CHAPTER-I

PROTEIN REQUIREMENT
INTRODUCTION

The nutritional requirements of crustaceans have been widely studied and reviewed, from time to time, by a number of research workers (Kanasawa et al., 1970; Subrahmanyam and Oppenheimer, 1970; Cowey and Forster, 1971; Kitabayashi et al., 1971d; Deshimaru and Shigueno, 1972; Hysmith et al., 1972; Balazs et al., 1973; Forster and Beard, 1973; Shewbart et al., 1973; Sick and Andrews, 1973; Deshimaru and Kuroki, 1974a,b,c; 1975a,b; Regnault et al., 1975; Pennucci and Zein-Eldin, 1976; Forster, 1976; New, 1976; Wickins, 1976; Hanson and Goodwin, 1977; Deshimaru and Yone, 1978; Conklin, 1980; Maguire, 1980). These studies, in general, have shown that crustaceans have all the dietary nutrient requirements usually associated with complex metazoa (Dall and Moriarty, 1983). However, knowledge of essential nutrient requirements of many species of crustaceans still remains incomplete and many of the avenues remain unscanned.

Nutritional studies in crustaceans, as such, is complicated by a number of abiotic and biotic factors which have tremendous influence on growth and utilization of food. Abiotic factors including temperature, pH, salinity, dissolved oxygen, depth, light and many others have been found to directly affect the growth and food utilization of crustaceans (Subrahmanyam, 1962; Teal, 1971; Buikema, 1972; Venkataramiah
Amongst biotic factors, molting which forms an important event in the life cycle has considerable influence on growth and feed utilization of crustaceans. Wide variations in the biochemical constituents of the body occur during the different phases of the molting cycle and thus, the growth in these forms shows discontinuity. It has also been established that each molting in crustaceans results in considerable energy loss, about 7.3% of molt in *Macrobrachium rosenbergii* (Nelson *et al.* 1977b) and potentially an average rate of 0.81%/day is lost. In the case of juvenile prawns, it amounts to a large quantum of energy, since at this stage a prawn molts every 8 to 10 days (Stern, 1976) or even earlier. In *Metapenaeus dobsoni*, it has been reported that the mean molt weight forms about 7.09% of dry wt. of the whole prawn (Thomas *et al.* 1984). Thus a crustacean body must efficiently function so as to recoup the lost nutrients, besides synthesizing and mobilising nutrients essential for growth, before the onset of the next molt.

Carr *et al.* (1977) observed that the nitrogen retention would be maximal in the young animals and tends to be zero in the mature and non-producing animals. Correspondingly, food intake in younger stages is found to be high compared to the adult stages in prawns (Sick *et al.*, 1973; Colvin and Brand, 1977; Clifford and Bricks, 1978) and according to Balazs and Ross (1976) better food conversion efficiency by the young stages results in high food intake.
The intake and utilization of feed not only depends upon the 'physiological state' of the organism, but also depends upon its quality and quantity. Qualitatively, the nutrients composition of the feed ingredients, their cohesive ability on long storage, stability of nutrients in the feed when introduced in the water and the attractability of the feeds are some of the factors which influence the growth performance of the crustaceans (Meyers et al., 1972; Meyers and Zein-Eldin, 1972; New, 1976; Biddle, 1977; Hanson and Goodwin, 1977; Fernandes et al., 1981).

The quality of a feed, in general, is primarily based on the energy nutrients, namely proteins, lipids and carbohydrates and non-energy nutrients comprising of minerals, vitamins, growth factors and binders. Thus, for proper physiological functioning and tissue synthesis, these nutrients should be proportionately added in the diets.

Among the energy nutrients, protein is the most important one, as it forms the major growth nutrient in animal tissues. Protein molecule as such exists in different shapes and these shapes directly reflect on the functional status of the proteins. Globular proteins are relatively soluble and readily go into colloidal suspension, performs all the enzymatic reactions and, transports nutrients and growth promoting factors. On the other hand, fibrous proteins, primarily, form the structural units because of their non-colloidal property.
Besides these functions, proteins serve as a source of energy under acute shortage of other dietary energy components. Various authors, based on the dose-response (growth) curve, have determined the minimal dietary protein level giving maximal weight gain in different species of crustaceans (Provasoli and D'Agostino, 1969; Kanamasa et al., 1970; Andrews and Sick, 1972; Deshimaru and Kuroki, 1975a; Colvin, 1976; New, 1976; Maguire, 1980; Veronica and Lim, 1983). Table I shows the recommended optimum levels of protein for different species and size groups of prawns. The optimum protein requirement for maximum growth varies from 22 to 60% in various prawn species (Venkataramiah et al., 1975; Forster, 1976; New, 1976). These variations can be attributed to both intrinsic and extrinsic factors which effect the organisms.

