None of the feeds exhibited any rancid odour.

Nylon-microencapsulated diet containing a whole chicken egg and either mysid extract or artififical diet sustained the growth of prawn larvae to post larval stages with considerable high survival rates (Kanazawa *et al*, 1982a). While *Artemia* fed controls produced the highest average survival rates (90%) for *Penaeus vannamei* in Ecuador, 70% replacement of *Artemia* by capsules gave an average survival of 81% (Jones *et al*, 1987). In the initial trial in Taiwan when capsules were used to replace *Artemia*, only 9% of *Penaeus monodon* larvae survived to PL- 7. Although this survival rate is close to the average performance of the hatchery using *Artemia*, it was obvious that the traditional practice of mild aeration is unable to suspend the encapsulated feed,which rapidly settles at the tank bottom. When the mechanical mixing device was employed, the survival rate of PL-7 increased to 47%. Despite the wide variation in survival rates to PL-5 for *Penaeus monodon* in the Philippine trials, replacement of all conventional live foods by microcapsules on a commercial scale was achieved for the first time. Survival rates (3-26%) are comparable with *Artemia* fed controls (4-29%) (Jones *et al*, 1987). Results for growth and survival indicated that all the microencapsulated diets investigated adequately supplemented algal diets and RDX 24 {experimental MED(Frippak feeds)}, proving to be a reasonable algal replacement (Kurmaly *et al*, 1989). Sudarsanam and Sukumaran

(1996) got recorded value of survival rate (91%) for *Penaeus monodon* larvae fed MED. Exclusive replacement with MED throughout the larval rearing period has not been successful, although Deru (1990) found that fresh water prawns could survive on MED alone from Zoea stage 6-7 to metamorphosis. In the present study also the survival rates observed were very poor for early larval stages (I-IV),compared to that fed with live *Artemia* nauplii. But from VI th stage onwards survival rates of *Macrobrachium rosenbergii* larvae fed MED (15 days acceptability study) were comparable with those observed using live food, *Artemia* nauplii. From VIII stage onwards all the experimental MED showed high rate (80%) of survival (Fig.3). Bell (1994) postulated that the problem of poor survival was not of nutritional inadequacy of the MED but of lack of assimilation by early larval stages. Unlike marine shrimp, fresh water prawns have no anterior mid gut diverticulae to produce enzymes before the hepatopancreas begins to function.. There is also a lack of any gastric armature. Jones *et al* (1993) demonstrated that *M.rosenbergii* trypsin levels increase dramatically from Z-5 and peak at Z-6, which explains the findings of Deru (1990). Fresh water prawns also have a very fast gastroevacuation time. Although the early larval stages have sufficient trypsin activity (or exogenous trypsin from the live feed) to digest *Artemia* nauplii, they cannot cope with MED until stage Z-6, when the relative hepatopancreas size has increased (Bell, 1994).. The observed differences in the survival rate of early larval stages fed *Artemia* nauplii and larvae fed microcapsules may be due to several cuases:- lack of digestive enzymes, the larval energy expenditure while eating

microcapsules is higher than while eating nauplii. Hence, the addition of digestive enzymes(exogenous enzymes), hormones or growth factors may contribute to a better assimilation of the microencapsulated diet and enhance larval production.

The microencapsulated diets used in the present work did not induce any noticeable variation in water quality.A daily exchange of 100% of the water seems to be adequate to maintain the water quality.

In the present experiment, only the acceptability study of the feed was conducted. More work in this area is essential. The replacement of *Artemia* by MED in the culture system will be of great help in reducing the cost of live feed.

The extraction of collagen and gelatin from fish waste materials and the preparation of MED using these waste materials will give a promising future to the culture industry.

6. SUMMARY

1. The objectives of the present investigation are:

   a. To formulate a microencapsulated feed to avoid leaching of nutrients

   b. To utilise the cheap sources of fish waste materials and to study the acceptance of the feed

   c. To prepare a water stable diet for nutritional studies and to reduce the cost of feed and

   d. To study the effect of microencapsulation on the quality and shelf life of the feed

2. Three microencapusulated feeds were prepared using clam meal as the protein source and the three encapsulating materials being gelatin and collagen extracted from fish skin waste and commercially available egg albumin.

3. The proximate analysis of the diets showed that the crude protein content of the basal feed was 53.22% and those of the encapsulated feeds were 49.8% for collagen encapsulated feed and 49.5% for gelatin encapsulated feed and 48.12% for egg albumin encapsulated feed.

4. All the three diets were analysed for water stability and acceptance by the larvae and found that the diets were stable (88-89%) even

after 8 hrs. in water and the feeds were well aceptable to the larvae from Vth stage onwards and the larvae had undergone molting during the period of acceptability study (15 days)

5. Three test diets were stored under different storage conditions (refrigerated and room temperature) using different packaging materials (polyethylene bag and polyethylene bottle) and Total Plate Count (TPC) and rancidity tests like Peroxide Value (PV) and Thio Barbituric Acid (TBA) value were analysed for every 15 days.

6. The statistical analysis of the microbial count of the feeds during the storage period revealed that there is significant difference between feed and between days of storage. The TPC of the collagen encapsulated diet (CED) and egg albumin encapsulated (EED)diets after 45 days of storage were within the permissible limit, while the gelatin encapsulated diet (GED) showed comparatively higher number of flora than the other two. However, after 15 days of storage the TPC observed was within the permissible limit.

7. The statistical analysis of the PV and TBA values of the tested diets showed that there is no significant difference between feeds.

8. The survival rate of the larvae fed encapsulated test diets were comparable(80-90%) with that of larvae fed live food *Artemia* nauplii (90%).

7. REFERENCES

Abdel-Rahman, S.H.1996. Evalulation of various diets for the optimum growth and survival of larvae of the penaeid prawn *Penaeus japonicus* Bate. *AquaCult.Nutr.*, **2**(3):151-155.

Abdel-Rahman, S.H., Ghoneim, S.I. and Abdel-Razek, F.A. 1993. Observations on propagation and mass rearing of penaeid shrimp larvae in Egypt. *Spec, Publ. Eur. Aquacult. Soc.* No.**19**. (Summary only).

Adisukresno, S., Escritor, G.L. and Mintardjo, K. 1982. Mass production of *Macrobrachium* post larvae in the Brackish water Aquaculture Development Centre (BADC),Jepra, Indonesia. In: New,M.B. (Ed.) Giant Prawn farming-Developments in aquaculture and fisheries science, Vol.**10**.pp:143-156.

Alam, M.J., Ang, K.J. and Cheah, S.H. 1993a. Use of *Moina macrura* as an *Artemia* substitute in the production of *Macrobrachium rosenbergii* post larvae. *Aquaculture*, **109**:337-349.

Alam, M.J., Ang, K.J., Cheah, S.H.,Ambak, M.A. and Saad, C.R. 1993b. Effects of *Moina macrura* from two different culture sources as a replacement of *Artemia* sp.in production of

Macrobrachium rosenbergii post-larvae. *Aquaculture and Fisheries Management*, **24**:47-56.

Alam, M.J.,Ang, K.J. and Cheah, S.H. 1993c. Weaning of *Macrobrachium rosenbergii* larvae from *Artemia* to *Moina macrura. Aquaculture*,**112**:187-194.

Ali,S.A. 1998. Water stability of prawn feed pellets prepared using different binding materials with special reference to tapioca. *Indian. J. Fish.*, **35**(1):46-51.

Amjad,S. and Jones,D.A. 1989b. A comparison of the stability of some artificial feeds used in penaeid larval culture. *Journal of the World Aquaculture Society*,**20**:12A.

*Amjad,S.and Jones, D.A. 1992. An evaluation of artificial larval diets used in the culture of penaeid shrimp larvae *Penaeus monodon* (Fabricius). *Pak. J. Zool.*, **24**(2):135-142.

*Amjad, S.,Jones, D.A. and Chitra Vadivelu, K. 1993. Advances in penaeid larval feed technology. In: Hock,C.S. and Saidin,T.H. (Eds.) New technologies in aquaculture. *Malaysian Fisheries Society Occasional Publications*, **6**:29-45.

