

**GROWTH RESPONSE OF GIANT FRESHWATER PRAWN *MACROBRACHIUM*  
*ROSENBERGII* (De Man) JUVENILES TO FEEDS BASED ON COMBINED  
ANIMAL PROTEIN SOURCES**

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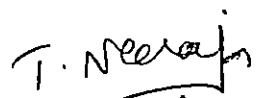
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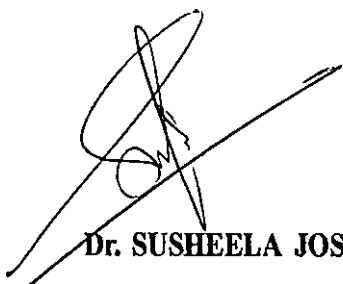
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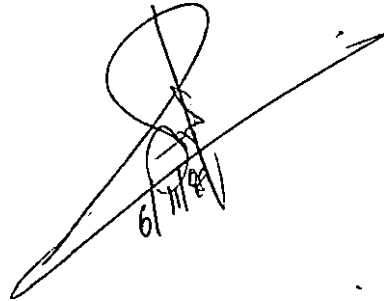
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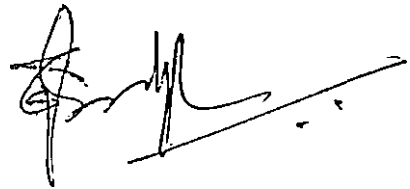


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## **INTRODUCTION**

## 1. INTRODUCTION

The giant freshwater prawn, *Macrobrachium rosenbergii* has proved to be an excellent organism for warm water aquaculture and interest in its cultivation has become widespread. The favourable attributes for the farming of the giant prawn, are its successful reproduction in captivity, established technique for larval rearing, faster growth rate, high tolerance to wide range of temperature and salinity, omnivorous feeding habit, compatibility in polyculture, absence of major disease problems, wide consumer acceptability, and high market value. During the last four to five years, *M. rosenbergii* culture has gained immense popularity in Indian aquaculture scenario due to the setback in the marine shrimp farming ever since the outbreak of viral disease.

Adequate nutrition is a prerequisite for the survival, normal development and proliferation of any animal species. In the natural environment, *M. rosenbergii*, is able to satisfy its nutritional requirements from a variety of sources which include aquatic worms, insects, small molluscs, crustaceans, plankton and organic detritus (Ling, 1969). However, under restricted controlled conditions of intensive and semiintensive culture systems, it is necessary to provide the prawn with appropriate levels of nutritionally balanced feeds and hence the success or failure of farming operations largely depend on the availability of a nutritionally balanced and palatable diet in adequate levels.

Effective formulation of a balanced diet for a particular species requires a detailed understanding of the nutritional requirements of the species. In intensive culture systems feed represents the largest input and feed cost often accounts for 50-60% of the operational cost (De Silva and Davy, 1990; Akiyama *et al.*, 1992; Sarac *et al.*, 1993) and may rise to as

high as 75% (Shang, 1981). Hence, the suitability and cost effectiveness of the ration are of paramount importance in making the farming operation a commercial success. Recent trends of intensification in the culture practices of *M. rosenbergii* necessitate the use of efficacious supplementary feeds which give maximum growth in limited time.

Since protein is the most expensive component of the diet, emphasis has been given to evaluate the protein requirement of cultured species both in quantitative and qualitative terms. Most of the feeds that are being currently used in intensive prawn culture, contains a single animal protein source and a single plant protein source. The single protein source may not contain all the essential amino acids in adequate levels required by the species (New, 1976; Gopal, 1986). A mixture of two or more protein sources have been reported to show better growth rate in cultured species than single protein source (Conklin *et al.*, 1977; Pascual, 1988; Sadhana and Neelakantan, 1997). Therefore if more than one protein source is included in the diet, the problem of inadequate levels of essential amino acids could be overcome.

With the expansion of crustacean aquaculture increased attention is being given to feeds as well as ingredients that are used to formulate the feeds, and several diets have been formulated for prawns in recent years both under laboratory as well as culture conditions. Though under culture conditions both freshwater and marine prawns accept a wide variety of food items, the flesh of molluscs and crustaceans have been found to be the most acceptable, producing the best growth (Deshimaru and Shigueno, 1972; Forster and Beard, 1973; Nair and Thampy, 1987; Rao, 1994). Crab meal and shrimp meal have been reported to be good protein sources (Gopal, 1986; Villarreal *et al.*, 1991; Das *et al.*, 1995) for use in prawn feeds, since their amino acid profile are close to the recipient prawns.



In the present study an attempt has been made to find out the effect of combined animal protein sources in the growth and survival of *M. rosenbergii* juveniles. Clam meat, crab meat and shrimp meat in various combinations have been tried to find out which formulation gives the highest growth rate, for freshwater prawn. A comparison has also been made between the diets based on combined animal protein sources over the diets with single animal protein sources for *M. rosenbergii* juveniles.

## **REVIEW OF LITERATURE**

## **2. REVIEW OF LITERATURE**

### **2.1. Nutritional requirements of freshwater prawns**

With the advent of prawn culture, several attempts have been made to formulate and evaluate diets for prawns to meet their nutritional requirements. In terms of nutritional research, the most widely studied species among palaemonid prawns is *Macrobrachium rosenbergii* since it has been identified as a candidate species for commercial aquaculture as early as 1960. Feeding studies carried out on *M. rosenbergii* are mostly concentrated on larval and juvenile stages (Fujimura, 1966; Ling, 1969; Wickins, 1972; Sick and Beaty, 1975; Fair and Fortner, 1981; D' Abramo and Reed, 1988; Raje and Joshi, 1992).

#### **2.1.1. Protein**

As a dietary constituent of major importance, protein is the most important limiting factor for growth. The dietary level, source and amino acid composition of proteins have received maximum attention in prawn nutrition, since the cost of the diet formulated is directly dependent upon the protein source and contents. The protein requirements of various prawn species have been investigated, using purified, conventional and unconventional sources of protein. Most protein requirement values for crustaceans are based on levels of protein that result in maximum growth with little or no index of protein utilization (Wilson, 1989). Growth rate in terms of weight is one of the most common criteria by which the diets and their protein levels are evaluated in prawns.

Since the early work of Subrahmanyam and Oppenheimer (1969), Kanazawa *et al.* (1970) and Deshimaru and Shigueno (1972), numerous studies have been conducted on growth rates and feeding efficiency of various crustaceans fed with different levels of

dietary protein. At one extreme, there are studies that indicate increased growth rates and feed efficiencies with increased protein levels, as high as 50%, while on the other extreme there are studies suggesting optimal dietary protein levels in the range of 20-30%. In the latter category several reports show no additional growth response with higher levels of protein and in some instances indicate decreased growth rate/efficiencies when protein level exceeds 40% of the diet. Among the different studies, most of the workers reported a protein level of 30-35% for the optimum growth and survival of *M. rosenbergii* juveniles. Table 1 shows the wide range of optimum dietary protein levels suggested by different workers with different sources of protein.

In general, the protein requirement of freshwater prawns (*Macrobrachium spp.*) is relatively low (Pandian, 1989). Balazs *et al.* (1974) prepared a series of diets for *M. rosenbergii* with protein levels 15, 25 and 35% from different dietary protein sources. He observed higher weight gain with increasing dietary protein level, with the exception of soy-tuna-shrimp diet, where weight gain decreased with an increased protein level. The results showed that prawns of different ages require varying protein levels from different sources in their diets. Balazs and Ross (1976) reported that a protein level in excess of 35% may be required for maximum growth in *M. rosenbergii*.

Millikin *et al.* (1980) used four different protein concentrations (23, 32, 40 and 49%) and found 40% protein level for maximum growth in *M. rosenbergii*. Boonyaratpalin and New (1980) evaluated the effect of three diets having 15, 25 and 35% protein on *M. rosenbergii* reared in outdoor concrete ponds and suggested 15% as the desirable protein level, since there was not much variation in the performance of these three diets. Further studies in

**Table 1. Summary of quantitative dietary protein requirements of freshwater prawns evaluated in feeding experiments with reference to protein source.**

Species	Optimum protein level (%)	Protein source	Reference
<i>Macrobrachium nobilli</i>	35	casein	Murugadas & Pandian (1987).
<i>M. rosenbergii</i>	35	fish meal, soybean meal, shrimp meal	Balazs <i>et al.</i> (1973).
<i>M. rosenbergii</i>	35	soybean meal, tuna meal	Balazs and Ross (1976).
<i>M. rosenbergii</i>	35	menhaden meal, soybean meal	Clifford & Brick (1979).
<i>M. rosenbergii</i>	40	menhaden meal, soy protein	Millikin <i>et al.</i> (1980).
<i>M. rosenbergii</i>	15	shrimp meal, fish meal, peanut meal, soy meal	Boonyaratpalin & New (1982).
<i>M. rosenbergii</i>	14	fish meal, shrimp meal	Antiporda (1986).
<i>M. rosenbergii</i>	25	fish meal, fish solubles, blood-meal, cotton seed meal	Perry & Tarver (1987).
<i>M. rosenbergii</i>	13-25	casein	Gomez <i>et al.</i> (1988).
<i>M. rosenbergii</i>	33-35	crab protein	D' Abramo & Reed (1988).
<i>M. rosenbergii</i>	30	crab protein	Freuchtenicht <i>et al.</i> (1988).
<i>M. rosenbergii</i>	30	casein	Sahadevan (1992).

asbestos asphalt or earthen bottom ponds by Bartlett and Enkerlin (1983) and in aquaria by Antiporda (1986) indicated favourable results at dietary crude protein levels as low as 14%. Gomez *et al.* (1988) reported lower protein requirements of 13-25% for *M. rosenbergii* juveniles by using casein based test diet. According to New (1990) the lower protein requirements shown by most of the experiments carried out in outdoor systems indicate the importance of natural food in prawn rearing. However, Murugadas and Pandian (1987) obtained an optimum protein level of 35% for *Macrobrachium nobilli* by using casein based diets. Studies using purified crab protein by D' Abramo and Reed (1988) and Fruechtenicht *et al.* (1988) indicated optimum protein levels of 33-35% and 30% respectively in *M. rosenbergii*.

Bingru *et al.* (1990) reported that optimum protein level for *Macrobrachium nipponensis* to be 36.8-42.27%. Without manure-induced natural feed, Law *et al.* (1992) found that a 40% protein diet supported optimal growth, compared to 25, 30 and 50%. Sahadevan (1992) reported an optimum protein requirement of 30% for juvenile *M. rosenbergii*, by using casein based diet. Pezzato *et al.* (1995) evaluated the influence of three protein levels (30, 40 and 50%) in the diet of post-larval *M. rosenbergii*. Higher protein levels in the diet were needed by post-larvae in the first ten weeks and after 20 weeks higher weight gain was obtained with a diet containing 41.38% crude protein.

The wide variations in the protein requirement is attributed to various factors such as the protein source and its amino acid composition which inturn influences protein digestibility and absorption by the prawns (Hepher, 1988), composition of other dietary nutrients such as fat and carbohydrate ratio (Teshima and Kanazawa, 1984), and the amount of

dietary organic salts (New, 1976). It may also vary with the changes in environmental factors, biotic factors and variation in the experimental procedures followed, including unstandardized way of expressing the proximate composition of test diets (New, 1976).

### 2.1.2. Amino acids

The essential amino acids for shrimps and prawns are qualitatively similar to those of other animals (New, 1976). Using radio isotopic analysis, Cowey and Forster (1971) and Shewbart *et al.* (1972) showed that arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine are essential for *Palaemon serratus* and *Penaeus aztecus* respectively. Similar results were obtained for *Macrobrachium ohione* by Miyajima *et al.* (1976) except for tryptophan. Watanabe (1975) and Watanabe *et al.* (1976) have shown that tyrosine is essential for *M. rosenbergii* in addition to histidine, methionine, leucine, isoleucine, valine, tryptophan and phenylalanine.

Miyajima *et al.* (1976) explained that all crustaceans require arginine primarily due to the lack of urea cycle in crustaceans. A conspicuous variation in *M. rosenbergii* is that in contrast to other crustaceans studied so far, lysine seems to be non-essential for *M. rosenbergii* (Watanabe, 1975). Similar observations by Stahl and Ahearn (1978) have shown that lysine is not essential for growth and survival of *M. rosenbergii*. These authors also investigated the essentiality of arginine, histidine and tryptophan, and reported that these three amino acids are found to be non-essential for *M. rosenbergii*, but cautioned that gut and/or tank bacteria may have supported the prawn with the above amino acids in sufficient amounts for growth.

Dall and Smith (1987) reported that dietary sources of amino acids regarded as

non-essential may be required if they have high rate of metabolic turnover. Alanine is thought to have an osmoregulatory function, while taurine is involved in cardiac and neural processes in *M. rosenbergii* (Smith *et al.*, 1987). Tidwell *et al.* (1993) have shown that, replacement of fish meal with soybean meal and distillers by-products in diets for *M. rosenbergii* caused significant increase in concentration of glutamine, proline, alanine, leucine and phenylalanine. Concentration of aspartic acid, glycine, lysine and arginine showed significant decrease with fish meal replacement.

Sparing action of amino acids has also been reported in prawns. For instance, methionine could be spared by cystine, and phenylalanine converts into tyrosine (Paulraj, 1993). Hence adequate levels of these amino acids must be ensured in the feed of prawns.

Studies on the quantitative requirements for amino acids in prawn feeds are scarce, because of the incapability of juvenile and adult prawn to utilize crystalline amino acids in the diets. However, few workers have studied the effects of amino acid supplementation. Farmanfarmaian and Lauterio (1979, 1980) supplemented the diet of *M. rosenbergii* with 1% arginine, lysine, phenylalanine, histidine, leucine, isoleucine, methionine or threonine individually. Arginine, phenylalanine, leucine, and isoleucine stimulated the growth conversion index, while other amino acids tested had no significant effect on growth.

### **2.1.3. Lipids**

Lipids are important in the diet of prawns, next to proteins. Besides acting as an energy source, it has been found that dietary lipids have the highest protein sparing action. Unlike fishes, crustaceans cannot tolerate high levels of dietary lipid (Andrews *et al.*, 1972; Forster and Beard, 1973; Deshimaru *et al.*, 1979; Davis and Robinson, 1986; Sheen and D'Abramo, 1991).



In comparison to penaeid shrimps, *M. rosenbergii* requires comparatively low lipid levels in diets. Joseph and Williams (1975) and Sandifer and Joseph (1976) reported best growth in post-larvae of *M. rosenbergii* fed on diets with 3% shrimp head oil. Hilton *et al.* (1984) indicated that the total lipid content in the diet should not exceed 10% for *M. rosenbergii*. Using 2:1 cod liver oil/corn oil mixture, Sheen and D' Abramo (1989) found that 6% inclusion rate was optimal, while 0, 10 and 12% levels depressed growth in *M. rosenbergii*. They also observed significant reduction in weight of *M. rosenbergii* juveniles when 10-12% of the same lipid sources were used. In commercial prawn feeds, a lipid level of 6-9% has been reported in Thailand (ASEAN/UNDP/FAO, 1988), 5-8% in French Guiana (IFREMER, 1989) and 2-4% in Taiwan (Hsieh *et al.*, 1989).

It is the profile of the essential fatty acids, phospholipids and sterols in the lipids that are more important than percentage of total lipids.