Protein requirement considerably depends upon the physiological state of the animal. During early growth phase, prawns require more of protein, about 60% in the case of Crangon crangon of size group 19-21 mm (Regnault and Luquet, 1974). Regnault and Luquet (1974) reported that with every 3 mm increase in length there was a 10% fall in protein requirement as the stage increased, and by the adult stage the requirement of protein was as low as 25-30%. The higher requirement of protein in early stages was attributed to the faster growth rate up to late juvenile stage and thereafter the growth becomes more or less slower, though molting continues.
<table>
<thead>
<tr>
<th>Species</th>
<th>Stage</th>
<th>Optimal level suggested (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penaeus japonicus</td>
<td>post-larva</td>
<td>30.0</td>
<td>Khannappa (1978)</td>
</tr>
<tr>
<td></td>
<td>juvenile</td>
<td>45.0</td>
<td>Lee (1971)</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>40.0</td>
<td>Khannappa (1977)</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>35.0</td>
<td>Lin <em>al.</em> (1981)</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>40.0</td>
<td>Veronica and Lim (1983)</td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>&quot;</td>
<td>55.0</td>
<td>Bages and Sloane (1981)</td>
</tr>
<tr>
<td>Penaeus setiferus</td>
<td>28 to 32</td>
<td>28 to 32</td>
<td>Andrews <em>al.</em> (1972)</td>
</tr>
<tr>
<td>Penaeus azetecus</td>
<td>22 to 30</td>
<td>22 to 30</td>
<td>Shewbart <em>al.</em> (1973)</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>40.0</td>
<td>Venkataramiah <em>al.</em> (1975)</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>36.5</td>
<td>Fenucci and Zein-Eldin (1976)</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>51.5</td>
<td>Zein-Eldin and Corliss (1976)</td>
</tr>
<tr>
<td>Penaeus duorarum</td>
<td>28 to 30</td>
<td>28 to 30</td>
<td>Sick and Andrews (1973)</td>
</tr>
<tr>
<td></td>
<td>28 to 30</td>
<td>28 to 30</td>
<td>Sick and Andrews (1973)</td>
</tr>
<tr>
<td></td>
<td>51.5</td>
<td>51.5</td>
<td>Zein-Eldin and Corliss (1976)</td>
</tr>
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Contd....
<table>
<thead>
<tr>
<th>Species</th>
<th>Stage</th>
<th>Optimal level suggested(%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrobrachium</td>
<td>Juvenile</td>
<td>35.0</td>
<td>Balass and Ross (1976)</td>
</tr>
<tr>
<td>roseboritii</td>
<td>&quot;</td>
<td>15-20</td>
<td>Sick (1976)</td>
</tr>
<tr>
<td>Penaeus indicus</td>
<td>Post-larva</td>
<td>40.0</td>
<td>Bhasker and Ali (1984)</td>
</tr>
<tr>
<td>&quot;</td>
<td>Juvenile</td>
<td>43.0</td>
<td>Colvin (1976)</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>42.9</td>
<td>Ali (1982 a)</td>
</tr>
<tr>
<td>Penaeus mekduensis</td>
<td>Juvenile</td>
<td>43.45</td>
<td>Aquacop (1978)</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>34-42</td>
<td>Sedgwick (1979)</td>
</tr>
<tr>
<td>Penaeus stylirostris</td>
<td>Post-larva</td>
<td>50.0</td>
<td>Colvin and Brand (1977)</td>
</tr>
<tr>
<td>Penaeus californiensis</td>
<td>&quot;</td>
<td>30-35</td>
<td></td>
</tr>
<tr>
<td>Penaeus veranensi</td>
<td>&quot;</td>
<td>30-35</td>
<td></td>
</tr>
<tr>
<td>Metapenaeus macleavy</td>
<td></td>
<td>27</td>
<td>Maquire and Hume (1982)</td>
</tr>
</tbody>
</table>
Variations in protein requirement of different species of prawns have also been attributed to the biological value of protein sources, which depends upon the amino acids composition of the protein (Harper, 1981; Kies, 1981). However, some proteins are biologically unavailable for the animals due to alterations in the amino acids composition during processing by combining with other compounds, thereby become resistant to proteolytic enzymes (Cowey and Sargent, 1972).

Protein requirements are also influenced by the composition of other dietary energy components, namely, fats and carbohydrates. Protein-lipid ratio and protein-carbohydrate ratio in the diets also significantly influence the protein requirements of prawns (Andrews et al., 1972; Sick and Andrews, 1973, Abdel-Rahman et al., 1979; Teshima and Kanazawa, 1984). Likewise, protein requirement of crustaceans has been reported to be influenced by the amount of organic salts (Sparks, 1971; Deshimaru and Kuroki, 1974a; New, 1976; Maguire, 1980; Ponat and Adelung, 1980) and composition of vitamin mixture (Adelung and Ponat, 1977).

From the foregoing review, it is clear that prawn species show marked differences in their dietary protein requirements and that protein requirement of a species is significantly influenced by both intrinsic and extrinsic factors. The present study was taken up on juveniles of Indian white prawn *P. indicus*, since very few studies have been carried out regarding its protein requirement. Earlier studies
on the protein requirement of this species were by Colvin (1976) and Ali (1982a) in juveniles and by Bhaskar and Ali (1984) in post-larvae. The first two works were carried out using compounded diets and therefore may not truly highlight the protein requirement of the species because of the interference of factors other than proteins. Therefore, the present study was undertaken to determine the optimal requirement of protein for juvenile *P. indicus* using purified diets and thus minimizing the influence of interfering components on growth.

**MATERIAL AND METHODS**

Experiments were conducted in the laboratory to study the efficiency of different levels of dietary protein and to determine the optimum requirements of protein in the diets of juvenile *P. indicus*. Data on survival, growth, feed conversion, protein efficiency ratio, and body composition (moisture, ash, protein, carbohydrate, lipid, RNA, DNA, calcium, magnesium and phosphorus) and ammonia excretion rates were obtained from these experiments.

**Experimental Aquaria:**

Experiments were carried out using plastic tubs of diameter, 54 cm and height, 24 cm. Earlier studies by Bernhard and Zattera (1970) have shown no harmful effect on the animals so held. The tubs were arranged on vertical steel racks and
were randomly distributed. Each of the tubs was provided with two rectangular aerator stones of 3 x 15 mm size, connected to a set of aerators through a plastic tube. The flow of air was maintained uniformly throughout the experimental period. The tubs were covered with nylon screen to prevent the escape of animals. Aeration was suspended for 2 hours every morning, while cleaning the tubs.