Andrew, J.W., Sick, L.W. and Baptist, G.P. 1972. The influence of dietary protein and energy levels on growth and survival of penaeid shrimp. *Aquaculture,* **1**:341-347.

*Andrew, T.F and Mc. Leod, W.C. 1970. Application and control of the algin- calcium reaction. *Food Prod. Dev.*, **4**(5):99-104.

Aniello, M.S. and Singh, T. 1982. Some studies on the larviculture of the giant prawn *Macrobrachium rosenbergii.* In:New,M.B.(Ed.) *Giant Prawn farming- Selected papers presented at "Giant prawn-1980"-An international conference on freshwater prawn farming held in Bangkok, Thailand, June 15-21, 1980.* Vol.**10**, pp:225-232.

Anil Kumar,P.1994. Evaluation of different protein sources in the formulated feed for *Macrobrachium rosenbergii* (de Man) juveniles. *M.F.Sc. Thesis. Submitted to Kerala Agricultural University, Faculty of Fisheries, Kochi, Kerala,* pp:141

Anonymous. 1994. FDA urged to approve fish gelatin as GRAS. *Food Chemistry News*, **36**:9-10

AOAC. 1983. Official Methods of Analysis. Horwitz,W., Senzel, W.A. and Reynolds,H. *Association of Official Analytical Chemists.* Washington, DC, pp:1094.

Ashraf, M., Simpson, K.L. and Begston, D.A. 1992. Formulation of semi-purified diets for striped bass, *Morone sexatillis*, larvae. *Aquaculture*-92 Growing toward the 21st century. pp:31-32.

Awaiss, A.1991. Mass culture and nutritional quality of the fresh water rotifer (*Brachionus calycifloras*) for gudgeon (*Gobio gobio*) and perch (*Perca fluviatilis*) larvae. *Spec. Publ. Eur. Aquacult. Soc.*, No.**15**.

*Azad,I.S.1996. Nutrition and health management in aquaculture. *Fishing Chimes*,**29**(2) :31-33.

Bautista, M.N., Millamena, O.M. and Kanazawa,A. 1989. Use of kappa-carrageenan microbound diet as feed for *Penaeus monodon* larvae. *Mar.Biol.*, **103**(2):169-173.

Bautista, M.N. and Subosa, P.F. 1997. Changes in shrimp feed quality and effects on growth and survival of *Penaeus monodon* juveniles. *Aquaculture*, **151**(1-4):121-129.

Bautista, M.N., Subosa, P.F. and Lavilla-Pitogo, C.R. 1992. Effects of antioxidants on feed quality and growth of *Penaeus monodon* juveniles. *J. Sci. Food. Agric.*, 60:55-60

*Bell, S. 1994. Growth, survival and trypsin activity for larval caridean shrimp *Palaemon elegans* and *Macrobrachium rosenbergii* when fed live and artificial diets. *Msc. Thesis., University of Wales, Bangor, U.K*, pp:44

Biddle,G.N. 1976. The nutrition of *Macrobrachium* species. *U.S. Dept. Commerce,NOAA.NMFS. Presented 1976 Workshop on the Aquaculture of Freshwater Prawns (Macrobrachium* species), *14-15 June 1976.* pp:36

*Boonyaratpalin,M.and New,M.B. 1993. On farm preparation and feeding strategies for marine shrimp and freshwater prawns. In: New,M.B., Tacon,A.G. and Csavas,I. (Eds.) Farm made Aquafeeds. *Proceedings of the FAO/AADCP Regional Expert Consultation on Farm-made Aquafeeds. 14-18 December 1992.* FAO/AADCP, Bangkok, Thailand.

*Boyd,C.E. 1979(Ed.). Water quality in warm water fish ponds. *Agric.Exper. Stn.*Auburn University, 359 pp.

Briggs,M.R.P., Jauncey,K.and Brown, J.H. 1988. The cholesterol and lecithin requirements of juvenile prawn (*Macrobrachium rosenbergii*) fed semi purified diets. *Aquaculture*, **70**:121-129.

Brown, J.H.1991. Freshwater prawns: In:Nash,C.E. (Ed.) *Production Aquatic Animals, World Animal Science*, C4. Elsevier Science Publishers, Amsterdam, Netherlands, pp:31-43.

*Canavate,P. and Marin,V. 1989. Experimental rearing of *Penaeus japonicus* larvae on microencapsulated diets using different containers. *Aquacult.Europe*-89. *Short communications and abstracts of review papers, Films Slide shows and Poster papers, Presented at the international Aquaculture Conference held in Bordeaux, France, 2-4 October, 1989.* Billiard,R., Pauw,N. Decomps. 1989 no.**10** pp:49-50.

Carpenter, K.J., Lea,C.H. and Parr, L.J. 1963. Chemical and nutritional changes in stored herring meal. IV Nutritional significance of oxidation of the oil. *Brit.J. Nutr.*,**17**:151-169.

Cary,S.C., Lovette, J.T., Perl, P.J., Huntley,M.E. and Vernet,M. 1992. A microencapsulation technique for introducing pure compounds in zooplankton diets. *Limnol.Oceanogr.*,**37**(2):404-413.

*Chang,T.M.S., Macintosh,F.C. and Mason, S.G. 1966. Semipermeable aqueous microcapsules. 1. Preparation and properties. *Can. J. Physiol.*, **44**:115-128.

Chen, H.Y. 1991. Larval and post larval nutrition of penaeid shrimp. *Spec. Publ. Eur. Aquacult.Soc.*, No.**15**. (Summary only).

Chen,H,Y. (1993). Recent advances in nutrition of *Penaeus monodon*. *J. World. Aquacult. Soc.*, **24**(2):231-240.

Chen,H.Y., Leu,Y.T.and Roelants,I. 1992a. Quantification of arginine requirements of juvenile marine shrimp, *Penaeus monodon*, using microencapsulated arginine. *Marine Biology*, **114**:229-233.

Chen,H,Y., Leu,Y.T.and Roelants,I. 1992b. Effective supplementation of arginine in the diets of juvenile shrimp, *Penaeus monodon*. *Aquaculture*. Amsterdam 106.

Chineah,V. 1982. Recent innovations in the larval rearing technique of *Macrobrachium rosenbergii* (de Man) in Mauritius . In: New, M.B. (Ed.) *Giant prawn farming-Seclected papers presented at 'Giant prawn 1980', an international conference on freshwater prawn farming held in Bangkok, Thailand, June 15-21, 1980.*

Chow, K.W. 1980a. Storage problems of feedstuffs. In: Fish Feed Technology. *FAO/UNDP Training Course, College of Fisheries, University of Washington, Seattle, 9 October-15 December 1978. ADCP/REP/80/11*, pp:216-224.

Chu,F.L.E. and Gibbons, M.C. 1988. Evaluation of the efficacy of a commercial microencapsulated feed for growth of juvenile American oyster.*J. Shellfish. Res.*, 7(3):572.

Chu, F.L.E., Webb, K.L., Hepworth, D.A. and Casey, B.B. 1987, Metamorphosis of larvae of *Crassostrea virginica* fed microencapsulated diets. *Aquaculture*, **64**:185-197.

*Chu,F.L.E., Webb,K.L., Hepworth,D.and Roberts, M. 1982b. The acceptability and digestibility of microcapsules by larvae of *Crassostrea virginica*. *J. Shellfish Res.*, **2**:29-34

*Clarke, A. 1987. A preliminary radio isotope study of the fate of igested palmitic acid in the Antarctic krill, *Euphausia superba* Dana. *Bull. Br. Antarct. Surv.* 1987. no.**77** pp:99-107.

*Cockerel,I. and Holiday,D. 1975. Quality control in animal feedstuffs manufacturing industry. *Rep. G97*, Trop. Prod. Institute, London, UK.

Common,R.H., Crampton,E.W., Farmer,F. and De Frietas, A.S.W. 1957. Studies to determine the nature of the damage to the nutritive value of menhaden oil from heat treatment. *J. Nutr.*, **62**:341-347.