Crustaceans are known to synthesize certain groups of fatty acids *de novo* in their body, viz., palmitic (16:0), palmetioleic (16:1), steric (18:0) and oleic (18:1 $\omega$ 1) acids (Kanazawa *et al.*, 1979 d). However the ability for the synthesis of long chain fatty acids with double bonds is absent or very limited in crustaceans (D' Abramo and Sheen, 1993; Kanazawa and Koshio, 1993). Post-larvae of *M. rosenbergii* are able to convert 18:2n-6 to 20:2n-6 and desaturate 20:2n-6 to 20:3n-6 (Reigh and Stickney, 1989). There is no evidence that prawns can synthesize 18:2n-6 or 18:3n-3, but the prawns apparently seem to have the ability to elongate or desaturate both. Polyunsaturated fatty acids (PUFA) of linolenic (n-3) and linoleic (n-6) families (18:2n-6, 18:3n-3, 20:5n-3, 22:6n-3) have been recognized as most indispensable nutrients for the growth of crustaceans (D' Abramo and Sheen,

1993). Growth of crustaceans has been improved through dietary additions of 18:3n-3 or 18:2n-6 and the levels of measured response of dietary 18:3n-3 generally exceed that to 18:2n-6 (Kanazawa *et al.*, 1977 a; Read, 1981). In contrast to these observations according to Reigh and Stickney (1989), *M. rosenbergii* appears to prefer dietary 18:2n-6 over 18:3n-3 in satisfying a C18 PUFA requirement.

Highly unsaturated fatty acids (HUFA) such as 20:5n-3 (eicosapentanoic acid, EPA) or 22:6n-3 (docosahexanoic acid, DHA) have higher nutritive value than 18:2n-6 or 18:3n-3 PUFA for *M. rosenbergii*. D'Abramo and Sheen (1993) found that the mean weight gain of the juvenile *M. rosenbergii* was 30-50% higher when fed on diet containing EPA and DHA over the control group fed with 18:3n-3 or 18:2n-6. The suggested requirement level of these fatty acids in the fresh water prawns ranges between 0.075 and 0.6%.

Dietary phospholipids (PL) play a critical role in crustacean nutrition. *M. rosenbergii* requires only very little quantity of PL in the diet, probably less than 0.1%. Though Hilton *et al.* (1984) noticed no additional benefits by the supplementation of soyllecithin in the diet, a trend towards enhancement of growth rate was observed by Briggs *et al.* (1988) when the lecithin content was increased from 0 to 5%. Koshio *et al.* (1992) found that inclusion of 0 to 2% of soyabean lecithin in diets showed no significant effect in prawns of 800 mg size, but growth enhancement was noticed in early post-larvae of 50 mg size. The results of the above study indicate that in *M. rosenbergii* phospholipids are essential only during the early phase of growth. Mahesh (1996) also found that supplementation of lecithin at a level of 2.5% in the diet accelerated growth and improved FCR during the early post-larval phase of the same species.

Among the sterols, cholesterol is the most important from nutritional point of view. *De novo* synthesis of sterols has been found to be absent in crustaceans. Teshima *et al.* (1997) reported that, in contrast to other crustaceans, *M. rosenbergii* juveniles were capable of *de novo* cholesterol synthesis but required a dietary source of about 0.1% cholesterol for maximum growth. As in marine crustaceans, no advantage has been demonstrated in *M. rosenbergii* by supplementing a semi-purified diet containing 0.12% cholesterol with 0.5 or 1% cholesterol (Briggs *et al.*, 1988). Sheen and D' Abramo (1991) indicated that no specific dietary lipid level was required for *M. rosenbergii*. Rather, the optimal amount of dietary lipid depends upon the amount and quality of dietary protein and dietary energy. Sherief *et al.* (1992) were unable to demonstrate any requirement for cholesterol in *M. rosenbergii* post-larvae above the endogenous 0.12% level present in the control diet contributed by cod liver oil. However, D' Abramo and Daniels (1994) found that by depriving *M. rosenbergii* of all sterols caused death within 48 days and cholesterol requirement was estimated as 0.3 to 0.6% dry weight of the diet.

#### **2.1.4. Carbohydrates**

Besides being an important source of energy component in the diet, carbohydrates play a significant role in glycogen storage, chitin synthesis and the formulation of steroids and fatty acids in prawns. Shrimps and prawns appear to have the ability to utilize complex carbohydrates better than simple ones like glucose (New, 1976).

Prawns have cellulase (Noborikawa, 1978) and chitinases, and carbohydrate can be used to spare protein. Gomez-Diaz and Nakagawa (1990) who examined the effect of various carbohydrate sources in purified diet, found that glucose gave the lowest weight gain in

juvenile *M. rosenbergii*, while the highest weight gain was achieved with diets containing alpha potato-starch or soluble starch. Briggs (1991) used a range of carbohydrate sources at 40% inclusion level in isonitrogenous and isolipidic semipurified diets for post-larvae of *M. rosenbergii*, and obtained best growth rate with wheat flour, followed by pure polysaccharide dextrin, alpha starch and disaccharide sucrose. Alpha cellulose, glucose and chitin were less effective and thus confirm that complex carbohydrates are utilized more effectively than simpler di- or monosaccharides. The main substrate used by *M. rosenbergii* post-larvae and juveniles to meet metabolic requirements was found to be carbohydrates (Diaz-Herrera *et al.*, 1992).

Anilkumar (1994) found that the protein level for *M. rosenbergii* juveniles could be lowered from 35 to 30% by increasing the carbohydrate level from 20 to 30% without affecting the growth, survival and feed efficiency. This suggests that a 5% reduction of protein can be achieved by enhancement of 10% carbohydrate, thus reducing the cost of the feeds without sacrificing the nutritional quality of the formulated feed.

#### **2.1.5. Fibre**

Little is known about the nutritional significance of fibre in the diet of crustaceans. In general, fibre refers to mixtures of cellulose, hemicellulose, lignin, pentosans and other undigestible fraction in the feed. It has been a common practice among crustacean nutritionists to include cellulose (primarily as a dietary filler) in experimental rations at levels ranging from 0 to as high as 75% of the total dry matter (New, 1990). Excess dry matter could also exert a negative influence on overall ingestion rates. Fair *et al.* (1980) found that dietary fibre levels of up to 30% does not produce any detrimental effect in *M. rosenbergii*

juveniles and levels of 5% and 20% in fact stimulated growth. In contrast to above findings, Gomez *et al.* (1988) reported poor growth and depressed locomotive activity in *Macrobrachium* fed on diet containing 20% cellulose. Zimmerman *et al.* (1992) incorporated four inexpensive forages (the legume *Clitoria ternalea* and the grasses *Bacharia purpurescens*, *B.arrecta* and *B.humidicola*) as fibre sources, which constituted 75% of the fibre content (6%) of the diets and found that the diet containing *B. purpurescens* gave highest average individual weight and biomass in *M. rosenbergii*.

#### 2.1.6. Vitamins

Vitamins are essential for normal growth, maintenance and reproduction of animals. Under high stocking densities of semiintensive and intensive culture systems where natural food is limited for sustaining the stocked species, vitamin supplementation in the diet seems essential. However, specific quantitative vitamin requirements are yet to be determined for most of the cultivable species.

The composition and levels of several vitamin premixes used in studies with *Penaeid* and *Macrobrachium* species were reviewed by New (1976). New (1980) reported that B group, C and E vitamins are required in crustacean diets. Vitamin D may be partly ingested in the diet but also can be synthesized. Vitamin K may be antagonistic to some species of crustaceans (New, 1980). The requirement of vitamin A in shrimp and prawn diets is suggested by the importance of its precursor substances, the carotenoids, to pigmentation (Castell, 1982). Dietary needs for vitamin C have been demonstrated in *M. rosenbergii* by many workers. Heinen (1988) reported vitamin C deficiency symptoms in *M. rosenbergii*, the most important being, the failure to moult. Dietary fat soluble vitamins or trace minerals

seem to be less important than water soluble vitamins, but this observation may be masked by leaching of the latter from pelleted diets. Omission of pyridoxine caused significant reduction in growth rate but deletion of riboflavin significantly increased growth rate. Vitamin C is required for hydroxylation of proline and lysine in the formulation of collagen. Vitamin C deficiency in crustaceans leads to the inhibition of alkaline phosphate activity, resulting in poor chitin synthesis (Paulraj, 1993), poor growth, reduced moulting frequency, high incidence of post moult-death, poor feed conversion, impaired collagen synthesis and wound healing, blackening of gills and in extreme cases black death disease which causes high mortality rate (Paulraj, 1993, He and Lawrence, 1993).

An important factor to be considered in the vitamin C requirement is its source and stability during processing and subsequent storage. Presently most of the commercial prawn feed manufacturers are using L-ascorbyl-2-polyphosphate, a derivative of vitamin C, which is relatively resistant to oxidation. It has manufacturing losses of only upto 20% with an additional loss of upto 30% after 180 days of feed storage in various types of feeds (Grant *et al.*, 1989). Water stable forms of ascorbic acid, the calcium salt of ascorbyl-2-monophosphate and ascorbyl-6-palmitate when used in feeds for *M. rosenbergii* juveniles showed an increased survival, as the level of vitamin C activity was increased from 0-100 mg/kg of diet (D' Abramo *et al.*, 1994). Mortality was observed in vitamin C deficient diets (50 mg/kg) with in 21 days. Gijo (1996) reported a maximum weight gain in *M. rosenbergii* juveniles when fed on diet containing 150 mg of vitamin C in the form of CVC-F90 (Hydrogenated vegetable oil form of vitamin C) per kg dry diet.

### 2.1.7. Minerals

There are only very few studies in cultured crustaceans regarding mineral nutrition. The experimental data on penaeid shrimps indicate that the ratio of phosphorus to calcium in the diet may be significant (Deshimaru and Shigueno, 1972; Huner and Colvin, 1977).

The dietary requirements of minerals in crustaceans is largely dependent upon the mineral concentration of water in which they are reared since shrimps and prawns can absorb or excrete minerals directly from or to aquatic environment through the gills and body surface. Vasquez *et al.* (1989) found that the optimal growth of *M. rosenbergii* at water hardness less than 53 mg/litre of  $\text{CaCO}_3$ . Growth rate did not change significantly at lower hardness but declined at higher levels.

Little was known about the dietary mineral requirements of prawns upto 1989. Zimmermann *et al.* (1994) demonstrated the important relationship between dietary calcium level and water hardness. Among the two dietary calcium levels (1.8 and 3.0%) both in diets with a 1.5:1 calcium : phosphorus ratio and three levels of calcium in water (23, 51 and 74 mg/ litre  $\text{CaCO}_3$ ), the highest survival, average weight and biomass was obtained with 3% dietary calcium and 51 mg/ litre  $\text{CaCO}_3$  in water. The 1.8% calcium diet performed better at the highest alkalinity level. High dietary calcium combined with high alkalinity was found to be detrimental. Rath and Dube (1994) studied the effect of the trace metal zinc in promoting growth and survival of *M. rosenbergii* and found that among the seven diets containing different levels of zinc (50, 70, 90, 110, 130, 150 and 0 mg/kg of diet), best growth, survival, FCR, SGR and condition factor were obtained with diet containing 90 mg zinc per Kg diet. Saju (1996) reported that phosphorus level in the diet and

Ca: P ratio significantly influenced FCR, PER, whole body calcium and phosphorus content of *M. rosenbergii* and suggested an optimum level of 1.8% dietary phosphorus and 1:1.68 ratio of Ca : P for *M. rosenbergii* juveniles.

## **2.2. Protein sources**

Growth rates of prawns are closely related to the quality of the diet, mainly protein source. Several workers have attempted to define optimum protein requirements using test diets containing purified sources of protein such as vitamin-free casein. However, it has been reported that casein is a poor protein source for crustaceans due to its qualitative inadequacy (Sick and Andrews, 1973; Lim *et al.*, 1979; Bowser and Rosenmark, 1981; Boghen and Castell, 1981; Deshimaru, 1982).

In recent years, efforts have been made to find out efficient protein sources to replace casein in crustacean diets (Boghen *et al.*, 1982; Castell *et al.*, 1989; Reed and D' Abramo, 1989; Sheen and D' Abramo, 1991). Among the various commercially available proteins and proteins extracted from locally available marine organisms tested, crab protein has been identified as an efficient replacement protein for casein in the reference diet. Optimum growth has been obtained in many crustacean species including marine shrimp (Koshio *et al.*, 1993), freshwater prawn, *M. rosenbergii* (Reed and D' Abramo, 1989), cray fish (Morrissy, 1989), and crab (Bordner, 1989) fed on crab protein based diet.

A variety of proteins from plant and animal sources and the substitution effect of one source with another in different species of shrimps and prawns, have been evaluated by several investigators with varying degree of success.

### **2.2.1. Plant protein sources**

Prawn feeds based exclusively on plant protein sources have often produced poor



results, since many of the essential amino acids like methionine, cystine, lysine and tryptophan are lacking in different sources of plant protein (Fetuga *et al.*, 1973; Felker and Bandurshi, 1977). But since animal protein sources are relatively more expensive, protein sources of plant origin have also been incorporated in formulated crustacean feeds by many workers to minimize the cost of production.

Balazs *et al.* (1973) reported that the diets with soybean meal gave results superior to that of fish-soybean diet but inferior to a fish-soybean-shrimp based diet for *M. rosenbergii*. Koshio *et al.* (1992) observed higher nutritive value of soybean protein for juvenile *M. rosenbergii* in comparison to crab protein concentrate. Tidwell *et al.* (1993) reported that fish meal can be partially or totally replaced with soybean meal and distillers by-products in the diets for freshwater prawn, *M. rosenbergii*.

Moore and Stanley (1982) suggested that corn silage can play a role in supporting pond production of *M. rosenbergii*. Pressed brewer's grain (by-product contained malted barley, corn, rice grit and hops) was evaluated as potential protein source for *M. rosenbergii* (Kohler and Kruger, 1985). Ashmore *et al.* (1986) evaluated the nutritive merit of four cereal grains (corn, milo, wheat and barley) in formulated diets and obtained higher growth in *M. rosenbergii* fed on barley based diet. Tidwell *et al.* (1992) reported that upto 40% of distillers dried grains with solubles can be used as a protein source in practical feeds of *M. rosenbergii*.

The effect of adding plant leaves including mangrove leaves and other plant materials in the diets for prawns have also been studied. Aquacop (1976) reported that acacia meal or copra meal can be used as substitute ingredients in the diet of *M. rosenbergii*, as the

mineral and carotenoid requirements of the prawn may be met by these ingredients. Addition of fresh leaves (*Ailanthus altissima* and *Malva parviflora*) to the diet of the prawn *M. rosenbergii* resulted in elimination of black death syndrome, and reduction in incidence of black spot (Harpaz and Schmalback, 1986). Garces and Heinen (1989) have shown that orange flesh, peeled sweet potatoes, frozen peeled bananas, turnip greens and carrot tops to be useful supplements with commercial trout chow for feeding prawns. Jayalakshmy and Natarajan (1993) reported that among four pelleted feeds prepared for *M. idella* using *Pandina*, *Elodea*, *Lemna* and *Bruguiera*, *Elodea* proved best. Vasudevappa *et al.* (1993) obtained best growth in *M. rosenbergii* fed on fish meal based diet incorporated with groundnut leaf powder than the diets either with fish meal alone or incorporated with mulberry leaf powder. Jeyalakshmi *et al.* (1997) obtained highest growth percentage in *M. malcolmsonii* fed on diet incorporated with leaf protein concentrate extracted from *Cajanus caryam* followed by *Chiloria ternala*.

### **2.2.2 Animal protein sources**

Various animal protein sources have been evaluated in the formulation of prawn feeds by several investigators, with varying degrees of success. Among the various animal protein sources of freshwater, marine and terrestrial origin crustacean and molluscan meals of marine origin were found to be better utilized by shrimps and prawns (Kanazawa *et al.*, 1970; Forster and Beard, 1973; Deshimaru, 1982; Gopal, 1986; Sherief, 1987; Kompaing, 1990; Villarrel *et al.*, 1991; Josekutty and Susheela, 1992; Cruz-Suarez *et al.*, 1993; Anilkumar, 1994).

Among the various crustacean meals, shrimp meal is one of the most commonly used protein source in the formulation of prawn feeds. Shrimp meal has high crude protein content

and several essential amino acids (Forster, 1976). Besides it is a good source of fatty acids (Sandifer and Joseph, 1976) and appear promising for compounding shrimp and prawn feed (Venkataramiah *et al.*, 1978; Ali, 1988; Zimmermann *et al.*, 1991; Das *et al.*, 1995).