Seawater (salinity: 32-35%) collected from the open sea off Cochin (depth 20-30 m), was transported to the laboratory in plastic jerry cans, filtered thrice using bolting silk (69 µ) and pooled into 500 l plastic pools. The salinity was adjusted to 20 ± 2.5% by diluting with tap water, since juvenile *P. indicus* prefer lower salinities (Colvin, 1976; Paul Raj, 1976; Paul Raj and Sanjewa Raj, 1980). This water was aerated for 3 to 4 days through a biological filter with sun dried sand and oyster shells. Daily, the water was irradiated for 2 hrs with UV rays using 125 % UV lamp as the bacterial load (Zobell and Feltham, 1938) was lowest in this treatment, when subjected to UV rays for different time periods, as shown in Table 2.

**Experimental Animals:**

Post-larvae of *P. indicus* belonging to the same brood-stock were obtained from Narakkal Prawn Culture Laboratory of
### TABLE 2: LOG BACTERIAL COUNT IN SEAWATER IRRADIATED AT DIFFERENT TIME PERIODS

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Mean Total Bacterial Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$1.95 \times 10^4$</td>
</tr>
<tr>
<td>15</td>
<td>$1.51 \times 10^4$</td>
</tr>
<tr>
<td>30</td>
<td>$1.75 \times 10^4$</td>
</tr>
<tr>
<td>45</td>
<td>$1.85 \times 10^4$</td>
</tr>
<tr>
<td>60</td>
<td>$1.50 \times 10^4$</td>
</tr>
<tr>
<td>90</td>
<td>$1.75 \times 10^4$</td>
</tr>
<tr>
<td>120</td>
<td>$1.25 \times 10^4$</td>
</tr>
<tr>
<td>180</td>
<td>$1.11 \times 10^4$</td>
</tr>
<tr>
<td>After 8 hrs of irradiation</td>
<td>$9.98 \times 10^4$</td>
</tr>
</tbody>
</table>
the Central Marine Fisheries Research Institute, Cochin and transported in polythene bags of 10 litre capacity, half-filled with fresh filtered sea water (salinity-25 ppt) and oxygen. These post-larvae were then introduced into 2 x 3 ft. perspex glass tanks, equally distributing about 50 to 60 animals per tank. The animals were then sorted out into different size groups, acclimated to laboratory conditions, and reared for 15-20 days with a compounded pellet diet to obtain the desired early juveniles (total length of about 20 mm) for experimentation.

Juvenile prawns of mean total length 20 ± 5 mm were used for the experiments. The total length of apparently healthy animals were measured to the nearest mm from the tip of the rostrum to the telson. The animals were then blotted dry carefully between the folds of filter paper (Bordner and Conklin, 1978), weighed on a Mettler electronic balance to the nearest mg, and were immediately transferred into the aquaria. The prawns were allowed to starve for 24 hrs to recover from handling stress prior to feeding. Before starting the experiment, about 15 prawns were measured, weighed and left for drying in an oven at 40°C for 48 hrs. The dried prawns were reweighed and the initial dry weight of the prawns were recorded.

Formulation and Preparation of Experimental Feeds:

Formulation and preparation of the feeds were done based on earlier nutritional studies carried out in crustaceans.
The ingredient composition of the formulated feeds, for determining optimum protein levels for juvenile prawns, is shown in Table 3. Casein has been widely used as a protein source for experimental studies in nutrition as it is the only protein source available in highly purified form (Halver, 1957; Kanazawa et al., 1971, 1976), though it is deficient in some of the amino acids (Halver, 1957; Ponat and Adelung, 1980). In the present study to determine the protein requirement of juvenile P. indicus, purified lipid-free casein was used as the major protein source. Gelatin and egg albumin were also used as protein sources as they supplement some of the deficient amino acids. Egg albumin in the feed is also reported to serve as a feed attractant for juvenile prawns (Clifford and Bricks, 1978). Gelatin, besides being a protein, serves as a binder for feeds (McLaren et al., 1947a).

The energy nutrients namely, proteins, lipids and carbohydrates were adjusted in the diets to obtain approximately a gross energy content of 4.2800 Kcals/g. The energy values for proteins, lipids and carbohydrates were calculated based on their gross calorific values of 5.65, 9.45 and 4.10 Kcals/g, respectively (Halver, 1957).
Test diets with graded levels of protein, ranging from 0 to 60%, were prepared by using casein as the principal protein source. The gross caloric content was adjusted to give approximately isocaloric diets using sucrose and starch as substitutes for protein. Carbohydrates were added both in the form of monosaccharides (glucose), disaccharides (sucrose) and complex polysaccharides (starch). Polysaccharides have been shown to be more efficiently utilized compared to simple sugars (Forster and Cabbott, 1971; Andrews et al., 1972; Sick and Andrews, 1973, Abdel-Rahman et al., 1979) and thus more quantity of starch was included in the diets.

Lipids were added in the form of corn oil (rich in linoleic acid) and cod liver oil (rich in polyunsaturated fatty acids of w3 series) to provide the w6 and w3 fatty acids which are essential for growth in prawns (Shewbart and Miss, 1973; Castell and Cowey, 1976; Colvin, 1976; Guary et al., 1976; Kanazawa et al., 1977a,b; Bottino et al., 1980). Since corn oil contains more of w6 fatty acids, which is detrimental to shrimps when in excess (Castell and Cowey, 1976), a mixture of corn oil and cod liver oil were used in the ratio of 1:2 and a lipid level of 9% was maintained in the diets based on earlier work (Hanson and Goodwin, 1977). Cholesterol was added in the diets, since crustaceans are incapable of sterol synthesis (Van Den Oord, 1964; Zandee, 1964; Whitney, 1970; Kanazawa et al., 1970; Deshimaru and Kuroki, 1974b), but
cholesterol is essential as a precursor for synthesis of steroid hormones, vitamin D and hypodermis pigmentation (New, 1976). Thus, in the diet 0.5% of cholesterol was added based on the recommendations of Kanazawa et al. (1970) for the prawn Penaeus japonicus and Castell et al. (1975) for the lobster Homarus americanus.