D'Abramo,L.R., Moncreitt,C.A., Holcomb,F.P., Montanez,J.L. and Buddington, P.R. 1994. Vitamin C requirement of the juvenile fresh water prawn, *Macrobrachium rosenbergii, Aquaculture*, **128**:269-275.

D'Abramo,L.R. and Sheen,H.Y. 1993. Polyunsaturated fatty acid nutrition in juvenile fresh water prawn, *Macrobrachium rosenbergii. Aquaculture*, **115**:63-86.

Daniels,W.H., D'Abramo,L.R. and De Parsevaall. 1992. Desgin and management of a closed,recirculating 'clear water' hatchery system for freshwater prawns, *Macrobrachiium rosenbergii. Journal of Shellfish Research*,**11**:65-73.

Das,K.M. 1993. Nutritional requirements of fresh water prawns and feed development. In: Tripathi,S.D.(Course Director) *Summer Institute on Recent Advances in Freshwater Aquaculture 20 May-8 June 1993. CIFA,(ICAR) Kausalyaganga,Bhubaneswar*, pp:81-85.

Dejarme,H.E., Dominisac, J.B. and Dejarme,S.M. 1982. Notes on spawner collection methods and larval rearing of giant prawns (*Macrobrachium rosenbergii* de Man) at MSU-IFFRD Station, Naawan. In: New, M. B. (Ed.) *Giant Prawn farming -Selected papers presented at 'Giant prawn 1980', an international conference on freshwater prawn farming held in Bangkok, Thailand, June 15-21, 1980.* pp:73-82.

De la Cruz,M.C., Erazo,G.and Bautista,M.N. 1989. Effect of storage temperature on the quality of diets for the prawn, *Penaeus monodon. Aquaculture*, **80**: 87-95.

Deru,J. 1990. Studies on the development and nutrition of the Caridiean prawn *Macrobrachium rosenbergii. PhD Thesis, University of Wales, Bangor, UK.* P.306.

Deshimaru,O.and Kuroki,K.1974. Studies on a purified diet for prawn II.Optimum contents of cholesterol and glucosamine in the diet. *Bull. Jap. Soc. Sci. Fish.*,**40**:421-424.

Devresse,B., Romdhane, M.S., Bazzi,M., Rasiwo,J., Leger,P.,Brown,J.and Sorgeloos,P.1990. Improved larviculture output in the giant fresh water prawn *Macrobrachium rosenbergii* fed a diet of *Artemia* enriched with n-3 HUFA and phospholipids. *World*

Aquaculture,**21**:123-125.

Dey,S.and Raghuvaran,A. 1988. Comparative studies on ascorbic acid status of the compound eye of a freshwater shrimp. *Comp. Physiol. Ecol.*, **13**:165-168.

Dharmaraj,S. and Kandasamy,D .1988. Microencapsulated diets as supplemental food for larvae and spat of the pearl oyster *Pinctada fucata*. *CMFRI bulletin*,**42**:358-363.

Divakaran,S., Leamaster,B.R. and Walsh,W.H. 1994. Long term storage stability of low-moisture shrimp feeds. *J. Aquacult. Trop.*, 9(1):55-66.

Djunaidah, I.S. 1993. Aquafeeds and feeding strategies in Indonesia.In: New, M.B., Tacon, A.G.J. and Csavas,I. (eds.) Farm-made aquafeeds. *Proceedings of the FAO/AADCP Regional Expert Consultation on Farm made Aquafeeds, 14-18 December 1997, Bangkok, Thailand, FAO-RAPA/AADCP, Bangkok, Thailand* pp:255-281.

Dugan,C.C.,Hagood,R.W. and Frakes,T.A. 1975. Development of spawning and mass larval rearing technique for brackish-freshwater shrimps of the genus *Macrobrachium*. *Fla. Mar. Res. Publ.*

No.**12**:1-28.

*Elendt,B.P. 1990. Nutritional quality of a microencapsulated diet for *Daphnia magna.* Effects on reproduction, fatty acid composition, and midgut ultrastructure.*Arch. Hydrobiol.*, **118**(4):461-475.

Fair,P.H.and Fortner, A.R. 1981. The role of formula feeds and natural productivity in culture of the prawn *Macrobrachium rosenbergii. Aquaculture,* **24**:233-243

Farman-farmain,A., Lauterio,T.and Ibe,M. 1982. Improvement of the stability of commercial feed pellets for the giant shrimp *Macrobrachium rosenbergii. Aquaculture,* **27**(1):29-41.

Fernandez-Diaz,C.and Yufera,M. 1995. Capacity of gilthead sea bream, *Sparus aurata*,L larvae to break down dietary microcapsules. *Aquaculture*,**134**(3-4):269-278.

Fernandez-Diaz,C. and Yufera,M.1997.Detecting growth in gilt head seabream , *Sparus aurata* L., larvae fed microcapsules. *Aquaculture*,**153** (1-2):93-102.

*Flower, L.G., Thorson, W.M.,Wallien, W.L., White,G.R. and Martin,P.E. 1990. Changes in Nutritive Contents of Fish Feeds at Five Federal Installations. *Technology Transfer Series No. 89-1.* AFF1-ATC-90-15. U.S. Fish and Wildlife Service, Abernathy Salmon Culture Technology Center, Longview, Washington, 68pp.

*Forster, J.R.M.. 1971. Some methods for binding prawn diets and their effects on growth and assimilation. *J. Cons. Perm. Int. Explor. Mar.*, **34**(2):40-57.

Forster,J.R.M. 1976. Studies on the development of compounded diets for prawns. In: Price,K.S., Shaw,W.N. and Danberg,K.S. (Eds.) *Proceedings of the First International Conference on Aquaculture Nutrition.* Univ. Delaware, Newark, DE, pp:229-248.

*Forster,J.R.M. and Beard,T.W. 1973. Growth experiments with the prawn *Palaemon serratus* (Pennant) fed with fresh and compounded diets. *Fish. Invest.*, Ser.II. **27**(7):16.

*Friedman,M.1975. In: Friedman,M.(Ed.) Protein Nutritional Quality of Foods and Feeds, Vol.1. Marcel Dekker, Inc., New York, 674pp.

Fujimura,T. 1966. Notes on progress made in culturing technique for *Macrobrachium rosenbergii. Indo-Pac. Fish. Counc. Proc., 12th Session* :1-4.

Gabaudan,J., Pigott,G.M. and Halver,J.E. 1980. The effect of processing on protein ingredients for larval diets: biological evaluation. *Proceedings of the World Mariculture Society*, **11**:424-432.

Gabbot,P.A., Jones,D.A. and Nicholas,D.H. 1976. Studies on the design and acceptability of microencapsulated diets for marine particle feeders. II Bivalve Molluscs. In: *Proceedings of the 10th European Symposium on Marine Biology, Ostend, Belgium, 17-23 September, 1975.* Vol.1,pp:127-141.

Gaither,W.S. 1984. Progress and problems with recirculating system aquaculture. Biotechnology in the marine sciences. *Proceedings of the first annual mit sea Grant lecture and seminar.* Col well,R.R., Sinsleey,A.J., Panser,E.R. (Eds.). *Masachusetts Inst. Of Technology, Cambridge USA, Sea Grant Coll. Program.* pp:61-80.

Garzon,A., Peragon,T., Hidalgo,M.C., Cardenete,G., Lupianez,J.A. and De-la-Higuera,M. 1994. Influence of dietary lysine availability on the fractional rates of protein synthesis and degradation in liver

and white muscle of rainbow trout. *Aquaculture*, **124**(1-4):64.

*Gatesoupe,F.J. 1986. The use of microparticles in aquaculture . *Training Session: Nutrition in marine aquaculture*, Lisbon 28-30 October 1986. Bruno,A.(Ed). *FAO-UNDP-Mediterranean Reg. Aquaculture-Prog.*,Tunis-Tunisia 1987.pp:281-301.