Nair and Thampy (1987) reported the superiority of diet based principally on shrimp meat (*Metapenaeus dobsonii*) over other sources of proteins for the larvae of *M. rosenbergii*. Zimmermann *et al.* (1991) compared the use of marine shrimp meal, fish meal and meat meal in 30% protein diets for the post-larvae of *M. rosenbergii* and obtained best biomass production with the shrimp meal based diet compared to other two diets. Jayalakshmy and Natarajan (1994) reported highest production, conversion efficiency and lowest FCR for *Macrobrachium idella* fed on diet containing prawn waste than diets based on fish meal and clam meat. Das *et al.* (1995) observed the highest performance of prawn meal based diet for *M. malcolmsonii* compared to mussel meat, fish meal and silkworm pupae based diets. Reena and Qureshi (1996) also reported superior performance of prawn meal based diet in terms of growth and FCR for *Macrobrachium dayanum*.

In addition, the use of shrimp head waste in compounded ration also appears promising since it contains several essential amino acids which induce high growth rate in prawns (Forster, 1976). It serves as a good source of fatty acids and pigments for use in prepared feeds for *M. rosenbergii* (Sandifer and Joseph, 1976) and many marine animals (Joseph and Williams, 1975). However, Law *et al.* (1990) reported lower digestibility of shrimp meal based diet than copra cake, soybean meal and wheat flour based diets for *M. rosenbergii*.

Crab protein has also been found to be a good protein source for the growth and survival of crustaceans (Boghen *et al.*, 1982, D' Abramo and Reed, 1988; Freuchtenich *et al.*, 1988; Castell *et al.*, 1989; Reed and D' Abramo, 1989; Bordner, 1989; Villarreal *et al.*, 1991; Koshio *et al.*, 1992). Protein extracted from crab has been reported to be an ideal source of protein for the preparation of standard reference diet (SRD) also for crustaceans. Feed formulations using protein concentrate from rock crab (*Cancer irroratus*) resulted in superior growth and survival of lobster *Homarus americanus*, when compared to several other proteins extracted from locally available marine organisms (Boghen *et al.*, 1982). Reed and D' Abramo (1989) reported that HFX CRD 84 (SRD prepared using protein concentrate from rock crab) appears appropriate to be used as reference diet for *M. rosenbergii*. However, Koshio *et al.* (1992) observed the superiority of dietary soybean protein (SBP) over the crab protein for *M. rosenbergii* and reported that the better utilization of SBP may be due to removal of antinutritional factors during purification process. Raje and Joshi (1992) have also obtained higher efficiency of crab meal in combination with other protein sources in the diet.

Another potential crustacean protein source which is inexpensive and locally available is the stomatopod - *Oratosquilla nepa*, the mantis shrimp. The use of squilla meal in the formulated feeds of *M. rosenbergii* has been reported by Anilkumar (1994).

Molluscs have been reported to be excellent feed for prawns (Sherief, 1989; Anilkumar, 1994). Minamizawa and Morizane (1970) obtained better results when *Artemia nauplii* were supplemented with chopped short necked clam for the larvae of *M. rosenbergii*. Sherief (1989) reported that dried clam meat as principal protein source gave

better growth and survival than fish meal based diet for *M. rosenbergii*. Anilkumar (1994) found highest growth rate and food conversion efficiency in *M. rosenbergii* juveniles fed on clam meat based diet in comparison to diets based on squilla, shrimp head waste and silkworm pupae as single protein sources. Contrary to this Jayalakshmy and Natarajan (1994) obtained poor FCR, production and conversion efficiency of clam meat based diet compared to diets with shrimp waste and fish meal as sole protein sources for *Macrobrachium idella*. Das *et al.* (1995) reported that for *M. malcolmsonii*, mussel meat based diet was found to be good but only second to the diet based on prawn meal.

Squid meal is another molluscan source of protein with high nutritive value. But though, it has been tried as a protein source in many penaeid shrimps (Deshimaru and Shigueno, 1972; Dokken and Lawrence, 1985; Cruz - Ricque and AQUACOP, 1987; Cruz - Ricque *et al.*, 1987), its use as a protein source in *M. rosenbergii* was evaluated only by Anilkumar (1994).

Although, fish meal is one of the most common ingredients used in the commercial shrimp feeds and is a high quality protein source for finfishes, it seems to have a lower nutritive value for shrimps and prawns, especially when used as the sole protein source. The poor performance of fish meal in latter case may be due to its inability to provide all essential amino acids, which are apparently essential for shrimps and prawns. Fish meal, in general is found to be poor in threonine, alanine, phenylalanine, arginine and histidine ( Lovel, 1989). While comparing different animal and plant protein sources, Sick and Beaty (1975) obtained poor growth rate in *M. rosenbergii* fed on fish meal based diet which was lower than diet based on soybean meal. Sherief (1989) also observed poor

performance of *M. rosenbergii* to fish meal based diet compared to clam meat. Das *et al.* (1995) reported that for *M. malcolmsonii* fish meal based diet produced lower growth rate, PER and higher FCR than prawn meal and mussel meat based diets.

### 2.2.3. Mixed protein sources

When any protein source is used singly in the diet of cultured species, it may not be able to support the nutritional demands of the species due to its deficiency in one or more amino acids. Hence, the most rational approach is undoubtedly, to combine a number of protein sources so as to formulate a diet which closely meets the amino acid requirement of the cultured species under consideration. Several workers have demonstrated better growth supporting effect of mixed protein sources over single protein source in formulated feeds of prawns.

In initial trials with *M. rosenbergii* a diet based on fish-soybean-shrimp gave superior growth compared to those based on either soybean alone or a combination of fish - soybean (Balazs *et al.*, 1973). Similarly Balazs *et al.* (1974) reported that better growth rates for *M. rosenbergii* could be obtained by combining tuna meal and soybean meal, than by using any of the above sources individually. Better growth performance in *M. rosenbergii* was observed by New (1976) also, when a mixture of two or more protein sources were used in place of single source. Nelson *et al.* (1977 b) showed that the assimilation rate of juvenile *M. rosenbergii* fed on mixed diet was low, hence lower specific dynamic effect and a lower non-growth component of energy utilization, while the rate was high for prawns fed on an exclusively plant based diet.

Boonyaratpalin and New (1980) successfully employed pelleted diets using shrimp meal : fish meal : peanut meal : soybean meal (5:2:1:1) as major protein sources for *M. rosenbergii*. Millikin *et al.* (1980) reported that a protein formulation consisting of menhaden meal and soy protein in 1.65 : 1 ratio at 40% protein level gave the best growth rate for *M. rosenbergii*. Ravishankar and Keshavanath (1988) reported that artificial pellet feed containing silkworm pupae plus shrimp waste as major protein sources was better utilized by juvenile *M. rosenbergii* than pellets containing either silkworm pupae or fish meal alone and silkworm pupae plus clam meat in combination.

Raje and Joshi (1992) obtained better survival and uniform growth of *M. rosenbergii* larvae by using combined protein sources such as crab meat + fish liver, clam meat + fish meat and clam meat + fish liver over feeds based on single protein sources like crab meat, fish liver, clam meat, fish meat and different live feeds. They reported that combination of crab meat and fish liver to be the most suitable diet followed by clam meat and fish meat combination in terms of high survival rate and early metamorphosis of *M. rosenbergii* larvae. Behanan *et al.* (1992) observed better FCR for *M. rosenbergii* post-larvae in a diet derived from combination of cat fish meat, prawn head, gluten and clam meat than feed based on clam meat powder alone and control feed (fresh clam meat).

#### **2.2.4. Other sources**

The potential use of silkworm pupae as a source of protein in prawn feeds has been investigated by a few workers. Most of them reported that silkworm pupae as a sole protein source produced poor growth, low digestibility and poor survival rate in prawns

(Ravishankar and Keshvanath, 1988; Anilkumar, 1994; Das *et al.*, 1995). Ravishankar and Keshavanath (1988) reported that diet containing silkworm pupae plus shrimp waste gave better growth for *M. rosenbergii*, in comparison to diet based on silkworm pupae alone, while Unnikrishnan *et al.* (1991) reported that the silkworm pupae based diet as good as clam meat based diet.

Various unconventional sources of protein like snail meat, earthworm meal, beef liver, and single cell protein (SCP) have been included in the formulated feeds of prawns by many workers. Costa (1980) reported that the flesh of African snail, *Achatina fulica* could be used as an exclusive food source for *M. rosenbergii*. Mossmann *et al.* (1990) reported that earthworm based diet produced ten times higher weight gain in *M. rosenbergii* than normal diets. Beef liver has been proved to be an excellent supplemental feed for *M. rosenbergii* at 15% inclusion level to commercial prawn feed (Garces and Heinen, 1989 & 1993; Rosa *et al.*, 1995). It has been surmised that the value of beef liver as a supplement for prawn might be due to its cholesterol content or B-vitamins.

Single cell protein (SCP) includes algal powders, yeast and bacterial protein. James *et al.* (1990) suggested that *Spirulina* can not serve as a sole protein for the post-larvae of *M. rosenbergii*, but can be effectively used as a supplemental protein. High amounts of PUFAs, vitamins and minerals present in marine yeast makes it an ideal protein source in shrimp and prawn feed. Zimmermann (1991) reported that dry sugarcane yeast can be used as much as 20% in growing-out diet for *M. rosenbergii* without causing serious losses. Akiyama *et al.* (1992) reported that the level of yeast should not exceed 5% in feeds unless the yeast product used is palatable to shrimp. Yet bacterial powder was found to be the best



assimilated protein in the diet of prawns. Manju and Devendran (1997) reported that *M. idella* fed on diet containing bacteria and actinomycetes gave significant growth, better conversion efficiency and increased body protein compared to fish meal based diet, suggesting that microbial SCP can replace the fish meal to a certain extent in formulated diets.

## **MATERIALS AND METHODS**

### 3. MATERIALS AND METHODS

The aim of the study is to find out the growth response of *M. rosenbergii* juveniles to feeds based on three animal protein sources in different combinations viz., clam meat, crab meat and shrimp meat over the feeds containing each of the three alone. The duration of the experiment was 60 days from 06-09-1997 to 04-11-1997.

#### 3.1. Preparation of the feed

##### 3.1.1. Feed ingredients

Animal and plant protein sources, tapioca flour, potato-starch, cellulose, cholesterol, sunflower oil and vitamin-mineral mixture were used for preparation of experimental feeds. Tapioca flour and potato-starch served as major carbohydrate sources as well as binders. Cholesterol and sunflower oil were used as supplementary lipid sources. Supplevite- M, a vitamin-mineral mixture (Sarabhai Chemicals, Bombay) was added to the experimental feeds. Cellulose was used as filler while preparing the feeds.

##### 3.1.2. Processing of the major protein sources

Various protein sources used for the feed formulation were processed using the following methods.

**Clam meat** : Meat of clam *Villorita cyprinoides* purchased from local market, was washed thoroughly and steamed in an autoclave at ambient pressure for 15 minutes and then dried in an electric dryer for 12 hours at 60°C.

**Shrimp meat** : Small sized shrimps, of *Metapenaeus dobsonii* was procured from the Instructional Farm of College of Fisheries. The heads and exoskeleton covering the body

were removed to obtain meat alone. This was steamed in an autoclave for 15 minutes at ambient pressure and dried in an electric dryer for 12 hours at 60°C.

**Crab meat** : Small sized crabs (*Portunus pelagicus*, *Charybdis cruciata*) were obtained from local market. The whole crabs were steamed in an autoclave for 15 minutes at ambient pressure and then all the appendages and carapace were removed. Thus the meat portion along with very small portion of soft shell were cut into small pieces, dried for 12 hours in an electric dryer at 60°C.

**Groundnut oil cake** : Fresh and mould free GOC was purchased from local market and dried in an electric dryer for 6 hours at 60°C.

The dried ingredients were powdered separately in a pulverizer and passed through a sieve of 250 microns. The powdered ingredients were packed separately in airtight plastic bottles and kept in a refrigerator along with other ingredients till they were used for feed preparation.

### **3.1.3. Proximate composition of feed ingredients**

Proximate composition of all the feed ingredients 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, 1990). Percentage of nitrogen obtained was multiplied by the factor 6.25 to get the crude protein content. Solvent extraction using petroleum ether (B.P. 40-60°C) in a soxhlet extraction apparatus

for 6 hours was carried out to estimate the crude fat. The crude fibre content was estimated by the method of Pearson (1976), while ash content was determined by burning the sample at  $550^{\circ}\text{C} \pm 10^{\circ}\text{C}$  for 6 hours in a muffle furnace. The carbohydrate content (nitrogen free extract, NFE) was determined by Hasting's (1976) difference method on dry weight basis.

$$\text{NFE} = 100 - (\% \text{ crude protein} + \% \text{ crude fat} + \% \text{ crude fibre} + \% \text{ ash}).$$

### 3.1.4. Formulation and processing of experimental diets

Seven types of pelleted feeds were formulated fixing their protein level at 35%, since this level has been reported to be optimal for the growth and survival of *M. rosenbergii* by several previous investigators (See Table 1). The feeds prepared were-

1. T<sub>1</sub> (clam meat, shrimp meat and crab meat as the major protein sources).
2. T<sub>2</sub> (shrimp meat and crab meat as major protein sources).
3. T<sub>3</sub> (clam meat and crab meat as major protein sources).
4. T<sub>4</sub> (clam meat and shrimp meat as major protein sources).
5. T<sub>5</sub> (clam meat as major protein source).
6. T<sub>6</sub> (shrimp meat as major protein source).
7. T<sub>7</sub> (crab meat as major protein source).

The proportion of the ingredients used for the preparation of pelleted feeds are given in Table 2.

The experimental feeds were prepared separately by mixing required quantity of ingredients. The respective ingredients were weighed accurately in an electronic balance and all the ingredients except supplevite-M and sunflower oil were mixed well in a dry mortar.

**Table 2. Proportion of various ingredients used in the preparation of pelleted feeds.**

Ingredients (%)	Feeds						
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>
Clam	14.99	-	22.50	22.50	44.90	-	-
Crab	14.46	21.69	21.69	-	-	-	43.39
Shrimp	10.13	15.19	-	15.19	-	30.37	-
GOC	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Tapioca	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Supplivit-M	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Cholesterol	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sunflower oil	2.76	3.83	2.0	2.43	0.60	4.27	3.40
Cellulose	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Starch	22.16	23.79	18.31	24.38	19.00	29.86	17.71
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>

The dry mixture was made into a dough by adding sufficient volume of distilled water (1 : 1.25 W/V) and mixed well in a mortar. The dough was transferred to glass bowl and steam cooked for 30 minutes in an autoclave at ambient pressure. The cooked dough was rapidly cooled and mixed well in a mortar along with supplevite-M and sunflower oil mixture.

The well mixed dough was pelletized using a hand operated extruder with a die of 2 mm diameter to a clean tray as a single layer and dried at 60°C for 12 hours in an electric dryer. The dried pellets with a diameter of 1 mm were crumbled into small pieces and packed in airtight plastic bottles and stored at 4°C in a refrigerator until use.

### **3.1.5. Proximate composition of experimental diets**

Proximate composition of the experimental diets was analysed to evaluate the nutrient status. Methodology employed was the same as that of ingredients. The gross energy value of each feed was determined by multiplying with the factors 5.65 for proteins, 9.45 for fat and 4.1 for carbohydrates (Koshio *et al.*, 1993). The energy values were expressed as K.cal/g.

### **3.2. Conditioning of experimental animals**

The post-larvae (PL 10) of *M. rosenbergii* were procured from a private hatchery at Cherai and were transported to the College hatchery in oxygen filled polyethylene bags under minimum stress. The larvae were introduced into an oval, flat bottom fibre glass tank of 1.2 ton capacity filled with filtered freshwater up to half the capacity and provided with gentle aeration. They were reared upto early juvenile stage within a period of two weeks. During this period the postlarvae were fed on finely chopped fresh clam meat once daily. The leftover feed and faeces were siphoned out daily prior to feeding with a water exchange rate of 20-30% daily.