Though specific mineral requirements have not been worked out for shrimps, considerable importance has been laid for Ca:P ratio (0.76: 1 to 4:1) as reviewed by New (1976) and Maguire (1980). In the present study, about 7.4% of mineral mixture was added (Table 3) in the diet, based on earlier studies in crustaceans (Kanazawa et al., 1970, 1976, 1977a, b; Adelung and Ponat, 1977; Ponat and Adelung, 1980). Vitamins were added in the diet as non-energy dietary nutrients based on the amounts administered by various earlier workers (Kanazawa et al., 1971, 1977a; Adelung and Ponat, 1977; Watanabe et al., 1977b; Ponat and Adelung, 1980).

Table 3 shows the quantities of different fat and water-soluble vitamins used in the experimental diets. Agar, starch and gelatin served as binders.

Finely ground, preweighed ingredients - casein, egg albumin, glucose, sucrose, mineral mixture, cholesterol, additives and agar agar were mixed in a waring blender. Fat soluble vitamins (A, D, E and K) were added into the mixture of codliver oil and corn oil. All the water-soluble vitamins were thoroughly ground and mixed using a mortar and pestle.
### Table 3: COMPOSITION OF EXPERIMENTAL DIETS

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>EXPERIMENT - I</th>
<th>EXPERIMENT - II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Casein (lipid-free)</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>Egg albumin</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Gelatin</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Glucosamine-HCl</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Sucrose</td>
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<td>19.9</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.19</td>
<td>7.19</td>
</tr>
<tr>
<td>Starch</td>
<td>43.45</td>
<td>32.59</td>
</tr>
<tr>
<td>Codliver-Oil</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sodium succinate</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Sodium citrate</td>
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<td>0.3</td>
</tr>
<tr>
<td>Mineral mixture*</td>
<td>7.41</td>
<td>7.41</td>
</tr>
<tr>
<td>Agar-agar</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cellulose</td>
<td>4</td>
<td>4</td>
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</table>

** TOTAL **

|          | 100.5 | 106.42 | 100.10 | 100.80 | 100.18 | 100.73 | 100.48 | 100.26 | 100.39 | 100.12 | 100.18 | 100.25 | 100.07 | 100.36 |

### Proximate Composition %

<p>| | | | | | | | | | | | | | |</p>
<table>
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<tbody>
<tr>
<td>Crude Protein</td>
<td>0.0</td>
<td>9.0</td>
<td>19.1</td>
<td>28.9</td>
<td>39.5</td>
<td>48.9</td>
<td>59.4</td>
<td>31.9</td>
<td>34.6</td>
<td>36.2</td>
<td>39.5</td>
<td>41.3</td>
<td>44.8</td>
</tr>
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<td>Total Lipid</td>
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<td>12.2</td>
<td>12.2</td>
<td>13.1</td>
<td>12.6</td>
<td>11.8</td>
<td>11.6</td>
<td>13.2</td>
<td>13.4</td>
<td>13.1</td>
<td>13.8</td>
<td>12.6</td>
<td>12.1</td>
</tr>
<tr>
<td>Ash</td>
<td>18.7</td>
<td>19.1</td>
<td>19.6</td>
<td>19.0</td>
<td>20.1</td>
<td>20.2</td>
<td>19.6</td>
<td>18.1</td>
<td>18.6</td>
<td>18.1</td>
<td>18.5</td>
<td>19.2</td>
<td>18.2</td>
</tr>
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</table>

* CaHPO$_4$·2H$_2$O 2.5, MgSO$_4$·7H$_2$O 2.0, KH$_2$PO$_4$ 1.5, Na$_2$HPO$_4$·12H$_2$O, MnSO$_4$·H$_2$O 0.14, FeSO$_4$ 0.10, ZnSO$_4$·7H$_2$O 0.10,
  Cr$_3$O$_7$·2H$_2$O 0.05, Co(NO$_3$)$_3$ 0.01, CuSO$_4$·5H$_2$O 0.01.

** 8-Carotene 0.014, Calciferol 0.002, a-Tocopherol acetate 0.032, Menadione 0.032, Ascorbic acid 2.424, Thiamine hydrochloride 0.01, Riboflavin 0.006, Nicotinic acid 0.032, Pyridoxine hydrochloride 0.016 Calcium pantothenate 0.06, Folic acid 0.001, p-Aminobenzoic acid 0.014, Choline chloride 0.10, Inositol 0.30, Biotin 0.004, Cyanocobalamin 0.001.
Gelatin was dissolved in cold double distilled water (Halver, 1978; 1980) and boiled over a water bath, along with cellulose and starch. After gelatinization, corn oil and cod liver oil containing fat soluble vitamins were added and the heating was continued for another 10 mins. in the water-bath at slightly lower temperature (70°C) and mixed thoroughly. To this mixture, added the powdered protein-mineral-agar mixture, again mixed thoroughly adding slowly double distilled water till the required consistency of moist dough was obtained. The whole mixture was then steamed at 115 lbs pressure for 5 minutes. The steamed feed was allowed to cool to room temperature and the water soluble vitamin mixture was added and mixed thoroughly. The pH of the diet was adjusted to 6.8 (Kanazawa et al., 1977a) using 0.1 N NaOH and was stored in polyethylene bags in a freezer. The moisture content in the feed was adjusted to about 30%. Each time feed required for 15 days was prepared so as to maintain the quality of the feed. Each day before feeding, the required amount of feed was thawed to room temperature and manually made into small balls, weighed and fed to the prawns.

**Feeding Level and Schedule:**

The juvenile prawns were fed with the experimental diets at the rate of 10% (by dry weight) of the live body weight/day as suggested by Subramanyam and Oppenheimer (1970). The feeding was done twice a day, in the morning and in the evening.
The amount of feed given was adjusted every 15 days of the experiment based on changes in the body weight.