Gawlicka,A., Mc Laughlin,L., Hung,S.S.O. and Noice. 1996. Limitations of carrageenan microbound diets for feeding white sturgeon, *Acipenser transmontanus* larvae. *Aquaculture*, **141**:245-265.

George, S. 1998. Hydrocolloids as food additives. In: Nambudiri(Course Director) *Short term training course on Additives in Sea Food Processing. Department of Processing Technology, KAU, College of Fisheries*, Panangad, Kochi.

Gijo,I. 1996. Vitamin C requirement and its deficiency syndromes in *Macrobrachium rosenbergii* juveniles. *M.F.Sc. Thesis. Submitted to Kerala Agricultural University, Faculty of Fisheries, Kochi, Kerala*,pp:30-51

Gill,C.1996. World feed panorama: more growth in key countries of Asia and Latin America. *Feed International*, **17**(1):4-5.

*Gill,C. 1997. World feed panorama: high cost of feed stuffs: global impact, response. *Feed International,* **18**(1):6-16.

Goulden,C.E. 1994. *37th Conference of the International Association for Great Lakes Research And Estuarine Research Federation:Program And Abstracts. International Assoc. For Great Lakes Research,* Buffalo-NY-USA BUFFALO,NY USA IAGLR:166.

*Groot, E.H. and Kleinobbink,H.J. 1953. Influence of heating cod-liver oil to 230 on growth of young rats. *Voeding* **14**:123-136.

*Grossman,S. and Beergman,M. 1992. Process for the production of gelatin from fish skins. U S Patent 5,093,474.

Gudmundsson, M. And Hafsteinsson,H. 1997. Gelatin from cod skins as affected by chemical treatments. *J.Food.Science*, **62**(1):37-39

Halver,J.E. 1976. Formulating practical diets for fish. *J. Fish.Res.Bd. Can.* **33**:1032-1039.

Hastings,W.H. 1976. Fish nutrition and fish feed manufacture. *FAO Technical Conference on Aquaculture*, Kyoto, Japan, FIR: *Aql. Conf/*76/R. **23**,pp:13.

Heinen,J.M. 1981. Evaluation of some binding agents for crustacean diets. *Prog. Fish Cult.*, **43**:142-145

Heinen,J.M. 1988. Vitamin requirements of fresh water prawn *Macrobrachium rosenbergii*. J. *World Aquacult. Soc.*, **19**(1) 36A:116.

Heras,H., Kean-Howie,J. and Ackman,R.G. 1994. The potential use of lipid microspheres as nutritional supplements for adult *Ostrea edulis*. *Aquaculture,* **123**(3-4):309-322.

Holland,D.L. and Jones,D.A. 1981. Microencapsulated feed enhances dietary value of *Artemia. Fish. Farming. International*,**8**(4):17.

* Hurrel,R.F. 1984. Reactions of food proteins during processing and storage and their nutritional consequences. In: Hudson,B.J.F.(Ed.) Developments in food proteins,3. *Elsevier Applied Science Publishers*, New York,pp:213-244.

*Hwang,D.F., Lim,J.H.and Cheng,H.M. 1995. Level of synthetic antioxidant in cultured fish and fish feed. *J.Food Drug Anal.*, **3**(1):27-32.

ICMSF.1978. Microorganisms in foods.1. Their significance and methods of enumeration , 2nd ed. *International Commission on*

Microbiological Specifications for foods (ICMSF) of the International Association of Microbiological Societies, University of Toronto Press, Toronto, 434pp.

*Itami,T., Takahashi,Y., Yoneoka,K. and Yan,Y. 1991. Survival of larval giant tiger prawns *Penaeus monodon* after addition of killed *Vibrio* cells to a microencapsulated diet. *J. Aquat. anim. health*.**2**:151-152.

*Jahangir,M. 1992. *Moina macrura* as a live food substitute for *Artemia* species in larval rearing of *Macrobrachium rosenbergii*. *PhD. Thesis. Faculty of Fisheries and Marine Science, University Pertanian Malaysia*. pp:44

Jones,D.A. 1985. Penaeid larval culture using microencapsulated diets. *Proceedings of the first International Conference on the culture of penaeid prawns, shrimps, Iloilo City, Philippines*, 4-7 December 1984. Taki,Y., Primavero,J.H., Llobrera,J.A.(eds). South east Asian Fish Dev Cent., IloiloCity, Philippines, *Aquaculture Dep*. pp:171.

Jones,D.A.1995. Frippak-The facts. A response to the article by P.R. Muir and D.C. Sulton (1994). *Journal of the World Aquaculture Society*,**26**(2):221-222.

*Jones,D.A., Amjad,S. and Chitravadivelu,K. 1989. Comparison of artificial feeds used in penaeid shrimp hatcheries. *Proceedings of the Third Egyptian-British Coference on Animals, Fish and Poultry production held in Alexandria, Egypt, 7-10 October 1989* :15-20.

*Jones,D.A. and Gabbott, P.A. 1976. Prospects for the use of microcapsules as food particles for marine particulate feeders. In: Nixon,J.R.(Ed.) *Proceedings of the Second International Symposium on Microencapsulation*.pp:77-91.

*Jones,D.A., Holland, D.L. and Jobborie,S. 1984. Current status of microencapsulated diets for aquaculture. *Appl.Biochem. Technol.*, **10**:275-288.

Jones,D.A., Kamarudin,M.S. and Vay,L.L. 1993. The potential for replacement of live feeds in larval culture. *Journal of the World Aquaculture Society*,**24**(2):199-210.

Jones.D.A.,Kanazawa,A. and Abdel Rahman,S. 1979a. Studies on the presentation of artificial diets for rearing the larvae of *Penaeus japonicus* Bate. *Aquaculture*, **17**:33-43.

Jones,D.A., Kanazawa,A. and Ono,K. 1979b. Studies on the nutritional requirements of the larval stages of *Penaeus japonicus* using microencaapsulated diets. *Marine Biology*,**54**:261-267.

Jones,D.A. and Kurmaly,K. 1987. Feeding and assimilation of Frippak and other feeds by crustacean larvae. *Journal of the World Aquaculture Society*, **18**:17A.

Jones,D.A.,Kurmaly,K. and Arshad,A. 1987. Penaeid shrimp hatchery trials using microencapsulated diets. *Aquaculture*,**64**:133-146.

Jones,D.A., Mumford,J.G. and Gabbot,P,G. 1974. Microcapsules as artificial food particles for aquatic filter feeders. *Nature* (London), **247**:233-235.

Kanazawa,A.1982a. Preparation of microencapsulated diet. *Manual of research methods for fish and shell fish nutrition. Central Marine Fisheries Research Inst.*, Cochin,India. *Cent. Adv. Stud. No.***8** pp:11-112.

Kanazawa,A.1986. New developments in fish nutrition. In: Maclean,J.L.,Dizon,L.B. and Hostillos,L.V. (Eds) *The First Asian Fisheries Forum.. Asian Fisheries Society, Manila, Philippines.* pp:9-14.

Kanazawa,A.,Koshio,S. and Teshima,S.I. 1989. Growth and survival of larval red seabream *Pagrus major* and Japanese flounder *Paralichthys Olivaceus* fed microbound diets. *Journal of the World Aquaculture Society*,20(2):31-27.

Kanazawa,A., Shimaya,M., Kawasaki,M. and Keshiwada,K.1970. Nutritional requirements of prawn I. Feeding on artificial diet. *Bull. Jpn. Soc. Sci. Fish.* **36**: 949-954.

*Kanazawa,A., Tanaka,N., Teshima,S. and Kashiwada,K. 1971. Nutritional requirements of prawn II. Requirements for sterols.. *Ibid.*, **37**;211-215.

Kanazawa.A.,Teshima,S.,Inamoru,S.,Sumida,S. and Iwashita,T. 1982b. Rearing of larval red sea bream and Ayu with Artificial diets. *Memoirs of Faculty of Fisheries, Kagoshima University* **31**: 185-192.