### 3.3. Experimental rearing facilities

The experiment was carried out in the wet lab of the Department of Aquaculture with adequate ventilation, roofed partially with translucent fibreglass reinforced plastic (FRP) sheets for moderate light conditions. Circular flat bottom fibre glass tanks with the following specifications were used for the experiments.

Capacity	-	83 litres
Diameter	-	55 cm
Height	-	35 cm
Rim Width	-	3 cm
Thickness	-	4 mm
Colour	-	Aquamarine

Clear filtered freshwater drawn from a well was used for the experiment and tanks were filled upto 20 cm height after subjecting to a fine filtration using nylon bolting cloth. Mild aeration was provided throughout the experimental period via diffuser stones and plastic tubes connected to the air distribution pipes from a roots air blower and was maintained uniformly in all the tanks by means of control valves.

Earthen tiles were provided as artificial substrate in each tank to reduce cannibalism among prawns. Tiles were placed in a slanting position over a small piece of stone.

### 3.4. Experimental design and procedure

210 numbers of healthy and uniform sized *M. rosenbergii* juveniles were selected from the stock population. Ten prawns were selected randomly from these and stocked in



each of the twenty-one experimental tanks. Seven treatments T<sub>1</sub> to T<sub>7</sub> were designed and for each treatment, three replicates were maintained in separate tanks, thus using 30 prawns for each treatment. The experiment was carried out using Completely Randomized Design for a period of 60 days.

The prawns were fed on experimental diets after they were acclimatized to tray feeding for a week. On the 8<sup>th</sup> day they were starved for 24 hours, prior to start of the actual experiment. Ten prawns from each tank were weighed collectively, using an electronic balance (Shimadzu-Labror AEU 130V) with an accuracy of 0.0001 g. The average weight of the juvenile prawns at the start of the experiment was  $77.15 \pm 0.99$  mg.

Prawns were provided with the feed *ad libitum* in petridishes twice daily, once between 9.00 and 10.00 a.m. and the other between 5.00 and 6.00 p.m. Petridishes were kept at the bottom of the tank close to the substratum provided. Every day before offering the feed, leftover feed was collected and dried at 60°C for estimation of FCR. Petridishes were cleaned thoroughly before next feeding. During morning hours before feeding, bottom and sides of the tanks were scrubbed and the suspended particles were allowed to settle. The settled particles were siphoned out and new filtered freshwater was used to fill the tanks. Complete water exchange was done in all the tanks once in a week to maintain water quality.

During the experimental period prawns were subjected to growth assessment every fortnight. At the end of the feeding study shrimps were starved for 24 hours and the number in each tank counted and weighed collectively. The body protein content of the prawns at

the beginning and at the end of the experiment was also estimated for all the treatments using microkjeldahl's method (AOAC, 1990).

### **3.5. Water quality parameters**

Water quality parameters in the experimental tanks were measured by the following methods.

1. Temperature - Mercury bulb thermometer with an accuracy of 0.1°C.
2. pH - Universal pH indicator method.
3. Dissolved oxygen - Standard Winkler method (Stickland and Parson, 1972).

Temperature and pH were measured daily and D.O. content once in a week.

### **3.6. Evaluation criteria**

The parameters like net weight gain, specific growth rate (SGR), percentage survival, food conversion ratio (FCR), protein efficiency ratio (PER), and productive protein value (PPV) were determined to evaluate the efficiency of combined animal protein sources over the individual animal protein sources, on the growth of *M. rosenbergii* juveniles.

#### **3.6.1. Net weight gain**

It gives the increase in the weight of prawns during the experimental period. It was calculated by using the formula.

$$\text{Net weight gain} = \text{final weight} - \text{initial weight}$$

### 3.6.2. Percentage growth

Percentage growth of the animal was calculated by using the following formula.

$$\text{Percentage growth} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100$$

### 3.6.3. Specific growth rate

Growth performance can be measured in terms of specific growth rate (SGR) since it is a more refined and improved growth index than absolute weight gain or percentage growth rate (Hepher, 1988). In the present study SGR was calculated by using the following formula.

$$\text{SGR} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

Where,

$W_1$  = Weight at day  $T_1$

$W_2$  = Weight at day  $T_2$

$T_2 - T_1$  = Duration of experiment in days.

### 3.6.4. Survival rate

It is expressed in terms of percentage.

$$\text{Survival percentage} = \frac{\text{initial number} - \text{number of dead prawns}}{\text{initial number}} \times 100$$

### 3.6.5. Food conversion ratio (FCR)

FCR is nothing but the ability with which an animal can convert the feed consumed into edible and other products (Devendra, 1989). It is the most commonly used index to measure the efficiency of different diets used in the experiment.

$$\text{FCR} = \frac{\text{feed intake on a dry matter basis}}{\text{weight gain on wet matter basis}}$$

### 3.6.6. Protein efficiency ratio (PER)

PER is defined as the weight gain per unit intake of protein (Paulraj, 1982).

$$\text{PER} = \frac{\text{gain in body weight}}{\text{protein intake}}$$

### 3.6.7 Productive protein value (PPV)

PPV gives the measurement of body protein deposition in the prawns with unit amount of protein consumed.

$$\text{PPV (\%)} = \frac{\text{final body protein} - \text{initial body protein}}{\text{protein consumed}} \times 100$$

## 3.7. Statistical analysis

The experiment was carried out by using the Completely Randomized Design (CRD).

All indices, such as growth, specific growth rate, survival rate, food conversion ratio, protein efficiency ratio, and productive protein value were subjected to comparison

using analysis of variance (Snedecor & Cochran, 1968) and treatment difference studied at 5% level of significance. Pairwise comparison of treatment values was done using critical difference based on students 't' distribution at 5% level of significance.

## **RESULTS**

## 4. RESULTS

The efficiency of diets based on combined animal protein sources over single animal protein sources was evaluated in *M. rosenbergii* juveniles. The crude protein level in all the feeds was fixed at 35% and crude fat level at 8%. The results of the experiment are presented below.

### 4.1. Proximate composition of feed ingredients and the formulated pelleted feeds.

#### 4.1.1. Feed ingredients

The data pertaining to proximate composition of the various ingredients used in formulating the pelleted feeds are given in Table 3.

The moisture content of the feed ingredients viz., clam meat powder, shrimp meat powder, crab meat powder, groundnut oil cake (GOC) powder and tapioca powder ranged from 7.63 - 8.20%, the maximum in GOC powder (8.20%) and minimum in tapioca powder (7.63%). Shrimp meat powder had the highest percentage of crude protein (80.45%), followed by crab meat powder (55.63%), clam meat powder (53.86%) and GOC powder (51.57%). Tapioca powder had the minimum crude protein content of 2.83%.

#### 4.1.2. Formulated pelleted feeds

The proximate composition of the formulated feeds are presented in Table 4.

The percentage of moisture in the seven feeds varied between 7.12% (Feed T<sub>7</sub>) to 9.23% (Feed T<sub>6</sub>), where as crude protein content ranged from 34.56% (Feed T<sub>2</sub>) to 35.06% (Feed T<sub>4</sub>). The crude fat content ranged between 7.69% (Feed T<sub>2</sub>) and 8.22% (Feed T<sub>7</sub>) while the percentage of nitrogen free extract was highest in feed T<sub>4</sub> (41.31%) and lowest in

**Table 3. Proximate composition of the ingredients used in the formulation of feeds.**

Ingredients	Moisture (%)	Crude protein (%)	Crude fat (%)	NFE (%)	Crude fibre (%)	Ash (%)
Clam meat	7.78	53.86	12.20	30.14	Nil	3.80
Crab meat	8.04	55.63	6.11	19.28	3.20	15.78
Shrimp meat	8.16	80.45	5.67	0.78	0.60	12.50
GOC	8.20	51.57	5.00	34.30	2.33	6.80
Tapioca	7.63	2.83	0.50	96.23	Nil	0.44

**Table 4. Proximate composition of pelleted feeds used in the experiment**

PARAMETERS (%)	FEEDS						
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>
Moisture	9.31	8.67	8.60	8.27	8.98	9.23	7.12
Crude protein	34.88	34.56	35.01	35.06	34.75	34.91	35.00
Crude fat	7.83	7.69	7.91	8.03	8.14	7.75	8.22
Crude fibre	0.99	1.25	1.16	0.56	0.47	0.65	1.86
NFE (Nitrogen Free Extract)	39.35	39.26	38.34	41.31	40.52	41.46	36.2
Ash	7.64	8.57	8.98	6.77	7.14	7.16	11.60
Calorific value K. cal/g.	4.33	4.29	4.30	4.43	4.39	4.40	4.24



feed T<sub>7</sub> (36.20%). Crude fibre values varied between 1.86% (T<sub>7</sub>) and 0.47% (T<sub>5</sub>). Caloric values of the seven experimental diets ranged from 4.43 to 4.24 K. cal/ g. The formulated feeds prepared were more or less isonitrogenous, isolipidic and isocaloric.

## 4.2. Efficiency of test diets

### 4.2.1. Growth

Data regarding average live weight gain of prawns fed on feeds containing different animal protein sources are presented in Table 5.

Analysis of variance (Table 6) and subsequent pairwise comparisons showed that the growth of prawns fed on feeds containing combined animal protein sources (T<sub>1</sub>, T<sub>3</sub>, T<sub>4</sub>) was significantly different ( $P < 0.05$ ) from that with feeds containing single animal protein sources (T<sub>5</sub>, T<sub>6</sub>, T<sub>7</sub>). But the diet T<sub>2</sub> which contained crab meat and shrimp meat was not seen to be significantly different from T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub>.

The average live weight gain of *M. rosenbergii* juveniles fed on feeds containing combined animal protein sources in treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> was found to be 234.07 mg, 188.83 mg, 265.30 mg and 236.43 mg respectively. The highest average live weight gain was obtained in treatment T<sub>3</sub> with crab meat and clam meat as the major protein sources, and the lowest live weight gain was obtained in the treatment T<sub>2</sub> containing crab meat plus shrimp meat as major protein sources. The treatments T<sub>3</sub> containing crab meat plus clam meat, T<sub>4</sub> with shrimp meat and clam meat, and T<sub>1</sub> with clam meat, crab meat and shrimp meat as major protein sources were not significantly different from each other but all the three treatments were found to be significantly different from the treatment T<sub>2</sub>.

The average live weight gain of prawns fed on feeds containing single animal protein source was found to be 156.15 mg, 182.17 mg and 177.90 mg respectively for treatments T<sub>5</sub> with clam meat, T<sub>6</sub> containing shrimp meat and T<sub>7</sub> with crab meat as major protein sources. The highest average live weight gain was observed in treatment T<sub>6</sub>, and lowest value was obtained in treatment T<sub>5</sub>. However, the treatments T<sub>6</sub>, T<sub>7</sub> and T<sub>5</sub> were not significantly different. Although the treatment T<sub>2</sub> produced higher average live weight gain in comparison to T<sub>6</sub>, T<sub>7</sub> and T<sub>5</sub>, it was not found to be significantly different from the latter three treatments. The growth of prawns in the treatments T<sub>3</sub>, T<sub>4</sub> and T<sub>1</sub> was found to be significantly greater than in treatments T<sub>6</sub>, T<sub>7</sub> and T<sub>5</sub>.

The graphical representation of growth observed in different treatments are given in Fig-1 and growth curves based on fortnightly growth are given in Fig-2.

The average daily weight enhancement was found to be 3.90 mg, 3.15 mg, 4.42 mg, 3.94 mg, 2.60 mg, 3.04 mg and 2.97 mg respectively for the treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub>. The average percentage weight increase of the juveniles from their initial size in the treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub>, and T<sub>7</sub> was 305.20, 246.40, 344.16, 304.46, 202.79, 236.61 and 228.40 respectively.

#### **4.2.2. Specific growth rate**

The data on the specific growth rate of prawns under various treatments are given in Table 7.

Analysis of variance of the data shows significant difference ( $P < 0.05$ ) in SGR values between treatments (Table 8).

**Table 5. Growth of *M. rosenbergii* fed on feeds containing various combined and single animal protein sources.**

Treatment	Replication	Av. initial weight (mg)	Av. final weight (mg)	Net gain in weight (mg)	Av. live weight gain	%weight gain	Av.% weight gain
T <sub>1</sub>	1	76.52	278.58	202.06	234.07	264.06	305.20
	2	77.13	334.77	257.64	±23.462	334.03	±29.861
	3	76.38	318.89	242.51		317.50	
T <sub>2</sub>	1	76.00	256.66	180.66	188.83	237.71	246.40
	2	78.32	248.57	170.25	±19.455	217.38	±27.931
	3	75.93	291.66	215.73		284.12	
T <sub>3</sub>	1	76.20	328.06	251.86	265.30	330.52	344.16
	2	78.23	336.64	258.41	±14.627	330.32	±19.432
	3	76.86	362.50	285.64		371.64	
T <sub>4</sub>	1	76.58	326.28	249.70	236.43	326.06	304.46
	2	79.23	303.46	224.23	±10.425	283.01	±17.575
	3	77.34	312.70	235.36		304.32	
T <sub>5</sub>	1	76.60	259.35	182.75	156.15	238.58	202.79
	2	75.93	204.29	128.36	±22.220	169.05	±28.423
	3	78.38	235.71	157.33		200.73	
T <sub>6</sub>	1	75.86	264.22	188.36	182.17	248.30	236.61
	2	78.31	265.71	187.40	±8.089	239.31	±10.813
	3	76.83	247.57	170.74		222.23	
T <sub>7</sub>	1	78.23	287.50	209.27	177.90	267.51	228.40
	2	77.21	242.11	164.90	±22.290	213.57	±27.226
	3	78.16	237.69	159.53		204.11	

**Table 6. Result of analysis of variance of the data on the growth of *M. rosenbergii* juveniles fed on feeds containing combined and single animal protein sources.**

Source	S.S	D.F	M.S.S	F
Diets	28100.1248	6	4683.3541	9.4709 <sup>★</sup>
Error	6922.9696	14	494.4978	
Total	35023.0944	20		

★Significant at 5% level

**Comparison of treatment means based on critical difference :**

Critical difference = 38.946

Treatments	T <sub>3</sub>	T <sub>4</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>5</sub>
mean	<u>265.30</u>	<u>236.43</u>	<u>234.07</u>	<u>188.83</u>	<u>182.17</u>	<u>177.9</u>	<u>156.15</u>

Underscored means are not significantly different

**Fig. 1 Growth of *M. rosenbergii* juveniles fed on diets containing various combined and single animal protein sources.**