Collection of Faecal Matter and Left-over Food:

The left-over food and faecal matter were daily collected from the aquaria by slow siphoning of the water through a narrow plastic tube and collected at the other end on a bolting silk. The faecal matter and left-over food, collected separately, were washed in distilled water to remove the adhering salts, transferred to pre-weighed aluminium foils and kept for drying at 70°C for 48 hrs. The dried samples were weighed and the dry weights were recorded. The samples were stored in a desiccator for subsequent analysis.

After 15 days of experiment, the animals were weighed and the tanks were thoroughly washed with detergent, rinsed with tap water and reintroduced the animals in fresh, dilute sea water of 20 ppt salinity. The experiment was terminated on the 30th day and the length and weight of the animals were recorded. The dried samples were powdered in a porcelain mortar and pestle and biochemical composition studies were performed.

Monitoring of Physico-Chemical Parameters in Water:

Water temperature was recorded twice a day morning (about 8 a.m.) and evening (about 6 p.m.), using a graduated mercury thermometer with an accuracy of 0.01°C. The dissolved
oxygen content in water samples was determined employing the Winkler's method (Strickland and Parsons, 1972; Spotte, 1979).

Sea water samples for ammonia estimation were collected from the experimental tanks just before and after changing the water and fixed with 4% phenol solution immediately, stored in refrigerator and analysed within 2 hrs. of collection (Spotte, 1979). The ammonia concentration was determined using phenol-sodium hypochlorite method (Solorzano, 1969). Salinity of the water in the experimental tanks was determined thrice a week using argentometric method (Strickland and Parsons, 1972; Riley et al., 1975). Standard sea water was obtained from IAPSO, Institute of Oceanographic Sciences, Surrey, England (chlorinity, 19.37).

pH of the water samples from the experimental tanks was determined thrice a week using Elico pH meter with an accuracy of 0.01. All pH determinations were done at room temperatures. During the experimental study, the prawns were maintained at 12L:12D photoperiod cycle. The mean temperature, salinity, pH, dissolved oxygen and ammonia levels maintained during the experiments are shown in Table 4, which were well within the established tolerance limits of prawns (Wickins, 1973; Colvin, 1976; Delistraty et al., 1977).

Recording of Data:
Survival Rate:

Daily the population of prawns was recorded from each of the experimental treatments and the mean number of surviving
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment I Meanvalues</th>
<th>Experiment II Meanvalues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>27.71 ± 1.94</td>
<td>27.63 ± 2.27</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>20.9 ± 2.5</td>
<td>21.23 ± 2.59</td>
</tr>
<tr>
<td>pH</td>
<td>8.36 ± 1.011</td>
<td>8.02 ± 0.49</td>
</tr>
<tr>
<td>Ammonia concentration in the water (\text{NH}_4-N\ mg/l/d)</td>
<td>0.0352 ± 0.0096</td>
<td>0.0236 ± 0.0032</td>
</tr>
<tr>
<td>Initial length (mm)</td>
<td>16.95 ± 1.122</td>
<td>20.06 ± 0.738</td>
</tr>
<tr>
<td>Initial weight (mg)</td>
<td>30.3 ± 0.0145</td>
<td>44.95 ± 2.193</td>
</tr>
</tbody>
</table>
prawns per week was determined. The final percent survival was determined as follows.

\[
\text{Percentage Survival} = \frac{\text{Initial number} - \text{Final number}}{\text{Initial number}} \times 100
\]

**Growth Rate:**

At the end of the experiment, the total length and weight of prawns were measured adopting similar procedures as for initial measurement of these prawns. The prawns were later killed by brief immersion in boiling water (Clifford and Brick, 1983) and left for drying for 48 hrs at 40°C in an oven. The dried samples were weighed and the final dry weight of the prawns was recorded. The mean percent gain in length and weight were determined as follows:

\[
\text{Mean percent gain in length/weight} = \frac{\text{Mean final length/weight} - \text{Mean initial length/weight}}{\text{Mean initial length/weight}} \times 100
\]

**Specific Food Consumption, Food Conversion Ratio and Protein Efficiency Ratio:**

The food consumption per body weight (wet weight) per unit time (Bordner and Conklin, 1981) was calculated as follows:

\[
\text{Specific food consumption(\%)} = \frac{\text{Total initial dry weight of food fed} - \text{Total final dry weight of food}}{\text{Number of animals surviving at the end of the experiment} \times \text{Time in days of animal} \times \text{Mean animal weight}} \times 100
\]
Food conversion ratio (FCR) was determined as follows:

\[
\text{Food conversion ratio} = \frac{\text{Total dry weight of food fed}}{\text{Final wet weight of food fed} + \text{Wet weight of prawns} - \text{Initial wet weight of prawns}} - \frac{\text{Total dry weight of leftover food}}{\text{Initial wet weight of prawns}}
\]

Protein efficiency ratio was determined as follows:

\[
\text{Protein efficiency ratio} = \frac{\text{Final wet weight of prawns} - \text{Initial wet weight of prawns}}{\text{Total protein intake}}
\]

All these parameters are apparent, since no correction factor was introduced for the exuviae and dead prawns eaten by the cohabitators during the experimental study.