Kanazawa,A.,Teshima,S., Kobayashi,T.,Iwashita,T.and Kawasaki,M. 1983. Rearing of the larval crab, *Portunus trituberculatus* with the artificial microparticulate diets. *Mem.Fac.Fish. Kagoshima. Univ.*,**32**:121-127.

Kanazawa,A., Teshima,S.I.,Sasada,H.and Abdel Rahman,S.1982a. Culture of the prawn larvae with microparticulate diets. *Bulletin of the Japanese Society of Scientific Fisheries*, **48**(2):195-199.

Kandasamy,D. 1987. Microparticulated and microencapsulated diets for feeding prawn and bivalve larvae. *Summer Institute In recent advances in finfish and shellfish nutrition. Tech.Paper*-**18**.

Kandasamy ,D.and Muthiah,P. 1988. Microencapsulated diet for larvae and spat of *Crassostrea madrasensis. CMFRI bulletin*, **42**:354-358.

Kaneda, T.,Sakai,H.,Ishii,S. and Arai,K. 1955. Studies on the nutritive value of Marine Animal Oils (in Japanese). *Bulletin 12. Tokai Regional Fisheries Research Laboratory*

*Kean,Howie, J.C, Silva,M.A.,and O'-Dor,R.K. 1989. The use of microparticulate diet for feeding studies on veliger larvae of *Placopecten magellanicus*.1.Evidence of acceptability. *Proceedings of the annual meeting 1989. Aquaculture Association of Canada Symposium -1989*. No.**89** pp:80.

*Ketola, H.G. 1976. Quantitative nutritional requirements of fishes for vitamins and minerals. *Feed stuffs*, **48**(8):42.

Kitayama,T., Kamata,T.,Shimaya,M.,Deshimaru,O. and Chichester,D.O. 1972. The biosynthesis of astaxanthin VIII. The conversion of ß-carotene-15-15'3H2 into astaxanthin in the prawn, *Penaeus japonicus*. *Bull.Jap. Soc. Sci. Fishe.* **38**:1171-1175.

Knauer,J.and Southgate, P.C. 1997. Assimilation of gelatin-acacia microencapsulated lipid by Pacific oyster (*Crassostrea gigas*) spat. *Aquaculture*,**153**(3-4):291-300.

Koshio,S., Ackman,R.G. and Lall.S.P. 1994. Effects of oxidised herring and canola oils in diets on growth, survival and flavour of Atlantic Salmon., *J. Agric. Fd. Chem.***42**:1164-1169.

Koshio,S., Kanazawa,A., Teshima,S.I .and Castel,J.D.1989. Nutritional evaluation of crab protein for larval *Penaeus japonicus* fed microparticulate diets. *Aquaculture*,**81**:145-154.

Kreeger,D.A.1996. Use of dual-labelled microencapsules to discern the physiological fates of assimilated carbohydrate, protein carbon, and protein nitrogen in suspension-feeding organisms, *Limnol. Oceanogr.* **41**(2):208-215.

Kumar,B.M. and Kesavanath,P. 1991. Formulation and use of microencapsulated feeds. *Fish. Chimes,* **11**(5):41-43

Kumlu,M. and Jones,D.A. 1995a. The effect of live and artificial diets on growth, survival and trypsin activity in larvae of *Penaeus indicus*. *J. World. Aquacult. Soc.*, **26**(4):406-415.

Kumlu,M.and Jones,D.A. 1995b. Feeding and digestion in the caridean shrimp larva of *Palaemon elegans* Rathke and *Macrobrachium rosenbergii* (De Man) on live and artificial diets. *Aquacult.Nutr.*, **1**(1):3-12.

Kumlu,M.,Sarihan,E.and Tekelioglu,N. 1992. Trypsin activity in larvae of *Penaeus monodon* Fabricius, in relation to their diet. *Isr. J. Aquacult. Bamidgeh.* **44**(4):103-110.

Kurmaly,K., Jones, D.A. and Yule,A.R. 1990. Acceptability and digestion of diets fed to larval stages of *Homarus gammarus* and their role of dietary conditioning behaviour. *Marine Biol.*,**106**:181-190.

Kurmaly,K.., Jones,D.A., Yule,A.R. and East,J. 1989. Comparative analysis of the growth and survival of *Penaeus monodon* (Fabricius) larvae from protozoea 1 to post larva 1, on live feeds, artificial diets and on combination of both. *Aquaculture*,**81**:27-45.

Laing,I. 1987. The use of artificial diets in rearing bivalve spat. *Aquaculture,* **65**(3-4):243-249.

*Lane,A. 1989. The effect of a microencapsulated fatty acid diet on larval production in the European flat oyster *Ostrea edulis* L. In: Pauw,ND., Jaspers,E.., Ackefors,H.and Wilkins,N. (Eds.) Aquaculture -a biotechnology in progress. *European Aquaculture Society*, Bredene, Belgium, pp:657-664.

Langdon,C.J. 1983. New techniques and their application to studies of bivalve nutrition. In: Pruder,G.D., Langdon,C.J. and Conklin,D.E. *Proceedings of the Second International Conference on Aquaculture Nutriton,* Lousiana State University, Baton Rouge, Lousiana,pp:305-320.

Langdon,C.J. 1989a. Preparation and evaluation of protein microcapsules for a marine suspension-feeder, the Pacific oyster *Crassostrea gigas. Marine Biol.* **102**:217-224.

Langdon,C.J. 1989b. Comparison of two capsule types for the delivery of dietary protein to the pacific oyster, *Crassostrea gigas. J.Shellfish.Res.*, **8**(2):414.

Langdon,C.J. and Bolton, E.T. 1984. A microparticulate diet for a suspension-feeding bivalve mollusc, *Crassostrea virginica* (Gmelin). *J.Exp.Mar. Biol. Ecol.*,**82**:239-258.

Langdon,C.J.and De Bevoise,A.E. 1990. Effect of microcapsule type on delivery of dietary protein to a marine suspension feeder, the oyster *Crassostrea gigas. Mar. Biol.*,**105**:437-443.

Langdon,C.J. and Levine, D.M. 1984. Technological innovations in the development of microparticulate feeds for marine suspension feeders. In: *Proceedings Ocean's 83, Vol.II, Institute of Electrical and Electronic Engineers, Piscataway, NJ, and Marine Technology Society, Washington,DC.*, pp:1005-1008.

Langdon,C.J. and Siegfried,C.A. 1984. Progress in the development of artificial diets for bivalve filter- feeders. *Aquaculture*, **39**:135-153.

Langdon,C.J. and Waldock,M.J. 1981. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. *Journal of the Marine Biological Association of the U.K*, **61**:431-448.

Leibovitz,H.E., Bengston,D.A. and Simpson, K.L.1991. An evaluation of microcapsule performance on delivering diets to silver side (*Menidia beryllina*) larvae.In: Lavens,P., Sorgeloos,P., Jespers,E.and Ollevier,F. (Eds.). *Larvi-91*. No. **15**:173-174

*Leuenberger,B.H. 1991. Investigations of viscosity and gelation properties of different mammalian and fish gelatins. *Food Hydrocolloids* **5**(4):353-361.

Levine, D.M. and Sulkin,S.D. 1984 a. Ingestion and assimilation of microencapsulated diets by brachyuran crab larvae. *Mar. Biol lett.* **5** (3): 147 - 153.

Levine,D.M. and Sulkin, S.D. 1984b. Nutritional significance of long chain polyunsaturated fatty acids to the zoel development of the brachyuran crab, *Eurypanopeus depressus* (Smith). *J. Exp. Mar. Biol. Ecol.*, **81**(3); 211-223.

*Levine,D.M., Sulkin,S.D. and Van Heukelem, L. 1983. The design and development of microencapsulated diets for the study of nutritional requirements of brachyuran crab larvae. In: Berg,C.J. (Ed.) The culture of Marine Invertebrates: *Selected Readings* .Hutchinson Ross Publ. Co., Stroudsburg,PA.pp:193-203.

Levine,D.M., Sulkin,S.D., Van Heukelem,L and Selzer, J.A. 1984. The use of calcium alginate microcapsules in the study of the nutritional requirements of larvae of the mud crab *Europanopeus depressus* (Smith). *J. Shellfish. Res* **4** (1): 93.