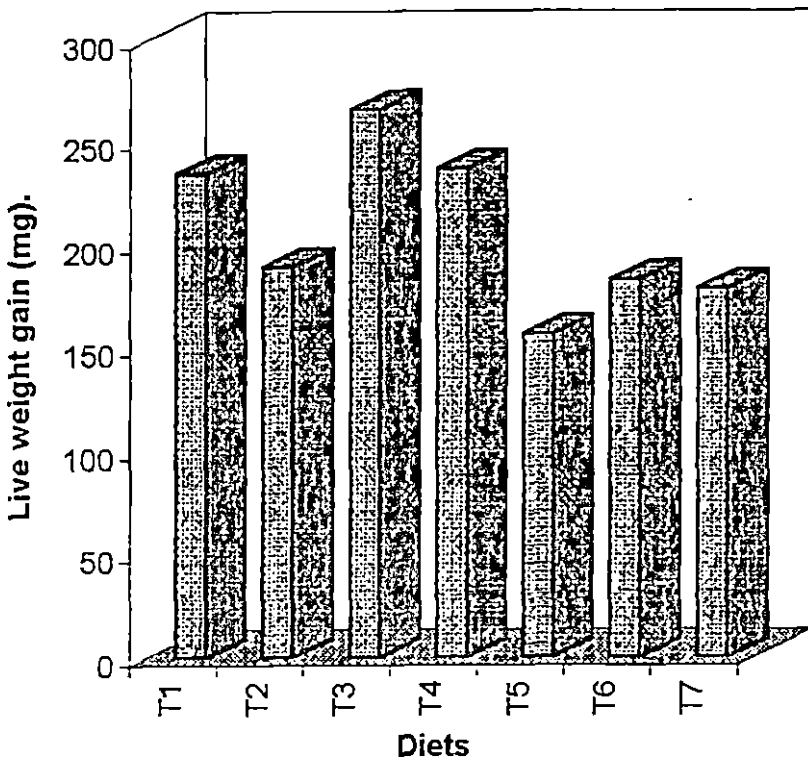
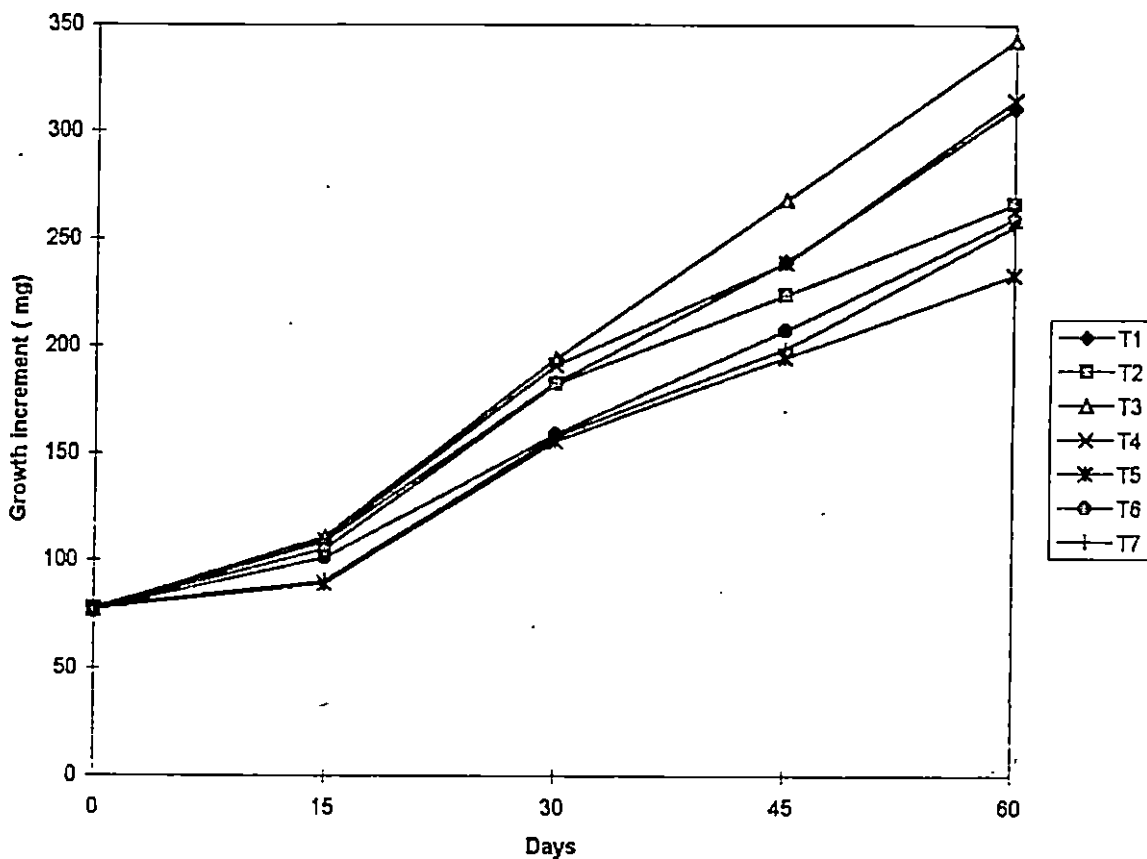


Fig. 2 The pattern of growth increment of *M. rosenbergii* juveniles fed on diets containing various combined and single animal protein sources.



The prawns in treatment T<sub>3</sub> gave the highest SGR (2.483), followed by those in treatments T<sub>1</sub> (2.328) and T<sub>4</sub> (2.327) though the latter two were not significantly different from the former. The SGR of prawns in the treatments T<sub>3</sub>, T<sub>1</sub> and T<sub>4</sub> were significantly higher than in treatment T<sub>2</sub>.

Among the prawns in the treatments T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub>, the highest SGR was obtained in the treatment T<sub>6</sub> (2.022), followed by those in T<sub>7</sub> (1.976) and T<sub>5</sub> (1.839), though the latter two were not seen to be significantly different from treatment T<sub>6</sub>. The treatments T<sub>3</sub>, T<sub>1</sub> and T<sub>4</sub> showed significantly higher SGR values over the treatments T<sub>6</sub>, T<sub>7</sub> and T<sub>5</sub>.

The SGR values of prawns fed on feeds containing different animal protein sources in various treatments are graphically represented in Fig-3.

#### 4.2.3. Survival

The data on survival percentage of *M. rosenbergii* juveniles fed on feeds with different animal protein sources in various treatments are given in Table. 9.

The analysis of variance (Table 10) of the data on survival percentage showed no significant difference among the treatments.

Highest average survival percentage of 96.67 was obtained in the treatment T<sub>3</sub>, followed by survival rates of 93.33% and 90.00% in treatments T<sub>4</sub> and T<sub>5</sub> and survival rate of 86.67% in treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>6</sub> and T<sub>7</sub> respectively. Graphical representation of percentage survival rates for different treatments are shown in Fig-4.

**Table 7. Specific growth rate of *M. rosenbergii* fed on feeds containing various combined and single animal protein sources**

Treatment	Replication	Av. initial weight (mg)	Av. final weight(mg)	Specific growth rate(%)	Mean $\pm$ S. D.
T <sub>1</sub>	1	76.52	278.58	2.154	2.328 $\pm$ 0.126
	2	77.13	334.77	2.447	
	3	76.38	318.89	2.382	
T <sub>2</sub>	1	76.00	256.66	2.028	2.065 $\pm$ 0.132
	2	78.32	248.57	1.925	
	3	75.93	291.66	2.243	
T <sub>3</sub>	1	76.20	328.06	2.433	2.483 $\pm$ 0.072
	2	78.23	336.64	2.432	
	3	76.86	362.50	2.585	
T <sub>4</sub>	1	76.58	326.28	2.416	2.327 $\pm$ 0.073
	2	79.23	303.46	2.238	
	3	77.34	312.70	2.328	
T <sub>5</sub>	1	76.60	259.35	2.033	1.839 $\pm$ 0.156
	2	75.93	204.29	1.650	
	3	78.38	235.71	1.835	
T <sub>6</sub>	1	75.86	264.22	2.080	2.022 $\pm$ 0.054
	2	78.31	265.71	2.036	
	3	76.83	247.57	1.950	
T <sub>7</sub>	1	78.23	287.50	2.170	1.976 $\pm$ 0.139
	2	77.21	242.11	1.905	
	3	78.16	237.69	1.854	



**Table 8. Result of analysis of variance of the data on the specific growth rates of *M. rosenbergii* juveniles fed on feeds containing combined and single animal protein sources.**

Source	S.S	D.F	M.S.S	F
Diets	0.97302	6	0.16217	8.3769 *
Error	0.27103	14	0.019359	
Total	1.24405	20		

\* Significant at 5% level

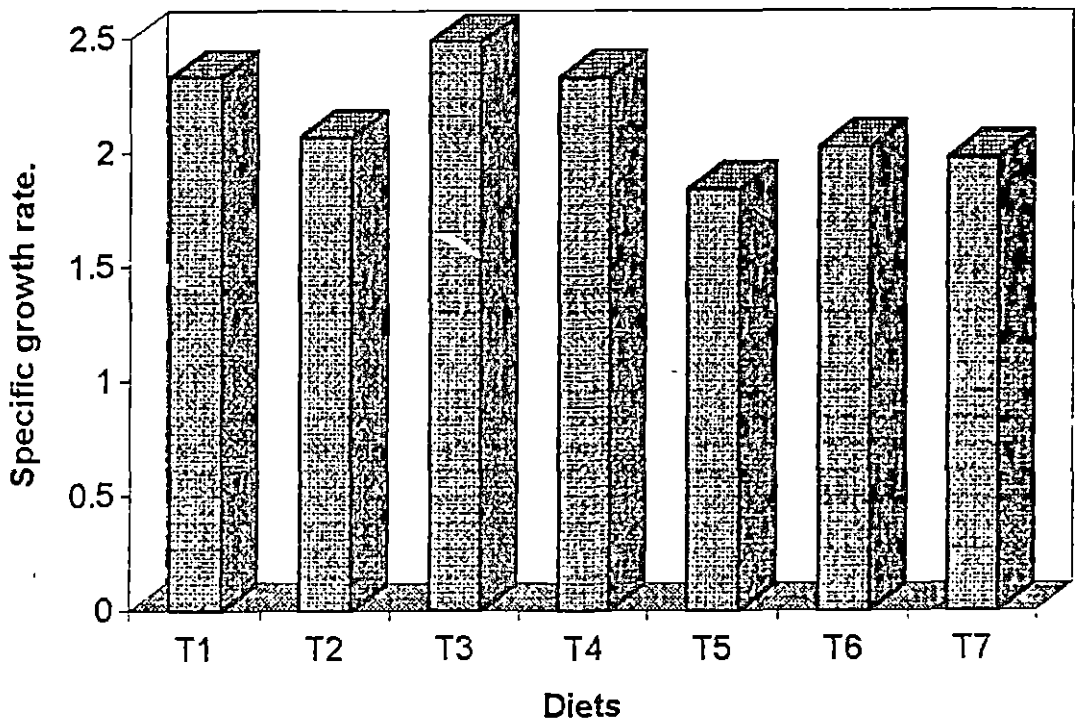
Comparison of treatment means based on critical difference :

Critical difference = 0.2437

Treatments	T <sub>3</sub>	T <sub>1</sub>	T <sub>4</sub>	T <sub>2</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>5</sub>
Mean	<u>2.483</u>	<u>2.328</u>	<u>2.327</u>	<u>2.065</u>	<u>2.022</u>	<u>1.976</u>	<u>1.839</u>

Underscored means are not significantly different

**Fig. 3 Specific growth rate of *M.rosenbergii* juveniles fed on diets containing various combined and single animal protein sources.**



**Table 9. Percentage survival of *M. reisenbergii* juveniles fed on diets containing various combined and single animal protein sources.**

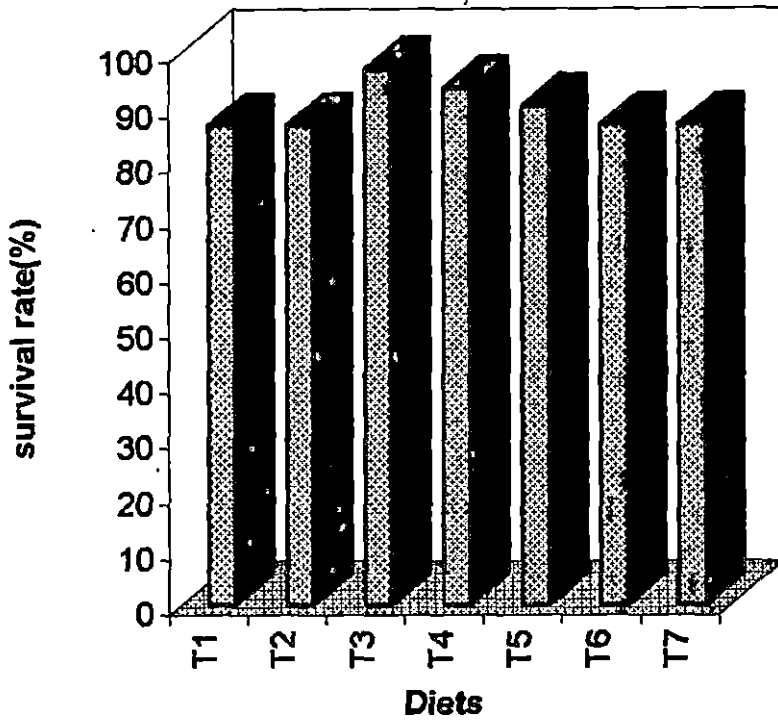
Treatment	Replication	Initial Stocking No.	Final Survival	% Survival No.	Mean (%) $\pm$ S.D.
T <sub>1</sub>	1	10	9.0	90	86.67 $\pm$ 4.714
	2	10	8.0	80	
	3	10	9.0	90	
T <sub>2</sub>	1	10	9.0	90	86.67 $\pm$ 4.714
	2	10	9.0	90	
	3	10	8.0	80	
T <sub>3</sub>	1	10	10.0	100	96.67 $\pm$ 4.714
	2	10	9.0	90	
	3	10	10.0	100	
T <sub>4</sub>	1	10	9.0	90	93.33 $\pm$ 4.714
	2	10	9.0	90	
	3	10	10.0	100	
T <sub>5</sub>	1	10	9.0	90	90.00 $\pm$ 8.165
	2	10	10.0	100	
	3	10	8.0	80	
T <sub>6</sub>	1	10	9.0	90	86.67 $\pm$ 4.714
	2	10	8.0	80	
	3	10	9.0	90	
T <sub>7</sub>	1	10	9.0	90	86.67 $\pm$ 4.714
	2	10	8.0	80	
	3	10	9.0	90	

**Table 10. Result of analysis of variance of data on the survival rates of *M. rosenbergii* juveniles fed on feeds containing combined and single animal protein sources.**

Source	S. S.	D. F	M. S. S	F
Diets	295.2381	6	49.2064	1.148
Error	600	14	42.8571	
Total	895.2381	20		

(F - value is not significant at 5% level.)

**Fig. 4 Percentage survival of *M.rosenbergii* juveniles fed on diets containing various combined and single animal protein sources.**



#### 4.2.4. Food conversion ratio

Food conversion ratio values of *M. rosenbergii* juveniles fed on various feeds with different animal protein sources are given in Table 11. The mean FCR values ranged from 2.52 to 3.87 in various treatments.

Analysis of variance of FCR values shows significant difference ( $P < 0.05$ ) between various treatments (Table 12). The lowest FCR was obtained in treatment  $T_3$  (2.52) followed by  $T_4$  (2.73),  $T_1$  (2.92) and  $T_2$  (3.44). The treatments  $T_3$ ,  $T_4$  and  $T_1$  were not significantly different among themselves in their FCR values. The treatment  $T_2$  showed greater FCR and was significantly different from  $T_3$ ,  $T_4$  and  $T_1$ . Among the treatments with single animal protein source in the feeds,  $T_6$  had lower FCR (3.54) than  $T_7$  (3.62) and  $T_5$  (3.87). Statistically these three treatments were not significantly different. It was also found that FCR values of  $T_3$ ,  $T_4$  and  $T_1$  were significantly different from FCR values of treatments  $T_6$ ,  $T_7$  and  $T_5$ .

Graphical representation of FCR values of juvenile prawns in various treatments are given in Fig-5.

#### 4.2.5. Protein efficiency ratio

The data on protein efficiency ratio of various treatments are given in Table 13. Analysis of variance (Table 14) of the data on PER values shows significant difference ( $P < 0.05$ ) among various treatments.