Chemical Composition of Feed and Carcass:

Moisture content in the feeds and prawns was determined gravimetrically by oven drying the samples at 100°C for feed samples and at 40°C for prawns, till concurrent dry weights were obtained. Percent moisture in the samples was calculated as follows:

\[
\text{Percent moisture} = \frac{\text{Wet weight of the sample} - \text{Dry weight of the sample}}{\text{Wet weight of the sample}} \times 100
\]

Weighed dried samples of feed and prawn were ashed in silica crucibles at 550°C for 6 hrs in a muffle furnace and the percent ash was determined as follows:

\[
\text{Percent ash} = \frac{\text{Weight of the ash}}{\text{Weight of the dried sample taken}} \times 100
\]
To determine the crude protein content, weighed samples of feed were digested in tubes with a catalyst mixture \( \text{K}_2\text{SO}_4; \text{CuSO}_4; \text{SeO}_2; \text{HCl} \) and concentrated sulphuric acid (Sp. gr. 1.84) for 3 hrs at 120°C. The total nitrogen content of the digested samples was determined using Kjeldahl method (AOAC, 1975). Crude protein content in the feeds was determined by using the conversion factor of 6.25 per unit of nitrogen.

To determine the protein content in prawns, known quantity of dry samples of prawns were homogenized in chloroform-methanol mixture and the supernatant was collected. To the residue added cold 15% TCA, homogenized and kept for 3 hrs in cold chamber (4°C) for complete extraction of carbohydrates. The samples was centrifuged at 1200 rpm for 10 mins and the supernatant was collected. Then washed the sample with cold 5% TCA, centrifuged and the collected supernatant was mixed with the first lot and kept for carbohydrate determination. The residue added 1N NaOH, homogenized and left overnight at 37°C for complete tissue protein dissolution. Tissue protein was determined using Biuret method (Gornali et al., 1949) and the optical density was recorded on ECIL-UV spectrophotometer at 530 nm. The protein content in the sample was determined from standard graph using Bovine serum as standard and the protein content was expressed on percent dry weight.

Lipid content in feeds was determined using Soxhlet extraction method (AOAC, 1970) using petroleumether (60-80°C) as solvent. Lipid extraction was carried out
for 16 hrs, and the total lipid in feed was determined
gravimetrically. Tissue lipid content was determined using
Bligh and Dyer (1959) method of chloroform-methanol-water
mixture, (2:2:1) modified by Ando et al., (1977). Weighed,
dried samples of whole prawn were homogenized for five
minutes with a mixture of chloroform-methanol(1:2). The
samples were then kept overnight at 4°C in dark for the
complete extraction of total lipids. The extracted lipids in
the chloroform-methanol mixture layer was centrifuged at 800
rpm for 10 mins in cold and the supernatant was collected.
To the residue added chloroform again, centrifuged for 5
minutes and the supernatant was collected. To the supernatant,
added double distilled water and the final solution had
chloroform:methanol:water in the ratio 2:2:1. The mixture
was thoroughly shaken and allowed to settle. Pipetted out the
layer of water:methanol and dried the chloroform-lipid layer
in a desiccator with concentrated sulphuric acid as desiccant
and the total lipid was estimated gravimetrically.

Tissue carbohydrate was determined from the TCA
supernatant extract using modified phenol-sulphuric acid method
(Dubois et al., 1956). The optical density (OD) was recorded
on ECIL-UV spectrophotometer at 490 nm.

Calcium in the whole prawn was determined by the
modified method of Clark and Collip (1925). To the weighed
dry samples of prawn about 4% ammonium oxalate was added,
mixed thoroughly and allowed it to stand for overnight. The
mixture was centrifuged at 1500 rpm and the supernatant was collected for magnesium determination. The precipitate was washed, centrifuged and washed three times with 2% ammonia solution. To the washed precipitate, added concentrated sulphuric acid (Sp. gr. 1.84) and mixed well. The tube was transferred to a boiling water bath for 1-2 minutes and titrated against 0.01N potassium permanganate to a definite pink color which persists for about a minute. The percent calcium determined in prawns was expressed in percent dry weight basis and was calculated as

\[
\text{Calcium (\%)} = \frac{(\text{Volume of } 0.01N - \text{Blank}) \times 0.2 \times 100}{\text{Weight of dried sample taken}}\]

where, 0.2 mg of calcium = 1 ml of 0.01N KMnO₄

The supernatant collected was used for determination of magnesium employing the modified method of Briggs (1922). Known volume of supernatant was mixed with 5% ammonium phosphate solution and concentrated NH₄OH. The mixture was left overnight for complete precipitation; centrifuged at 1500 rpm and the supernatant was discarded. The precipitate was washed first with 33% ammonium hydroxide solution, two to three times centrifuged and siphoned off the solution and finally washed with alcoholic ammonia solution and decanted the same. Ammonia was completely evaporated from the sample by placing the tube in hot air oven at 50-60°C for 1 hour. The precipitate was dissolved with molybdate solution and to this added aminonaphtholsulphonic acid, allowed to stand for 5 minutes, and the optical density (OD) was measured in a ECIL spectrophotometer.
at 680 nm. The magnesium content was expressed as percent dry matter and calculated as follows.

\[
\text{Magnesium (\%)} = \frac{\text{OD of unknown}}{\text{OD of standard}} \times \frac{0.03 \times 100}{\text{Weight of dried sample}}
\]

Total phosphorus was estimated by the method of Lowry et al. (1954) using phosphomolybdate and ascorbic acid. Weighed samples were added to the ashing mixture containing 70% HClO₄ and 20% H₂SO₄. Heated the mixture in an oven at 95°C for 2 hrs, followed by heating at 165°C for another 2 hrs. The mixture was cooled to room temperature and added the mixture of ammonium molybdate and ascorbic acid. Immediately mixed thoroughly and placed the tubes at 37°C for 2 hrs. After cooling, the optical density was recorded in a ECIL-UV spectrophotometer at 820 nm. The phosphorus was expressed on percent dry weight basis and calculated as follows.

\[
\text{Phosphorus (\%)} = \frac{\text{OD of sample}}{\text{OD of standard}} \times \frac{\text{Concentration of standard}}{\text{Weight of sample}} \times 10
\]