Ling,S.W. 1962. Studies on the rearing of larvae and juveniles and culturing of adults of *Macrobrachium rosenbergii* (de Man). *FAO/IPFC. Curr.Aff. Bull.*, **35**:1-11.

Ling,S.W. 1969a. The general biology and development of *Macrobrachium rosenbergii* (deMan). *FAO Fish. Rep.* **57**(3):589-606.

Ling, S.W. and Merican,A.B.O. 1961. Notes on the life and habits of the adults and larval stages of *Macrobrachium rosenbergii* (de Man). *IPFC. Proc.*, **9**(2):55-61

*Lobao,V.L., Pazinatto,A.C., Roverso,E.A., Marques,H.L.A. and Hortencio,E. 1993. Efficiency evaluation of binders employed in shrimp feed. *Biol. Inst. Pesca.*, Vol.**20**,pp:87-94.

Lopez-Alvarado,J.and Kanazawa,A.1993. Utilization of crystalline acids by Japanese flounder larvae, *Paralichthys olivaceus*, fed on zein-microbound diets.*Spec.Publ.Eur.Aquacult. Soc*, no.**19** (Summary only).

Lopez-Alvarado,J.and Kanazawa,A. 1995. Optimum levels of crystalline amino acids in diets for larval red seabream (*Pagrus major*). Mass rearing of juvenile fish. In: Pittman,K., Batty,R.S., Vereth,J.(Eds.). *Selected papers from a symposium held in Bergen,21-23 June 1993.*Vol.**201**pp:100-105.

Lopez-Alvarado,J., Langdon,C.J.,Teshima,S.I. and Kanazawa,A.1994. Effects of coating and encapsulation of crystalline amino acids on leaching in larval feeds. *Aquaculture,* **122**(4):335-346.

Mahesh,S. 1996. Effects of different dietary levels of lecithin on growth, survival, moulting and body phospholipid levels in *Macrobrachium rosenbergii* post larvae. *M.F.Sc. Thesis . Submitted to Kerala Agricultural University, Faculty of Fisheries,* Kochi, Kerala, pp:92.

Marin-Magain,V.and Canavate,J.P. 1995. Fluorometric determination of selectivity between live and inert food by *Penaeus japonicus* larvae. *Aquaculture,* **134** (3-4):307-311.

*Marte,C.L. and Duray,M.N. 1991. Microbound larval feed as supplement to live food for milk fish (*Chanos chanos* Forskal) larvae. In: Lavens,P., Sorgeloos,P., Jaspers,E. and Ollevier.F. (Eds.) *Larvi 91. Symposium on fish and crustacean larviculture.*

European Aquaculture Society, Gent, Belgium:175-177.

*Martinez,Tapia,C., Fernandez-Pato,C.and Chereguini,O. 1990. Comparative study of larval survival and growth in seabream using microencapsulated or living food. *Copenhagen Denmark Ices*.5pp.

*Matsuo,N. 1954a. Toxicity of fish oil (in Japanese). *J. Biochem.* (Japan), **41**:481-487.

*Matsuo,N. 1954b. Toxicity of fish oil (in Japanese). II. *J. Biochem.*(Japan), **41**:647-652.

Merchie,G., Lavens,P., Nelis,H., Sorgeloos,P. and De Leenheer,A. 1993. Effect of feeding vitamin C-enriched live food on the hatchery production of *Macrobrachium rosenbergii*: *Special Publication European Aquaculture Society*, **19**:149

Meyers,S.P. and Brand,C.W. 1975. Experimental flake diets for fish and crustacea. *Prog. Fish.Cult.*,**37**(2):67-72.

Meyers,S.P. and Zein-Eldin,Z.P. 1972. Binders and pellet stability in development of crustacean diets. *Proc. World Maricult. Soc.*, **3**:351-364.

Muir, P.R. and Sulton, D.C. 1994. Bacterial degradation of micro encapsulated foods used in larval culture. *J. World. Aquacult. Soc.*, **25**(3):371-378.

Murthy,H.S. 1996. Farming the giant fresh water prawn, *Macrobrachium rosenbergii in India. Fishng Chimes*,**29**(2) :43-47

Murthy,H.S. and Devaraj,K.V.1992. Formulation, stability and effect of storage on the quality of three artificial feeds used in carp culture. *Fish. Technol., Soc. Fish. Technol.*,Kochi.**16**(2):107-110.

Mylvaganam, R. 1988. Shrimp feed -- Meeting the increase in consumer demand. *Info fish. Int.*, No.**1**,pp:44.

Nair,C.M. and Hameed,M.S. 1992. Commercial production of Scampi seed using synthetic brackish water. In: Silas,E.G.(Ed.) *Freshwater Prawns*, Kerala Agricultural University, Thrissur, India, pp: 152-155.

Nair, C.M. and Sherief,P.M. 1993. Effects of diets containing different proportions of clam meal and tapioca on growth of *Macrobrachium rosenbergii. J. Aquacult. Trop.*8:239-244.

*Nell, J.A. 1993. The development of oyster diets. *Aust. J. Agric.Res.* 44(3):557-566.

New,M.B. 1976a. A review of dietary studies with shrimp and prawns. *Aquaculture,* 9:101-144

New, M.B. 1976b. A review of shrimp and prawn nutrition. *Proc. 7th Annual Meeting World Mariculture Society*:277-287.

New, M.B. 1982. Biology . In: New,M.B.and Singholla,S. (Eds.) Freshwater prawn farming - *Macrobrachium rosenbergii. FAO Fisheries Technical Paper No.***225**. pp.2-3.

New,M.B. 1995. Status of freshwater prawn farming: *A review:Aquaculture Research,***26**:1-54.

*Norland,R.E. 1987. Fish gelatin. Technical aspects and applications. In: S.J. Band (Ed.) *Photographic Gelatin,* Royal Photographic Society, London.p.266.

*Norland,R.E. 1990. Fish gelatin. In: M.N. Voight and J.K. Botta (Ed.) *Advances in Fisheries Technology and Biotechnology for Increased Profitability,,* Technomic Publishing Co., Lancaster,PA.pp:325.

*Osborne,K., Voight,M.N. and Hall,D.E. 1990. Utilisation of lumpfish carcasses for production of gelatin. In: Voight,M.N. and Botta,J.K. (Ed.) Advances in Fisheries Technology and Biotechnology for Increased Profitability. Technomic Publishing C., Lancaster,PA. pp:143.

Ottogali,L. 1991. Total substitution of microparticles for algae for *Penaeus stylirostris* larval rearing in New Caledonia. *Journal of the World Aquaculture Society,* **22**:46A.

Patterson,H.B.W. 1989. Handling and storage of Oilseeds, Oils, Fats and Meal. *Elsevier Applied Science*, New York,394pp.

Paulraj,R. 1993. Aquaculture feed. *Handbook on Aqua farming, The Marine Proudct Export Development Authority*, Kochi, India.pp.34.

Pearson,P. 1976. The Chemical analysis of food. Churchill, London. pp.575.

Petitjean,M. and Csengeri,I. 1993. Microencapsulation of hydrosoluble additives to artificial diets for cyprinid larvae to avoid leaching. *The carp proceedings of the second "Aquaculture" sponsored symposium Held in Budapest, Hungary, 6-9 September, 1993.* Billard,R., Gall. G.A.E. (Eds). 129(1-4):254.

Pike,J.H.1998. Future supplies of fish meal and fish oil: Quality requirements for aquaculture. In: International Aquafeed Directory and buyers guide,1998.pp:39-49.

Rao,K.J.1991. Breeding and larval rearing of the freshwater prawn, *Macrobrachium malcolmsonii. Journal of Aquaculture in the Tropics*, **6**:99-106.

Rasheed,A.A., Oldfield,J.E.,. Kaufmes,J. and Sinnhuber,R.O. 1963, Nutritive value of marine oils. 1. Menhaden oils at varying oxidation levels with and without antioxidants in rat diets. *J.Nutr.* **79**:323-332.