The highest PER value was obtained in the treatment  $T_3$  (1.137) and the least in  $T_5$  (0.745). The average PER values obtained in various treatments  $T_1$ ,  $T_2$ ,  $T_3$ ,  $T_4$ ,  $T_5$ ,  $T_6$  and

**Table 11. Food conversion ratio of *M. rosenbergii* fed on feeds containing various combined and single animal protein sources.**

Treatment	Replication	Av. initial wt. (mg)	Av. final wt.(mg)	Av. live wt. gain(mg)	Av. wt. of feed Consumed (mg)	FCR	Mean $\pm$ S D
T <sub>1</sub>	1	76.52	278.58	202.06	673.23	3.33	2.92
	2	77.13	334.77	257.64	687.34	2.67	$\pm$ 0.292
	3	76.38	318.89	242.51	668.51	2.76	
T <sub>2</sub>	1	76.00	256.66	180.66	643.23	3.56	3.44
	2	78.32	248.57	170.25	631.32	3.71	$\pm$ 0.278
	3	75.93	291.66	215.73	659.18	3.06	
T <sub>3</sub>	1	76.20	328.06	251.86	663.29	2.63	2.52
	2	78.23	336.64	258.41	671.30	2.60	$\pm$ 0.135
	3	76.86	362.50	285.64	665.54	2.33	
T <sub>4</sub>	1	76.58	326.28	249.70	639.23	2.56	2.73
	2	79.23	303.46	224.23	663.21	2.96	$\pm$ 0.168
	3	77.34	312.70	235.36	631.00	2.68	
T <sub>5</sub>	1	76.60	259.35	182.75	621.11	3.40	3.87
	2	75.93	204.29	128.36	561.00	4.37	$\pm$ 0.397
	3	78.38	235.71	157.33	602.23	3.83	
T <sub>6</sub>	1	75.86	264.22	188.36	631.12	3.35	3.54
	2	78.31	265.71	187.40	643.25	3.43	$\pm$ 0.215
	3	76.83	247.57	170.74	656.00	3.84	
T <sub>7</sub>	1	78.23	287.50	209.27	643.72	3.08	3.62
	2	77.21	242.11	164.90	628.61	3.81	$\pm$ 0.333
	3	78.16	237.69	159.53	632.23	3.96	

**Table 12. Result of analysis of variance of the data on food conversion ratios of *M.rosenbergii* juveniles fed on feeds containing combined and single animal protein sources.**

Source	S.S	D.F	M.S.S	F
Diets	4.6296	6	0.7716	6.427*
Error	1.6807	14	0.12005	
Total	6.3103	20		

\* Significant at 5% level

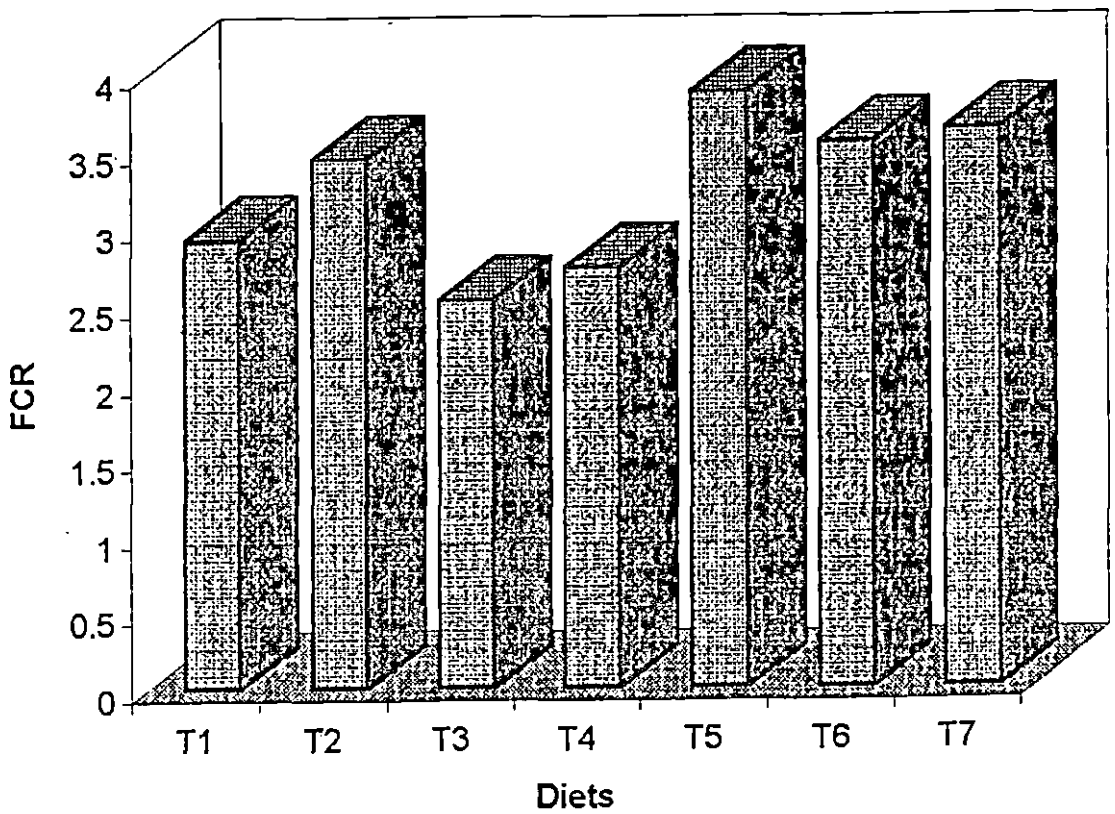
**Comparison of treatment means based on critical difference:**

critical difference = 0.6068

Treatments	T <sub>3</sub>	T <sub>4</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>5</sub>
Mean	<u>2.52</u>	<u>2.73</u>	<u>2.92</u>	<u>3.44</u>	<u>3.54</u>	<u>3.62</u>	<u>3.87</u>

Underscored means are not significantly different

**Fig. 5 Food conversion ratio of *M.rosenbergii* juveniles fed on diets containing various combined and single animal protein sources.**





**Table 13. Protein efficiency ratio of *M. rosenbergii* fed on feeds containing various combined and single animal protein sources.**

Treatment	Replication	Av.initial wt.(mg)	Av.final wt.(mg)	Av.live wt. gain(mg)	Av. wt. of protein consumed (mg)	Protein efficiency ratio	Mean $\pm$ SD
T <sub>1</sub>	1	76.52	278.58	202.06	235.63	0.858	0.988 $\pm$ 0.093
	2	77.13	334.77	257.64	240.57	1.071	
	3	76.38	318.89	242.51	233.98	1.036	
T <sub>2</sub>	1	76.00	256.66	180.66	225.13	0.802	0.836 $\pm$ 0.071
	2	78.32	248.57	170.25	220.96	0.771	
	3	75.93	291.66	215.73	230.71	0.935	
T <sub>3</sub>	1	76.20	328.06	251.86	232.15	1.085	1.137 $\pm$ 0.063
	2	78.23	336.64	258.41	234.96	1.100	
	3	76.86	362.50	285.64	232.94	1.226	
T <sub>4</sub>	1	76.58	326.28	249.70	223.73	1.116	1.049 $\pm$ 0.624
	2	79.23	303.46	224.23	232.12	0.966	
	3	77.34	312.70	235.36	220.85	1.066	
T <sub>5</sub>	1	76.60	259.35	182.75	217.39	0.841	0.747 $\pm$ 0.076
	2	75.93	204.29	128.36	196.35	0.654	
	3	78.38	235.71	157.33	210.78	0.746	
T <sub>6</sub>	1	75.86	264.22	188.36	220.89	0.853	0.81 $\pm$ 0.047
	2	78.31	265.71	187.40	225.14	0.832	
	3	76.83	247.57	170.74	229.60	0.744	
T <sub>7</sub>	1	78.23	287.50	209.27	225.30	0.929	0.80 $\pm$ 0.092
	2	77.21	242.11	164.90	220.01	0.750	
	3	78.16	237.69	159.53	221.28	0.721	

**Table 14. Result of analysis of variance of the data on protein efficiency ratio of *M. rosenbergii* fed on feeds containing combined and single animal protein sources.**

Source	S.S	D.F	M.S.S	F
Diets	0.39387	6	0.06565	8.027*
Error	0.11449	14	0.00818	
Total	0.50836	20		

\* Significant at 5% level

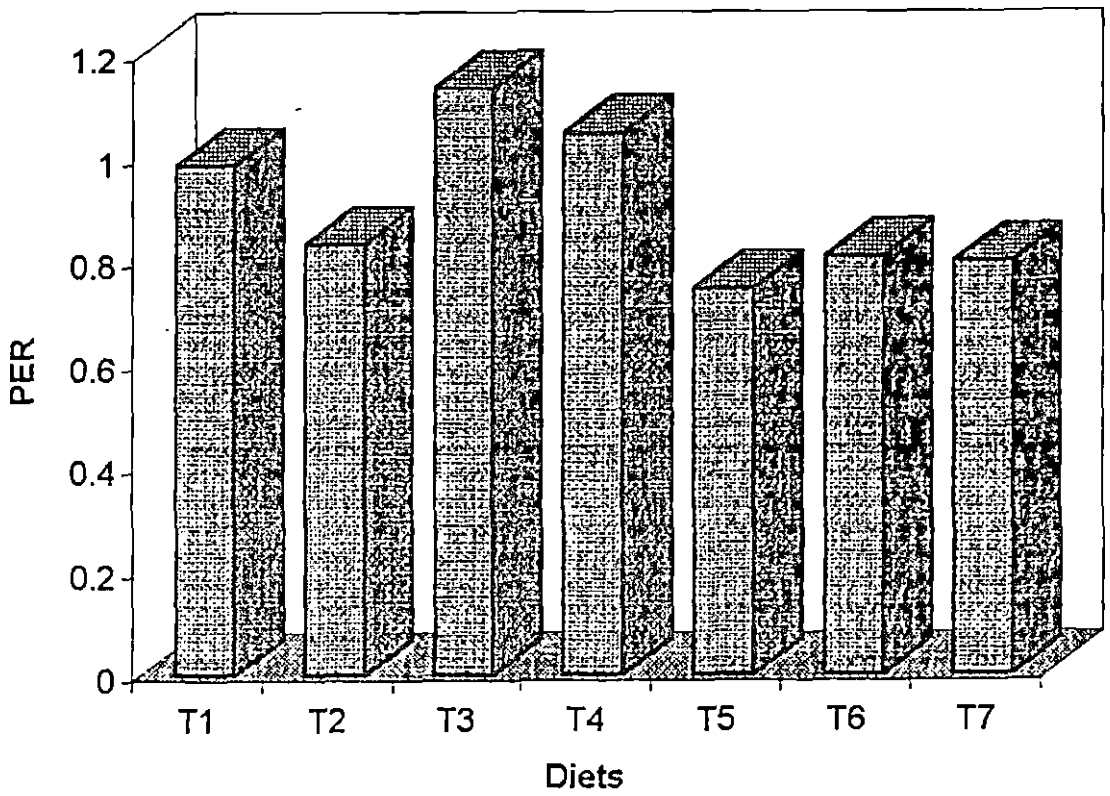
**Comparison of treatment means based on critical difference :**

critical difference = 0.1583

Treatments	T <sub>3</sub>	T <sub>4</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>5</sub>
Mean	<u>1.137</u>	<u>1.049</u>	<u>0.988</u>	<u>0.836</u>	<u>0.81</u>	<u>0.8</u>	<u>0.747</u>

Underscored means are not significantly different

**Fig. 6 Protein efficiency ratio of *M. rosenbergii* juveniles fed on diets containing various combined and single animal protein sources.**



T<sub>7</sub> were 0.988, 0.836, 1.137, 1.049, 0.745, 0.81 and 0.80 respectively. There was no significant difference between treatments T<sub>3</sub>, T<sub>4</sub> and T<sub>1</sub>. The treatments T<sub>2</sub>, T<sub>6</sub>, T<sub>7</sub> and T<sub>5</sub> were also not significantly different. However, the treatments T<sub>3</sub>, T<sub>4</sub> and T<sub>1</sub> were significantly different from the treatments T<sub>2</sub>, T<sub>6</sub>, T<sub>7</sub> and T<sub>5</sub>. The PER values obtained in various treatments are graphically represented in Fig-6.

#### 4.2.6. Productive protein value

The data on productive protein values of juvenile prawns from different treatments are presented in Table 15. Analysis of variance shows significant difference ( $P < 0.05$ ) between various treatments (Table 16).

The productive protein values of juvenile prawns obtained in the treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub> were 16.086, 13.333, 18.807, 17.271, 11.929, 14.293 and 12.934 respectively. The highest PPV was obtained in the treatment T<sub>3</sub> (18.807) and the lowest in the treatment T<sub>5</sub> (11.929).

The prawns in treatments T<sub>3</sub>, T<sub>4</sub> and T<sub>1</sub> showed no significant difference in their PPV values, while the PPV values of T<sub>3</sub> and T<sub>4</sub> were significantly different from that of treatment T<sub>2</sub>. Among the treatments T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub>, highest PPV was observed in T<sub>6</sub> and lowest in T<sub>5</sub>, but all the three treatments were not significantly different. Treatments T<sub>3</sub> and T<sub>4</sub> showed a significant difference from treatments T<sub>6</sub>, T<sub>2</sub>, T<sub>7</sub> and T<sub>5</sub>, while the treatment T<sub>1</sub> was not found to be significantly different from T<sub>6</sub> and T<sub>2</sub>. The productive protein values observed in various treatments are represented graphically in Fig-7.

**Table 15. Productive protein values of *M. rosenbergii* fed on feeds containing various combined and single animal protein sources.**

Treatment	Replication	Av. initial wt.(mg)	Initial body protein (mg)	Av. final wt.(mg)	Final body protein (mg)	Gain in protein (mg)	Av.protein consumed (mg)	PPV	Mean $\pm$ SD
T <sub>1</sub>	1	76.52	11.16	278.58	44.18	33.02	235.63	14.013	16.086 $\pm$ 1.482
	2	77.13	11.25	334.77	53.09	41.84	240.57	17.392	
	3	76.38	11.14	318.89	50.57	39.43	233.98	16.852	
T <sub>2</sub>	1	76.00	11.08	256.66	40.39	29.31	225.13	13.019	13.333 $\pm$ 0.810
	2	78.32	11.42	248.57	39.12	27.70	220.96	12.536	
	3	75.93	11.07	291.66	45.90	34.83	230.71	14.445	
T <sub>3</sub>	1	76.20	11.11	328.06	52.82	41.71	232.15	17.967	18.807 $\pm$ 1.019
	2	78.23	11.41	336.64	54.20	42.79	234.96	18.212	
	3	76.86	11.21	362.50	58.36	47.15	232.94	20.241	
T <sub>4</sub>	1	76.58	11.17	326.28	52.19	41.02	223.73	18.335	17.271 $\pm$ 0.998
	2	79.23	11.55	303.46	48.54	36.99	232.12	15.936	
	3	77.34	11.28	312.70	50.02	38.74	220.85	17.541	
T <sub>5</sub>	1	76.60	11.17	259.35	40.30	29.13	217.39	13.30	11.929 $\pm$ 1.130
	2	75.93	11.07	204.29	31.75	20.68	196.35	10.532	
	3	78.38	11.43	235.71	36.63	25.20	210.78	11.756	
T <sub>6</sub>	1	75.86	11.06	264.22	41.52	30.46	220.89	13.790	14.293 $\pm$ 2.056
	2	78.31	11.42	265.71	41.75	38.33	225.14	17.025	
	3	76.83	11.20	247.57	38.90	27.70	229.60	12.064	
T <sub>7</sub>	1	78.23	11.41	287.50	45.11	33.70	225.30	14.958	12.934 $\pm$ 1.442
	2	77.21	11.26	242.11	37.98	26.72	220.01	12.145	
	3	78.16	11.40	237.69	37.29	25.89	221.28	11.700	

**Table 16. Result of analysis of variance of the data on the productive protein values of *M. rosenbergii* fed on feeds containing combined and single animal protein sources.**

Source	S.S	D.F	M.S.S	F
Diets	113.345125	6	18.8909	7.0672*
Error	37.422376	14	2.67303	
Total	150.767501	20		

\* Significant at 5% level.