The weighed samples (10 mg dry weight) were individually homogenized in 4 ml ice-cold distilled water with a tissue grinder. The RNA was extracted by the Halliburton and Thompson (1965) method and measured at 260 nm on a SCIL-UV spectrophotometer. RNA obtained from Sigma Chemical Company was used to prepare the RNA standard curve. The DNA content of the sample was determined by the indole method (Ceriotti, 1952, 1955). Highly polymerized calf thymus DNA (Type I, Sigma Chemical Co.) was used to prepare the DNA standard curve. The detailed
Ammonia Excretion Rates:

Ammonia excretion rates in prawns were studied individually using 3 l conical flask containing 2.1 l of fresh diluted irradiated seawater (20 ppt). Each of the conical flasks was provided with an aerator stone (3 x 15 mm size) connected with a plastic tube to the aerator. An additional tube was provided with a stop pinch cork at one end, for collection of water sample. The whole apparatus was plugged with rubber cork and the mouth was covered with aluminium foil with a provision for the two plastic tubes to enter into the conical flasks.

Prior to the introduction of the animals, the seawater was aerated. The volume of seawater taken for the experimental study in each of the conical flask was so adjusted that at the end of 24 hrs, the flask had about 1.5 l of seawater. The temperature, pH, salinity and dissolved oxygen were recorded at the start and end of the experiment. The photoperiod was maintained at 12L:12D and no artificial light was provided other than natural day-light during light period.

Intermolt prawns fed for 30 days on different diets were selected in triplicate from each of the treatment for experimental study. Animals selected for the experimental study were almost of the same size and weight. Prior to the introduction in the experimental flasks, the prawns were fed with respective protein level purified diets. After two hours of
TABLE 5: ANALYTICAL PROCEDURE FOR MEASUREMENT OF RNA AND DNA (FLOW CHART)

Prawn sample
↓
Homogenised in 5 ml, ice-cold distilled water for 4 mins.
↓
Homogenate + 2.5 ml cold 0.6N PCA. Allowed it to stand for 10 mins.
↓
Centri. at 10,000 rpm, 4°C, 15 mins.
↓
Super. discarded
↓
Washed twice with 5 ml of 0.2 N PCA
↓
Centri. 10,000 rpm, 4°C, 5 mins
↓
Super. discarded
↓
Added 4 ml 0.3 N KOH
↓
incubated at 37°C for 2 hrs and cooled it in ice bath (15 mins)
↓
Centri. 10,000 rpm, 4°C, 15 mins
↓
Super. 1.
↓
Washed twice with 5 ml cold 0.2 N PCA
↓
Super. 2, added to 1 and read at 260 nm for RNA
↓
Dissolved in 5 ml, 0.3 N KOH
↓
In incubated overnight at 37°C
↓
Diluted to 15 ml with distilled water.
↓
0.5 ml diluted sample, added 0.5 ml Indole and HC1 reagent
↓
Shook, heated in water bath for 10 mins, cooled
↓
Extracted thrice in 1 ml, amyl acetate or CHCl3
↓
Centri. 1000 rpm
↓
Upper layer of Amyl acetate or CHCl3 discarded
↓
Aqueous layer
↓
Read at 490 nm for DNA

ppt = precipitate; Super. = supernatant,
PCA = Perchloric acid; Centri. = centrifuge.
feeding, the prawns were transferred into the individual conical flasks. A control was kept without any prawns.

After introduction, water sample was taken for determination of ammonia concentration. Water samples were collected every four hours from each of the flask and quickly analysed for ammonia. The experiment was carried out for 24 hrs. Ammonia concentration in water was analysed by the method of Solorzano (1969). The ammonia concentration excreted by the prawns was expressed as \( \text{NH}_4^- \text{N mg/ day/g prawn} \).

**Statistical Analysis of the Data:**

The data obtained on various parameters from the experiment were statistically analysed. Analysis of variance (ANOVA) was carried out to test the difference between the treatments. Least significant difference (LSD) method was followed to compare the means of the treatments (Snedecor and Cochran, 1973).

**RESULTS AND OBSERVATIONS**

Two sets of experiments were conducted to study the effect of different levels of dietary protein on growth, feed efficiency and body composition and to determine the optimum dietary protein requirement of the juveniles of the Indian white prawn, *P. indicus.*
EXPERIMENT - I

In this experiment, protein levels ranging from 0 to 60%, with an interval of 10% were used for compounding purified diets. The diets were fed to the prawns for a period of 30 days and the results of the experiment are presented here.

Survival:

Data on percent survival recorded from different treatment groups are shown in Fig. 1. Although, the protein content in the diet, had apparent effect on the percent survival, analysis of variance of the data did not show any significant influence of dietary protein level on the survival of prawns. However, the percent survival of prawns increased with protein content in the diet up to 40% and thereafter it showed a gradual decline. The maximum survival (73.3%) was recorded at 40% protein level in the diet and the minimum (26.7%) in the protein-free diet. In all other treatments, the percent survival ranged between 51.1 and 66.7%. While an abrupt decline in survival rate of prawns was observed during the second week in the protein-free dietary treatment (0%), not much variation in the survival rate was observed in the other treatment groups. However, a steady decrease in the survival rate of prawns was observed from the fourth week onwards in treatment groups of prawns fed diets with protein levels ranging from 10 to 30%. 
Fig. 1. Weekly percent survival of prawns fed with different levels of protein in the diets (0-60%)
Growth of prawns fed on the protein-free diet (0% protein level) showed marked difference with that of other treatments. Data on mean percent gain in length (37%), wet weight (200%) and dry weight (138%), shown in Fig. 2, indicate that poor growth has occurred in prawns fed on the protein-free diet, and the growth achieved can mainly be attributed to the cannibalism by the cohabiting species on the post-molted and dead prawns, before they were removed from the aquaria.