Reddy,A.K., Prakash,C.and Sinha,P.S. 1991. Larval rearing of *Macrobrachium rosenbergii* in artificial sea water. *Journal of the Indian Fisheries Society*, **21**:1-4.

Reigh,R.C and Stickney,R.R. 1989. Effect of purified dietary fatty acids on the fatty acid composition of fresh water shrimp *Macrobrachium rosenbergii, Aquaculture*, **77**:157-174.

Rimmer, M. A.and Reed,A.W. 1989 In:Barret,J. (Ed.) Effects of nutritional requirement of live food organisms on growth and survival of barramundi/seabass *Lates calcarifer* larvae. *Advances in*

tropical Aquaculture: Workshop held in Tahiti, French-Polynesia, February20- March 4. no.9,pp:611-623.

Rimmer, M.A., Reed,A.W. and Levitt,M.S. 1992.Allan, G.I., Dall.W. (Eds.) Effects of nutritional enhancement of brine shrimp, *Artemia salina* on survival of barramundi, *Lates calcarifer*, larvae. *Proceedings of the Aquaculture Nutrition Workshop,.* Salamander Bay, NSW-Australia. NSW-Fisheries,pp:199.

Robert,R,J. 1978. In. Tindall,B.(Ed.) Fish pathology. . New york.318 pp.

Romdhane,M.S., Devresse,B., Leger,P.and Sorgeloos,P. 1994. Effects of feeding (n-3) HUFA enriched *Artemia* during a progressively increasing period on the larviculture of the freshwater prawn *Macrobrachium rosenbergii. Journal off the World Aquaculture Society,* pp.24

Ruyet,J.P.L., Alexandre,J.C., Theband,L. and Muganier,C. 1993. Marine Fish larvae feeding: Formulated diets or live prey? *Journal of the World Aquaculture Society.* **24**(2):211-215.

Saju,M.S. 1996. Dietary Phosphorus requirement and deficiency syndromes in *Macrobrachium rosenbergii juveniles. M.F.Sc. Thesis. Submitted to Kerala Agricultural University, Faculty of*

Fisheries, Kochi, Kerala,pp:*47-66*.

Sakamoto,M. Holland,D.L. and Jones, D.A. 1982. Modification of the nutritional composition of *Artemia* by incorporation of polyunsaturated fatty acids using microencapsulated diets. *Aquaculture*, **28**:311-320.

*Samara Singhe,R.P., De-Silva,O.S.S. and Fernando, D.Y. 1993. The culture of *Biddulphia longicuris* and its use as a food for *Penaeus monodon larvae. Asean. Fish.Sci.*, **6**(2):223-227.

Sandifer,D.A. and Joseph,J.D. 1976. Growth responses and fatty acid composition of juvenile prawns (*Macrobrachium rosenbergii*) fed a prepared ration augmented with shrimp head oil. *Aquaculture*, **8**:129-138.

Sato,K., Yoshinaka,R.,Sato,M.and Ikeda,S. 1986. A simplified method for determining collagen in fish muscle. *Bull. Jap. Soc. Sci.Fish.*, **52**:889-893.

Sherief,P.M. 1989. Clam meal as animal protein source in the diet of fresh water prawns. In: Balachandran,K. Perigreen, P.A.,Madhavan, P. and Surendran,P.K. (Eds.) *Recent Advances in processing of low cost fish*; Society of Fisheries Technologists (India),

Cochin, pp:327-334.

Sherief,P.M. 1990. Nutritional requirement of *Macrobrachium rosenbergii* larvae. In: *Training on "Hatchery production of Macrobrachium rosenbergii* seed" *to officers of the Fisheries* Department, *Govt.of Kerala. KAU, College of Fisheries,* Panangad, pp:69-75.

Sherief, P.M., Nair, C.M. and Malika, V. 1992. The cholesterol requirement of larval and post larval prawn *Macrobrachium rosenbergii* In: E.G. Silas (Ed.). Fresh Water Prawns. *Proc. Nat. Symp. Fresh water prawn (Macrobrachium* spp) 12-14, Kochi, pp:213-217.

Shewbart,K.L. and Mies,W.L. 1973. Studies on nutritional requirements of Brown shrimp. The effect of linolenic acid on growth of *Penaeus aztecus. Proc. World. Maricult. Soc.*,**4**:277-287.

*Shudo, K., Alakamura,K., Ishikawa,S.and Kitabayashi,K.1971. Studies on formula feed for Kuruma prawn, IV. On the growth promoting effect of both squid liver oil and cholesterol. *Bull. Tokai Reg. Fish. Res. Lab*, **65**:129-137.

*Shudo,K., Nakamura,K., Ishikawa,S. and Kitabayashi,K. 1971. Studies

promoting effects of both squid liver oil and cholesterol. *Bull. Tokai Reg. Fish. Lab.* (Tokyo), **65**;129-138.

Sick,L.V. 1975. Selected studies of protein and amino acid requirements for *Macrobrachium rosenbergii* larvae fed neutral density formula diets. *Proc. First International Conf. Aquaculture Nutrition.* pp:215-228.

Sick,L.V. and Andrews,J.W. 1973. The effect of selected dietary lipids, carbohydrates and proteins on the growth, survival and body composition of *Penaeus duorarum. Proc. World Maricult. Soc.*, **4**:263-276.

*Sick,L.V., Andrews,J.W. and White,D.W. 1971. Preliminary studies of selected environmental and nutritional requirements for the culture of penaeid shrimps. *Fisheries Bulletin*, **70**(1):102-108.

*Sick,L.V. and Baptist, G,H. 1973. The effects of selected physical and nutritional factors on the rate of ingestion of pelleted food by post larval penaeid shrimp. *Journal Mistchell Sci, Soc.*,**89**(3):320-329.

*Sick,L,V. and Beaty,H. 1974. Culture techniques and nutrition studies for larval stages of the giant prawn,*Macrobrachium rosenbergii* . University of Georgia, Science Center, *Technical Report Series* **74-5**.pp:1-30.

*Sick,L,V. and Beaty,H. 1975. Development of formula foods designed for *Macrobrachium rosenbergii larval and juvenile shrimp. Proc. 6th Annual Meeting World Mariculture Society*:89-102.

Sick,L.V. and Milliken,M.R. 1983. Dietary and nutrient requirements for culture of the Asian prawn, *Macrobrachium rosenbergii*. In: Mc Vey ,J.P. (Ed.). CRC Hand Book of Mariculture, Vol.I. Crustacean Aquaculture, CRC Press, Inc. Boca Raton,pp:381-390.

Singh,B.N. and Kumar,K. 1989. A new freeze dried microencapsulated egg diet for fish larvae and fry. *J. Zool. Res.*, **12**(1 &2):91-97.

Sinnhuber, P.O. and Yu,T.C. 1958. 2-thio barbituric acid method for the measurement of rancidity in fishery products 2. Quantitative determination of malonaldehyde. *Food Technol.***12**(1):9-12.

*Smith,T.I.J., Sandifer,P.A. and Trimble,W.C. 1976b. Progress in developing a recirculating synthetic seawater hatchery for rearing larvae of *Macrobrachium rosenbergii*. *Proc. Food. Drugs from the Sea Conf.*, Mayaguez, Puerto Rico, 1974. *Mar. Technol. Soc.*, pp:167-181.

*Sorgeloos,P. 1976. The brine shrimp, *Artemia salina*: a bottle neck in mariculture? *FAO Technical Conference on Aquaculture*, Kyoto, Japan. 5pp.

Sorgeloos,P.and Leger, P. 1992. Improved larviculture outputs of marine fish, shrimp and prawn. *Journal of the World Aquaculture Society*, **23**:251-264.

Southgate, P.C., Lee,P.S. and Lucas, J.S. 1992. Development of artificial diets for bivalve larvae. Allan, G.L.and Dall,W. (Eds.) *Proceedings of the Aquaculture Nutrition Workshop*. Salamander Bay, NSW, Australia NSW Fisheries.pp.156-164.