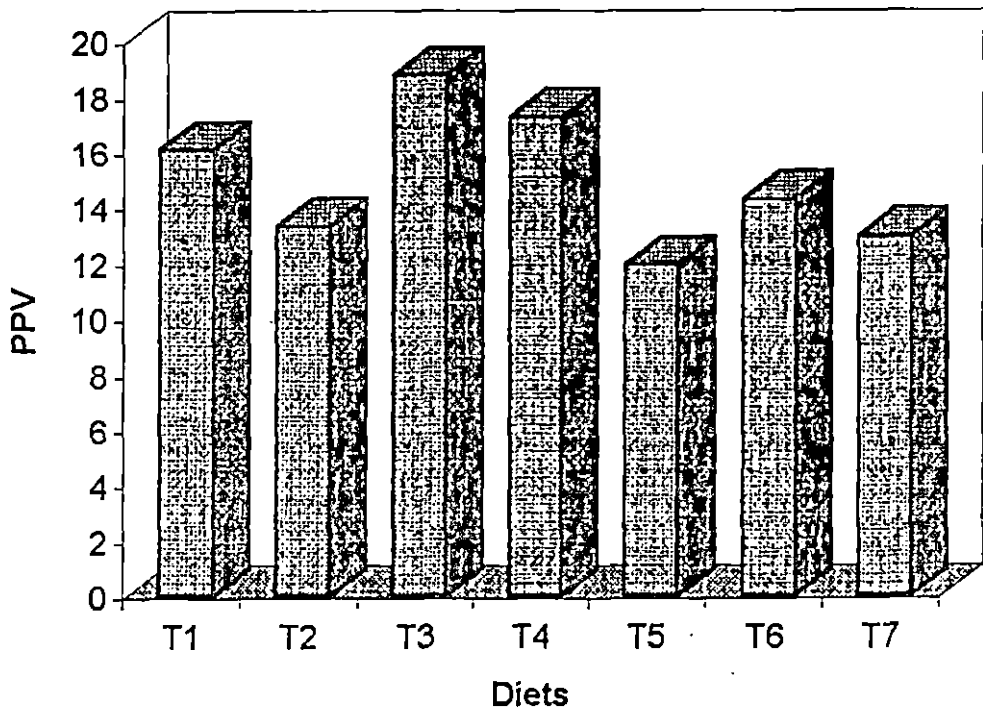
**Comparison of treatment means based on critical difference :**

critical difference  $\approx$  2.863

Treatments	T <sub>3</sub>	T <sub>4</sub>	T <sub>1</sub>	T <sub>6</sub>	T <sub>2</sub>	T <sub>7</sub>	T <sub>5</sub>
Mean	18.807	17.271	<u>16.086</u>	<u>14.293</u>	13.333	12.934	11.929

Underscored means are not significantly different

**Fig. 7 Productive protein value of *M.rosenbergii* juveniles fed on diets containing various combined and single animal protein sources.**



### **4.3. Water quality parameters**

#### **4.3.1. Water temperature**

The range of water temperature in the experimental tanks during the study period are given in Table 17. Minimum temperature recorded was 27<sup>o</sup>c and maximum temperature was 30<sup>o</sup>c. Weekly mean temperature values ranged from 27.4 to 29.58<sup>o</sup>c.

#### **4.3.2. pH**

Range of pH in the experimental tanks are given in the Table 18. Minimum and maximum pH values observed during the study period were 7.0 and 8.5 respectively. Weekly mean pH values ranged from 7.71 to 8.09.

#### **4.3.3. Dissolved oxygen**

Range of dissolved oxygen (D.O) in the experimental tanks are given in Table 19. A minimum of 6.2 ppm , and a maximum DO content of 8.39 ppm were obtained during the study period. Mean values ranged from 6.67 to 7.8 ppm.



**Table 17. Water temperature in the experimental tanks during the study period.**

Temp-erature	WEEKS								
	1	2	3	4	5	6	7	8	9
Mean	27.63	29.09	27.84	28.40	27.90	27.4	27.56	29.58	27.67
± SE	0.41	0.61	0.48	0.47	0.41	0.45	0.53	0.94	0.55
Range	27.2- 28.2	28.3- 30.0	27.3- 28.3	27.8- 29.2	27.2- 28.3	27.1- 28.3	27.0- 28.3	29.0- 30.0	27.0- 28.5

**Table 18. pH of water in the experimental tanks during the study period.**

pH	WEEKS								
	1	2	3	4	5	6	7	8	9
Mean	7.80	7.71	8.05	8.07	8.00	7.83	7.65	8.09	7.87
± S.E.	0.43	0.26	0.52	0.29	0.23	0.45	0.32	0.31	0.41
Range	7.0- 8.3	7.5- 8.1	7.5- 8.5	7.5- 8.3	7.8- 8.3	7.1- 8.5	7.2- 8.3	7.5- 8.3	7.2- 8.2

**Table 19. Dissolved oxygen in the experimental tanks during the study period.**

D.O	WEEKS								
	1	2	3	4	5	6	7	8	9
Mean	7.8	7.3	7.8	6.67	6.84	7.59	7.46	7.53	6.82
± S.E.	0.33	0.31	0.24	0.29	0.73	0.52	0.68	0.38	0.38
Range	7.5- 7.8	7.1- 7.8	7.5- 8.1	6.3- 7.01	6.20- 8.24	7.08- 8.28	6.62- 8.39	7.00- 8.16	6.34- 7.25

## **DISCUSSION**

## 5. DISCUSSION

### 5.1. Proximate analysis of formulated feeds

Proximate composition of experimental diets was analysed to evaluate the nutrient status. Most of the previous workers have reported that 30-35% protein may be optimum for the growth and survival of *M. rosenbergii* juveniles. According to Balazs and Ross (1976) a protein level above 35% may be required for maximum growth in *M. rosenbergii* juveniles. But Clifford and Brick (1979) reported that a protein level of 35% is optimum for best growth and survival of *M. rosenbergii*. In 1983, Sick and Millikin estimated the protein requirement of early juvenile *M. rosenbergii* to be around 40 % and of larger prawns to be 25-30%. In studies using purified crab protein D'Abramo and Reed (1988) reported that 33-35% is the optimum dietary protein level for *M. rosenbergii* while 30% was found to be optimum by Freuchtenichet *et al.* (1988). Proximate analysis of the diets in the present study revealed that they contained crude protein in the range of 34.56-35.06% which is the optimum value suggested by various investigators.

Carbohydrate was in the range of 36.20-41.31% in the test diets. Shrimps and prawns appear to utilize complex carbohydrates more efficiently than simple ones (New, 1976). Fair *et al.* (1980) reported that dietary fibre levels upto 30% do not appear to suppress growth in *M. rosenbergii*. Briggs (1991) successfully employed a series of carbohydrate sources at 40% inclusion level in semipurified diets for post-larvae of *M. rosenbergii*.

Hilton *et al.* (1984) have indicated that the total lipid content in the diet should not exceed 10% for *M. rosenbergii*. In commercial feeds of *M. rosenbergii*, a lipid level of 6-9% has been reported in Thailand (ASEAN/ UNDP/ FAO, 1988), 5-8% in French Guiana

(IFREMER, 1989) and 2-4% in Taiwan (Hsieh *et al.*, 1989). The lipid content of the diets used in the present study was in the range of 7.69-8.22% which is almost the same as employed in various commercial grow out feeds of *M. rosenbergii*.

## **5.2. Evaluation of efficiency of protein sources**

The present study was carried out to evaluate the nutritional quality of combined animal protein sources over single animal protein sources in formulated feeds for *M. rosenbergii* juveniles based on growth, specific growth rate, survival, food conversion ratio, protein efficiency ratio and productive protein value.

### **5.2.1. Growth**

Among the different formulated feeds tested in the present study, combined animal protein sources gave superior growth performance in comparison to the sources tested individually. Among the combined protein sources, the diet (T<sub>3</sub>) with crab plus clam meat produced highest growth rate of 265.30 mg, closely followed by T<sub>4</sub> with shrimp plus clam meat and T<sub>1</sub> with clam plus crab plus shrimp meat. Growth rate obtained with crustacean meat diets namely shrimp plus crab meat (T<sub>2</sub>) was seen to be next best followed by two diets based on shrimp meat (T<sub>6</sub>) and crab meat (T<sub>7</sub>) as single protein sources. Lowest growth rate was recorded for the diet based on clam meat (T<sub>5</sub>) alone, though not significantly different from the latter three diets.

Early works on shrimps and prawns by Deshirmaru and Shigueno (1972), New (1976) and Conklin *et al.* (1977) have shown that a mixture of two or more protein sources invariably give better growth than single protein source. The improved performance of mixed diet is mainly because of the fact that single protein source may not be able to provide all

the essential amino acids in adequate levels and the deficiency may be overcome by mixing a number of protein sources so as to formulate a diet which closely meets the amino acid requirement of the test species. In the present study also, all the diets based on combined protein sources gave superior growth performance in *M. rosenbergii* juveniles in comparison to the diets based on either shrimp meat, crab meat or clam meat alone. Balazs *et al.* (1973), in their initial trials with *M. rosenbergii* reported that a diet based on fish-soybean-shrimp gave superior growth performance compared to those based on either soybean alone or a combination of fish-soybean. Similarly Balazs *et al.* (1974) obtained better growth rate in *M. rosenbergii* with a diet based on tuna meal and soybean meal compared to the diets with either of these protein sources alone. New (1976) also reported better growth performance in *M. rosenbergii* using a mixture of two or more protein sources than when used individually. Millikin *et al.* (1980) have shown that a diet based on menhaden meal and soy protein in 1.65 : 1 ratio gave the best growth rate for *M. rosenbergii*. Ravishankar and Keshavanath (1988) found that the diet containing silkworm pupae plus shrimp waste as major protein sources produced better growth rate in *M. rosenbergii* juveniles followed by silkworm pupae plus clam meat diet; while feeds containing fish meal or silkworm pupae alone gave poor growth rates. Raje and Joshi (1992) obtained faster growth rate (as observed by early moulting) in *M. rosenbergii* larvae by using prepared feeds based on combined animal protein sources such as crab meat + fish liver, followed by clam meat + fish meat and clam meat + fish liver diets over feeds based on either of these protein sources when used singly. Behanan *et al.* (1992) found almost equal growth rates (0.1743 mg) in *M. rosenbergii* post larvae with a diet at 33% protein level containing catfish meat, prawn head, gluten and clam meat, to the diet based on clam meat alone at 44% protein level (0.1752 mg).

Among the combined protein sources tested in the present study, diets T<sub>3</sub>, T<sub>4</sub> and T<sub>1</sub> based on both crustacean and molluscan meats gave superior growth rates of 265.30 mg, 236.43 mg and 234.07 mg respectively over the diet T<sub>2</sub> (188.83 mg) based on combination of crustacean meats alone. Although crab plus clam meat based diet (T<sub>3</sub>) gave highest growth rate, it was closely followed by shrimp plus clam meat diet (T<sub>4</sub>), as well as clam plus crab plus shrimp meat based diet (T<sub>1</sub>). The relatively lower growth rate produced by shrimp + crab meat based diet (T<sub>2</sub>) might be due to the incorporation of both crustacean meats alone which may not have provided all essential amino acids in adequate quantities required by prawns in comparison to the diets based on combination of either of the crustacean meats with clam meat or combination of the both crustacean meats with clam meat.

Crustacean meals have been tested by many previous workers as protein source in prawn feeds anticipating better results due to similarity of their amino acid profile with the body protein of prawns. Shrimp meal is high in crude protein content and several amino acids (Forster, 1976) and appears promising for compounding prawn feed (Zimmermann *et al.*, 1991; Jayalakshmy and Natarajan, 1994; Das *et al.*, 1995; Reena and Qureshi, 1996). Balazs *et al.* (1973) reported better growth in *M. rosenbergii* when shrimp meal was incorporated in fish meal-soybean meal based diet. Shrimp meal based diet gave best growth rate in *M. rosenbergii* post-larvae in comparison to fish meal or meat meal based diets according to Zimmermann *et al.* (1991). Jayalakshmy and Natarajan (1994) reported that prawn waste based diet gave highest biomass production in *Macrobrachium idella* than fish meal or clam meal based diets. Das *et al.* (1995) observed the highest growth performance in *M. malcolmsonii* with prawn meal based diet in comparison to mussel

meat, fish meal and silkworm pupae based diets. Reena and Qureshi (1996) also reported superior performance of prawn meal based diet in terms of growth in *Macrobrachium dayanum*. However, Richards (1990) has attributed the high fibre and chitin content of the shrimp meal as a major disadvantage in shrimp meal based diet.

Crab protein has also been found to be a good source of protein for the growth and survival of crustaceans. Bogen *et al.* (1982) reported that the feed formulations using protein concentrate from rock crab resulted in superior growth rate and survival in juvenile lobster, *Homarus americanus*, in comparison to several other marine animal protein sources. Reed and D' Abramo (1989) reported that crab protein based diet produced better growth performance in *M. rosenbergii* than casein based diet. However, Koshio *et al.* (1992) observed superior performance of soybean protein over crab protein for *M. rosenbergii*.

Various molluscan meals are also reported to be excellent protein sources for prawn feeds and available literature suggests that clam is a rich source of basic amino acids especially arginine. Sandifer and Joseph (1976) reported that apart from the quality of protein, clam meal is also rich in polyunsaturated fatty acids (PUFA), especially rich in n-3 PUFA which is reported to have a growth enhancing effect on *M. rosenbergii*. Sherief (1989) obtained better growth performance in *M. rosenbergii* juveniles with clam meat based diet than fish meal based diet. Anilkumar (1994) also found that clam meat based diet gave superior growth rate in *M. rosenbergii* juveniles in comparison to the diet based on shrimp head meal. However, according to Jayalakshmy and Natarajan (1994) the performance of clam meat based diet was inferior to shrimp waste and fish meal based diets in *M. idella*. Das *et al.* (1995) reported that mussel meat based diet gave good growth rate in *M. malcolmsonii* but only second to the diet based on prawn meal.

In the present study, superior growth performance of *M. rosenbergii* to shrimp meat (T<sub>6</sub>), followed by crab meat (T<sub>7</sub>) based diets was observed, which may be due to the removal of exoskeleton of shrimp used in the diet, which if present in excess quantities in diets, would have depressed the growth rate of prawns. Even though, clam meat based diet (T<sub>5</sub>) produced lower growth rate, it does not vary significantly from the two former diets based on shrimp meat and crab meat.

### 5.2.2. Specific growth rate

Specific growth rate can be considered as an index of growth in the evaluation of diets since it is a more refined and improved growth index than absolute weight gain or percentage growth rate (Hepher, 1988). The results of the present study indicate the highest SGR with the diet T<sub>3</sub> containing crab meat plus clam meat as major protein sources, closely followed by the diets T<sub>1</sub> with clam + crab + shrimp meat and T<sub>4</sub> with shrimp meat + clam meat, though statistically there was no significant difference ( $P < 0.05$ ) between these three diets. However, the diet T<sub>2</sub> with crab meat + shrimp meat produced significantly lower SGR than the former three diets. All the diets with combined animal protein sources produced superior SGR values than the diets based on either shrimp meat (T<sub>6</sub>), crab meat (T<sub>7</sub>) or clam meat (T<sub>5</sub>) alone.

Revishankar and Keshavanath (1988) reported highest specific growth rate of 1.1923 for *M. rosenbergii* juveniles with pelleted feed containing silkworm pupae and shrimp waste followed by the diet with silkworm pupae and clam meat, though statistically there was no significant difference between these two diets. However, the diets based on either silkworm pupae or fishmeal alone gave significantly lower SGR values. Anilkumar (1994)



obtained a maximum SGR of 2.97 for clam meal based diet while shrimp head meal based diet gave a lower SGR of 2.37 for *M. rosenbergii* juveniles. Das *et al.*(1995) reported highest SGR of 1.64 in *M. malcolmsonii* juveniles with formulated feed based on shrimp meal as major protein source whereas the mussel meat based diet gave a SGR of 1.59, though the latter is not statistically different ( $P<0.05$ ) from the former. The diets based on fish meal and silkworm pupae produced poor specific growth rates. In the present study also, better SGR values were obtained for combined protein sources in comparison to single protein sources in formulated feeds. Among the single protein sources shrimp meat based diet ( $T_6$ ) gave highest SGR, followed by crab meat ( $T_7$ ) and clam meat ( $T_5$ ) based diets, though the latter two were not seen to be significantly different from diet  $T_6$ .