Stansby, M.E. 1990.Oxidation of fish oils. In: Stansby,M.E. (Ed.) Fish Oils in Nutrition. pp:251-252.

*Stephenson,M. and Simmons,M.A. 1976. Section V. Respiration and growth of larval *Macrobrachium rosenbergii*. In: Knight,A.W.

(Principal Investigator) Laboratory studies on selected nutritional, physical and chemical factors affecting the growth, survival, respiration and bioenergetics of the giant prawn *M. rosenbergii*. *Water Sci. & Eng. Paper 4501.* Univ. Calif. Davis. pp:38-46.

Stroemgren,T., Nielsen, M.V. and Ueland,K. 1986. Short term effect of microencapsulated hydrocarbons on shell growth of *Mytilus edulis*. *Mar. Biol.*, 9(1):33-39.

Sudarsanam,K. and Sukumaran,N. 1996. A simple microencapsulated egg diet for the post larvae of *Penaeus monodon*. *Seafood export journal*, 27(3):5-9.

*Sumeru,S.U. 1988. Status of shrimp feed development at BADC Jepra, Indonesia. *Report of the Workshop on shrimp and fin fish feed development. Johore Bahru, Malaysia, 25-29, October, 1988.*,pp:45-51.

Swaminathan,M. 1979. Proteins. In: Swaminathan, M. (Ed.) Food Science and Experimental foods. pp:38-39.

Tarladgis,B.G., Pearson,A.M. and Dugan,L.R. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity

in foods.II. Formation of the TBA-malonaldehyde complex without acid- heat treatment. *J.Sci. Food. Agric.*, **15**:602-607.

Tarladgis,B.G., Watts,B.M.and Yonathan,M.T. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J.Amer. Oil Chemists' Soc.*, **37**:44-48

Tereza-Ventura,M., Susana-Grazina-Freitaz, M.and Cruz-E-Eilva,M.P. 1987. Lipoid liver degeneration in trout- a diet related condition. *Aquaculture*, **67**(1-2):213

Teshima,S.I. 1982. Sterol metabolism . In:. Pruder,G.D., Langdon C.J and Conklin. D.E.(Eds.). *Proceedings of the 2nd International Conference on Aquaculture Nutrition:Biochemical physiological approaches to shell fish Nutrition*. Louisiana State University Baton Rouge, pp:205-216.

*Teshima, S.I., Kanazawa,A., Hitott Sumatsu,K.I., Kim,K.S., Oshida,K.and Koshio,S. 1992a. Tissue uptake and bioconversion of eicosa penta enoic acid and phosphatidyl choline in prawns *Penaeus* and *Macrobrachium. Comp. Biochem.Physiol.* 102B: 885-890.

*Teshima,S.I., Kanazawa,A.and Koshio,S. 1993. Recent developments in nutrition and microparticulate diets of larval prawns. *J.*

Aquacult. Bamidgel., **45**:175-184.

Teshima, S.I.,Kanazawa,A. and Sakamoto,M. 1981. Attempt to culture the rotifers with microencapsulated diets. *Bull. Japn Soc. Sci. Fish.*, **47**(12):1575-1578.

*Teshima,S.I., Kanazawa,A. and Sakamoto,M. 1982. Microparticulate diets for the larvae of aquatic animals. *Min. Rev. Data file.Fish. Res.*,**2**:67-86.

Teshima, S.I., Kanazawa,A.and Sasada,H. 1983. Nutritional value of dietary cholesterol and other sterols to larval prawn, *Penaeus Japonicus* Bate. *Aquaculture,* **31**:159-167.

Thompson,R.K.,Douce,H. and Huron,J.M. 1992. Overview of the *Macrobrachium rosenbergii* industry in the private sector in Mauritius (Indian Ocean) In: Silas, E.G. (Ed.) *Freshwater prawns. Kerala Agricultural University, Thrissur*, India, pp:23-31.

*Toth,E.O. and Papp,Z.G.1986. Microencapsulated egg diets for wels (*Silurus glanis* L.) *Aquacult. Hung.*, Vol.**5** pp:65-70.

*Uno,Y.and Soo,K.C. 1969. Larval development of *Macrobrachium rosenbergii* de Man reared in the laboratory. *J. Tokyo Univ.* **55** (2):179-190.

*Vergara Martin,J.M..,Robaina Robaina, L.E., Hernandez-Cruz,C.M., Fernandez-Palacios,H. and Izquierdo,J.E. 1990. *Estudios sobre el valor nutricional de una dieta microencapsulada para larvas de Dorada (Sparus aurata, Linn). Actas III Congreso Nacional Acuicultura*:**39**-44.

Verreth,J., Storch,V.and Segner,H. 1987. A comparative study on the nutritional quality of decapsulated *Artemia* cysts, microencapsulated egg diets and enriched dry feeds for *Clarias gariepinus* larvae. *Aquaculture,* **63**(1-4):269-282.

Villamar, D.F. and Brusca,G.J. 1987. Survival and growth of *Crangon nigricauda* larvae reared on experimental diets. *J. World Aquaculture Soc.* **18**(1):11-25.

Villamar, D.F. and Langdon,C.J. 1993. Delivery of dietary components to larval shrimp (*Penaeus vannamei*) by means of complex microcapsules. *Mar. Biol.*,**115**:635-642.

Walford,J. and Lam,T.J. 1987. Effect of feeding with microcapsules on the content of essential fatty acids in live foods for the larvae of marine fishes. *Aquaculture,* **61**: 219-229.

Walford,J., Lim,T.M. and Lam,T.J., 1991. Replacing live foods with microencapsulated diets in the rearing of seabass (*Lates calcarifer*) larvae: do the larvae ingest and digest protein-membrane microcapsules? *Aquaculture*, **92**:225-235.

*Walker,A. 1975. Crustacean aquaculture. *Proc. Nutr. Soc.*,**34**:65-73.

*Watanabe,W.O. 1975. Identification of the essential amino acids of the fresh water prawn, *Macrobrachium rosenbergii. Msc. Thesis, Dept. Zool. U. of Hawai*, pp:26

Yufera,M., Diaz,C. and Pascual,E. 1995. Feeding rates of gilthead seabream (*Sparus aurata*) larvae on microcapsules. *Aquaculture*, **134**:257-268.

Zein-Eldin,A.P. and Meyers,S.P. 1973. General considerations of problems in shrimp nutritions. *Proc. World Maricult. Soc.*,**4**:299-317.

* not referred in original

MICROENCAPSULATION AND ITS EFFECTS ON QUALITY AND SHELF LIFE OF PRAWN LARVAL FEED

BY

REMA M.K

ABSTRACT

Submitted in partial fulfilment of the requirement for the degree

MASTER OF FISHERIES SCIENCE

FACULTY OF FISHERIES

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF PROCESSING TECHNOLOGY

COLLEGE OF FISHERIES

PANANGAD - KOCHI

1998

ABSTRACT

Three microencapsulated diets have been prepared using clam meal as the protein source and collagen and gelatin (extracted from fish skin waste) and commercially available egg albumin as the encapsulating material. Attempts were made to rear the larval prawn, *Macrobrachium rosenbergii*, with these diets and to study the effect of encapsulation on quality and shelf life of the encapsulated feeds. The results of the 15 days feeding trials showed that all the three encapsulated diets were acceptable to the larvae from Vth stage onwards with survival rate ranging from 80-90 % which is comparable with that of live *Artemia* nauplii (90%) and the larvae also undergone moulting during the rearing period. The microbial analysis and the rancidity tests conducted to evaluate the shelf life of the feed showed that all the feeds remain in good condition after 45 days of storage except that the gelatin encapsulated diet showed a comparatively higher number of microflora. However, the gelatin encapsulated diets showed permissible limit of microflora after 15 days of storage. The peroxide and malonaldehyde content of the prepared diets were below the toxic level to affect the survival of the larvae. A definite tolerable level of rancidity in prawn feeds is not known. Hence, to find the lethal level of toxicity of oxidised feed further work has to be done in this field.