### 5.2.3. Survival

The juvenile prawns under all the seven treatments in the present study showed fairly good survival rates ranging from 86.67% to 96.67%, suggesting that the different protein sources tested either individually or in various combinations did not produce much variation on survival rates. New (1976), in his review on the nutritional studies of shrimps and prawns has opined that mortalities in nutritional studies are rare, unless the diet is grossly deficient in nutrients. Behanan *et al.* (1992) reported a survival of 76% in *M. rosenbergii* post-larvae with the diet based on cat fish meat, prawn head, gluten and clam meat based diet. Raje and Joshi (1992) obtained survival rates of 66% (clam meat + fish meat based diet) to 69% (crab meat + fish liver based diet) in *M. rosenbergii* larvae. Anilkumar (1994) reported a high survival rate (>90%) in *M. rosenbergii* juveniles fed on diets based on different animal protein sources, and according to him the higher survival rates were due

to better nutritional quality of the feeds. Survival ranging from 83.3 to 100% was obtained by Das *et al.* (1995) in *M. malcolmsonii* juveniles fed on diets with different protein sources viz., shrimp meal, fish meal, mussel meal and silkworm pupae. Although, variations in growth and feed efficiency were observed with test diets having different protein sources, the high survival rates observed in the present study, irrespective of the protein sources may be due to the fulfilment of dietary requirements, which might have helped to overcome the moulting stress in juvenile prawns.

#### **5.2.4. Food conversion ratio**

Food conversion ratio (FCR) is the ability with which an animal can convert food for the growth process and is reflected in the ratios of food consumed to the live weight it has gained. Thus higher efficiency in food utilization indicates lower food conversion ratios. In the present study, lower food conversion ratios were registered for diets with combined animal protein sources over single animal protein based diets. Among the combined protein sources, lowest FCR (2.52) was obtained for diet T<sub>3</sub> containing crab meat plus clam meat followed by the diets T<sub>4</sub> (2.73) with shrimp meat plus clam meat and T<sub>1</sub> (2.92) with shrimp plus crab plus clam meat, though T<sub>4</sub> and T<sub>1</sub> were not significantly different ( $P < 0.05$ ) from T<sub>3</sub>. However, the diet T<sub>2</sub> based on shrimp meat plus crab meat gave significantly higher FCR (3.44) than the former three diets. Among the single protein sources shrimp meat based diet (T<sub>6</sub>) gave better FCR (3.54) followed by crab meat diet (T<sub>7</sub>) with FCR of 3.62 and clam meat diet (T<sub>5</sub>) with FCR 3.87. Though clam meat based diet registered highest FCR, it was not seen to be significantly different from diets T<sub>7</sub> and T<sub>6</sub>. According to Colvin (1976), protein source that is deficient in essential nutrients in adequate quantities produces less efficient

feed conversion ratio. In the present study, the better food conversion ratios obtained for diets based on combined protein sources in comparison to single protein sources might be due to the fact that even at lower diet intake level, combined protein sources provided essential nutrients in required proportion for better growth of prawns.

Ravishankar and Keshavanath (1988) obtained a better absolute conversion rate of 6.84 in *M. rosenbergii* juveniles fed with pelleted diet based on silkworm pupae plus shrimp waste followed by silkworm pupae plus clam meat diet, though statistically there was no significant difference between these two pelleted diets. Behanan *et al.* (1992) reported a feed conversion ratio of 4.13 in *M. rosenbergii* post-larvae with compounded diet based on cat fish meat, shrimp head, gluten and clam meat, while the feed conversion ratio of 4.19 was registered for diet based on clam meat alone. Jayalakshmy and Natarajan (1994) reported a FCR of 2.8 with prawn waste based diet and 4.1 with clam meat based diet in juvenile *M. idella*. However, Anilkumar (1994) reported better feed conversion ratio of 2.69 with clam meal diet than shrimp head meal based diet (3.5) for *M. rosenbergii* juveniles. Das *et al.* (1995) found that diet based on prawn meal gave better FCR of 3.07 in *M. malcolmsonii* followed by mussel meal diet (3.2), though the latter was not seen to be significantly different from prawn meal diet. In the present study lowest FCR of 2.52 was registered with crab meat plus clam meat based diet (T<sub>3</sub>) and highest FCR of 3.87 with diet (T<sub>5</sub>) containing clam meat alone.

#### **5.2.5. Protein efficiency ratio**

Protein efficiency ratio (PER) is used to evaluate the quality of dietary protein, those with high PER can be considered as better quality and those with low values as poor qual-

ity. Behanan *et al.* (1992) reported a PER of 0.73 in *M. rosenbergii* post-larvae fed with clam meat based diet while the diet with catfish meat, shrimp head, gluten and clam meat gave lower PER of 0.555. However, from the point of highest survival and feed conversion efficiency the latter diet with combined protein sources was found to be ideal for feeding *M. rosenbergii* postlarvae. Anilkumar (1994) obtained higher PER of 1.07 with clam meat based diet and 0.817 for shrimp head meal based diet for *M. rosenbergii* juveniles. But a better PER of 0.9 was reported by Das (1995) with shrimp meal based diet closely followed by mussel meat based diet (0.83) in *M. malcolmsonii*, though there was no significant difference between these two diets.

In the present study it was found that, *M. rosenbergii* juveniles fed on diet containing crab meat plus clam meat ( $T_3$ ) was more efficient in converting dietary protein with PER of 1.137, followed by those fed on diets based on shrimp meat plus clam meat ( $T_4$ ) with PER of 1.049 and shrimp plus crab plus clam meat ( $T_5$ ) with PER of 0.988. However, there was no significant difference ( $P < 0.05$ ) in protein efficiency ratios between these three diets. The diets based on shrimp meat plus crab meat ( $T_2$ ) and single animal protein sources gave significantly lower PERs in comparison to the former three diets. Among the individual protein sources highest PER of 0.81 was obtained for shrimp meat based diet ( $T_6$ ), followed by diets with crab meat ( $T_7$ ) with PER of 0.8 and clam meat ( $T_8$ ) with PER of 0.745, though the latter two diets were not seen to be significantly different ( $P < 0.05$ ) from shrimp meat diet. The results of the present study indicate that the diets based on combined animal protein sources are more efficient in converting dietary protein for growth than those diets with single animal protein sources.

### 5.2.6. Productive protein value

Protein utilization can also be expressed in terms of productive protein value (PPV). Very high productive protein values ranging from 23.2 to 51 have been reported in case of different fish species. (Takeuchi *et al.*, 1978; Reinitz *et al.*, 1978; Steffens, 1981; Degani *et al.*, 1989). However studies on PPV of prawns and shrimps are scarce (James *et al.*, 1990; Boby and Susheela, 1996; Vinod and Susheela, 1998; Sreeja, 1998).

James *et al.* (1990) reported PPV of 6.67 for *Spirulina* based diet and 9.42 for casein based control diet for *M. rosenbergii* post-larvae. Boby and Susheela (1996) obtained a PPV of 13.48 in *M. rosenbergii* post-larvae when 10 ppm of the growth promoter, oxytetracycline was incorporated in a casein based purified diet; while the PPV of control diet was 8.99. Vinod and Susheela (1998) in their studies on the growth promoters in *P. monodon* juveniles reported a PPV value of 16.6 and 14.38 when glucosamine was incorporated in clam meal based diet at 0.25 and 0.5% respectively, which are significantly different from control diet with a PPV of 9.97. The results of the present study in *M. rosenbergii* indicate that the diets based on combined animal protein sources T<sub>3</sub> (crab meat plus clam meat) with a PPV of 18.807, T<sub>4</sub> (shrimp meat and clam meat) with a PPV of 17.271 and T<sub>1</sub> (shrimp plus crab plus clam meat) with a PPV of 16.086 were superior to diets containing single animal protein sources where the range of PPV was 14.293 to 11.929. The diet based on shrimp meat plus crab meat (T<sub>2</sub>) gave significantly lower PPV than the diets T<sub>3</sub>, T<sub>4</sub> and T<sub>1</sub>. Among the single protein sources, shrimp meat based diet (T<sub>6</sub>) produced higher PPV of 14.293 and it was seen to be significantly different from productive protein values of diets with crab meat (T<sub>7</sub>) and clam meat (T<sub>5</sub>) as major protein sources.

### 5.3. Water quality parameters

#### 5.3.1. Temperature

Water temperature is an important factor which influences the survival and growth of any organism. *M. rosenbergii* can survive in a wide range of temperature (18-33°C) without any deleterious effect, provided temperature fluctuations are not severe, sudden and of long duration (Farmanfarmaian and Moore, 1980). New (1990) also reported that *M. rosenbergii* adults can tolerate wide temperature range of 18-34°C, while for larvae the optimum range is 26-31°C. Temperature below 14°C or above 35°C are reported to be lethal for post-larvae, optimum being 29-31°C. The weekly range of temperature observed during the present experimental period was 27.4°C to 29.58°C. The values recorded are within the optimum range suggested for the growth of *M. rosenbergii* juveniles.

#### 5.3.2. pH

pH of water is another factor which has been reported to affect the growth of prawns. In the present study, pH of water was almost uniform in all experimental tanks and varied between 7.6 to 8.09. New and Singholka (1982) and Sandifer and Smith (1985) reported a pH range of 7.5 to 8.5 as optimum for culture of *Macrobrachium spp.* Malecha *et al.* (1980) and Sandifer and Smith (1985) observed that high pH values were not favourable for the growth of *M. rosenbergii*. Ideal pH for *M. rosenbergii* is reported to be 7-8.5 by Hsieh *et al.* (1989).

#### 5.3.3. Dissolved oxygen

Temperature of water and metabolic rate of prawn influence the physiological need for oxygen. New and Singholka (1982) reported that an oxygen concentration of 75% satu-

ration was optimum for the growth of *Macrobrachium spp.* Vasquez *et al.* (1989) found that the optimum level of dissolved oxygen in pond conditions for *Macrobrachium* culture is 6-8 ppm. During the present study, weekly dissolved oxygen values in the experimental tanks ranged from 6.67 to 7.8 ppm, since mild aeration was provided to the tanks. These values were found to be optimum for rearing of *Macrobrachium rosenbergii* juveniles.

## **SUMMARY**



## 6. SUMMARY

An experiment has been carried out to evaluate the efficiency of pelleted feeds formulated from different animal protein sources viz., clam meat, shrimp meat and crab meat in various combinations on the growth and survival of *Macrobrachium rosenbergii* juveniles. A comparison has also been made between the diets based on combined animal protein sources and those with single animal protein source.

1. Proximate analysis of various feed ingredients used in the formulation of test diets showed that shrimp meat contained highest crude protein content of 80.45%, followed by crab meat (55.63%) and clam meat (53.86%).

2. Seven isonitrogenous test diets, T<sub>1</sub> to T<sub>7</sub> with 35% crude protein were prepared for the study. They were diet T<sub>1</sub> with clam meat + shrimp meat + crab meat, diet T<sub>2</sub> with shrimp meat + crab meat, diet T<sub>3</sub> with clam meat + crab meat, diet T<sub>4</sub> with clam meat + shrimp meat, diet T<sub>5</sub> with clam meat, diet T<sub>6</sub> with shrimp meat and diet T<sub>7</sub> with crab meat. The other ingredients included were groundnut oil cake, tapioca powder, potato starch, cholesterol, sunflower oil and supplevite-M (a Vitamin-mineral mixture).

3. Proximate analysis of the formulated test diets showed that crude protein content of the diets ranged between 34.56% and 35.06%, crude fat content ranged between 7.69% and 8.22%, carbohydrate content between 36.20% and 41.31%, and the caloric content between 4.24 K. cal/ g and 4.43 K. cal/ g.

4. The various observations in the water quality parameters were found to be well within the tolerance limits for the optimum growth of *M. rosenbergii* juveniles.

5. At the end of 60 days of rearing, better growth rates were recorded in *M. rosenbergii* juveniles fed with diets based on combined animal protein sources than those with single animal protein sources.

6. Higher specific growth rates were recorded in prawn juveniles fed on diets T<sub>3</sub> (2.483), T<sub>1</sub> (2.328), T<sub>4</sub> (2.327) and T<sub>2</sub> (2.065) based on combined animal protein sources than the diets with single animal protein sources T<sub>6</sub> (2.022), T<sub>7</sub> (1.976) and T<sub>5</sub> (1.839).

7. The survival rate of *M. rosenbergii* juveniles was not found to be influenced substantially by the various protein sources tried and the survival rate recorded in various treatments ranged between 96.67% and 86.67%.

8. Low food conversion ratios were obtained in prawns fed on the diets with combined animal protein sources viz., T<sub>3</sub> (2.52), T<sub>4</sub> (2.73), T<sub>1</sub> (2.92) and T<sub>2</sub> (3.44) over the diets T<sub>6</sub> (3.54), T<sub>7</sub> (3.62) and T<sub>5</sub> (3.87) based on single animal protein sources.

9. High protein efficiency ratios were recorded with diets T<sub>3</sub> (1.137), T<sub>4</sub> (1.049), T<sub>1</sub> (0.988) and T<sub>2</sub> (0.836) based on combined animal protein sources than those diets T<sub>6</sub> (0.81), T<sub>7</sub> (0.80) and T<sub>5</sub> (0.747) with single animal protein sources.

10. High productive protein values were also obtained with the diets T<sub>3</sub> (18.807), T<sub>4</sub> (17.271), and T<sub>1</sub> (16.086) based on combined animal protein sources over the diets T<sub>6</sub> (14.293), T<sub>7</sub> (12.934) and T<sub>5</sub> (11.929) with single animal protein sources.

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**GROWTH RESPONSE OF GIANT FRESHWATER PRAWN *MACROBRACHIUM*  
*ROSENBERGII* (De Man) JUVENILES TO FEEDS BASED ON COMBINED  
ANIMAL PROTEIN SOURCES**

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**ABSTRACT OF A THESIS**

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## 8. ABSTRACT

The present study aimed to evaluate the efficiency of test diets formulated from different animal protein sources viz., clam meat, shrimp meat and crab meat in various combinations on the growth of giant freshwater prawn, *Macrobrachium rosenbergii* juveniles was done for a period of 60 days. A comparison has also been made between the diets based on combined protein sources over the diets with single protein sources.

Seven isonitrogenous test diets, T<sub>1</sub> to T<sub>7</sub> were prepared with 35% crude protein. They were diet T<sub>1</sub> with clam meat + shrimp meat + crab meat, diet T<sub>2</sub> with shrimp meat + crab meat, diet T<sub>3</sub> with clam meat + crab meat, diet T<sub>4</sub> with clam meat + shrimp meat, diet T<sub>5</sub> with clam meat, diet T<sub>6</sub> with shrimp meat and diet T<sub>7</sub> with crab meat. The other ingredients used were GOC, tapioca powder, potato starch, cholesterol, sunflower oil and supplevite-M (a vitamin-mineral mixture).

Results showed better growth rates in prawn juveniles fed with diets based on combined animal protein sources than those with single animal protein sources. Among the combined protein sources the diet T<sub>3</sub> recorded highest growth rate of 265.30 mg though it was not seen to be significantly different from T<sub>4</sub> and T<sub>1</sub>. Specific growth rate and protein efficiency ratio also showed better performance of prawn juveniles fed on combined animal protein sources. The highest SGR (2.483) and PER (1.137) were recorded in prawns fed on diet T<sub>3</sub> and lowest SGR (1.839) and PER (0.747) were obtained with diet T<sub>5</sub>. The survival rate of juvenile prawns was not found to be significantly influenced by the various test diets used and the survival ranged from 86.67% to 96.67%.

Productive protein value (PPV) was found to be highest in prawn juveniles fed on diet T<sub>3</sub> (18.807) and the lowest with diet T<sub>5</sub> (11.929). The PPV of prawns fed on diets T<sub>3</sub> and T<sub>4</sub> were found to be significantly higher than those fed on diets T<sub>2</sub>, T<sub>6</sub>, T<sub>7</sub> and T<sub>5</sub>.