Fig. 2 shows the mean percent gain in length of prawns fed diets differing in the percent protein. Results of analysis of variance showed that the protein levels in the diets have significant (P<0.05) effect on the mean percent gain in length of prawns. The percent gain in length of prawns increased with increasing protein levels in the diet from 10% upto 30% and showed a gradual decline, thereafter, with further increase in protein level of the diet. The maximum mean percent gain in length was observed at 30% protein level (74%) and the minimum at 10% protein level (35%). There were no significant differences between the mean percent gain in length of prawns fed diet with 30, 40 and 50% protein.

The diets containing various protein levels also had highly significant (P<0.01) effect on the mean percent gains in wet weight and dry weight of prawns. The mean percent wet
Fig. 2. Percent gain in length and weight, and total biomass (g) of prawns fed with different levels of protein in the diets (0-60%).
FIG. 2

TOTAL BIOMASS (g)

MEAN GAIN IN LENGTH (%)

MEAN GAIN IN WEIGHT (%)

PROTEIN (% in Dry diet)

△ WET WEIGHT

▲ DRY WEIGHT
weight and dry weight increased with increasing protein levels in the diet. However, the increase was observed up to 40% protein in the diet and thereafter a gradual decline was observed as the protein level in the diet increased to 60%. The maximum percent wet weight gain (597%) and dry weight gain (521%) were recorded at 40% protein level and the minimum at 10% protein level (wet weight gain 190% and 213% dry weight gain). There was pronounced increase in the percent gains in wet weight and dry weight of prawns fed diets from 20% protein level up to 40% protein in the diet, indicating the significant influence of protein in the diet on the prawns' growth.

**Specific Food Consumption (SFC):**

Highly significant ($P < 0.01$) differences were observed among the specific food consumption of prawns fed diets containing different levels of protein. Very high value of SFC (33.7%) was obtained in the case of prawns fed on the protein-free diet (Fig. 3). Between 10% and 60% protein level in the diet, the highest SFC was observed at 10% and 20% protein levels (11.7% and 11.5%, respectively) and the lowest was recorded at 40% protein level (4.3%). In all other treatment groups SFC ranged between 5.5 and 7.3%.

**Food Conversion Ratio (FCR):**

Food conversion ratios obtained from the experiment are shown in Fig. 3. Highly significant ($P < 0.01$) differences were observed in the FCRs obtained by feeding different protein
Fig. 3. SFC, PCR and PFR for different levels of protein in the diets (0-60%).
concentrations in the diet. While the maximum FCR was obtained with 10% protein level (4.2), the minimum was obtained with 30% protein level (0.96). In other treatments (20, 40, 50 and 60%), the FCR values ranged between 1.35 and 3.4.

**Protein Efficiency Ratio (PER):**

Analysis of the data showed that the protein levels in the diets, significantly (P < 0.01) influence the protein efficiency ratio (Fig. 3). The maximum PER was recorded at 30% protein level (3.7) and the minimum at 60% protein level (1.1). There were no significant differences among PER obtained from treatments with protein levels between 40 and 60%. The PER in other treatments ranged between 1.3 and 2.4.

**Biochemical Composition:**

The moisture, ash, protein, lipid and carbohydrate contents of prawns were also found significantly (P < 0.01) affected by the level of protein in the diet. It was observed (Fig. 4) that as the protein level in the diet increased, the moisture content of prawns declined up to 40% protein level and thereafter the moisture content increased with further increase in protein concentration in the diet. The prawns fed diets containing 10% and 20% protein had significantly higher moisture content than most other groups.

The prawns fed diets containing 40% protein had significantly (P < 0.05) less ash (10.3%) and more protein (66.9%)
Biochemical composition of prawns fed with different levels of protein in the diets (0-60%).
FIG. 4

ASH (%)

CARBOHYDRATE (%)

LIPID (%)

PROTEIN (%)

MOISTURE (%)

PROTEIN (% IN DRY DIET)
than prawns from other treatments. The highest ash content was recorded at 60% protein level in the diet (21.2%).

Although, the minimum protein content was recorded in prawns fed with the 20% protein diet (58.9%), it was not significantly different from the protein content of prawns fed diet with 10% protein (59.9%). Similarly, there were no significant differences between the protein contents of prawns from treatments with 30, 50 and 60% protein levels in the diet.

The lipid content was significantly higher in prawns fed with 10% protein diet (18.5%). Similarly, lipid contents in prawns fed diets containing 50 and 60% protein (10.4%) were significantly less than the lipid content of prawns from other treatments, which varied between 11.2 and 17.3%. There was a steady decline in the total lipid content of prawns with the increasing protein level in the diets, however, there was a slight increase in the lipid content of prawns fed with 40% protein diet which can be correlated with the low moisture content of prawns recorded at this treatment. An inverse relationship between total lipid and moisture content of the prawns was observed.

Maximum significant \((P < 0.05)\) differences in carbohydrate content were observed between 10% and 20% protein levels. The maximum carbohydrate content of prawns was recorded at 10% protein level (3.4%) and minimum at 40% protein level (2.4%). In all other treatments, the carbohydrate content of prawns varied between 2.5 and 2.9%. There were no significant
differences among the carbohydrate content of prawns fed diets containing protein levels ranging from 30 to 60%. However, a declining trend in carbohydrate content of prawns was observed with the increase in dietary protein level up to 40% and thereafter a gradual rise in carbohydrate content occurred as the concentration of protein in the diet increased.

The moisture, ash, protein, lipid and carbohydrate contents of prawns fed the protein-free diet showed significant ($P < 0.05$) differences with that of prawns fed on other diets.

There was high incidence of mortality and cannibalism in this group, which significantly ($P < 0.05$) influenced the composition. It is felt that comparison of the result with that of other treatments may not provide any meaningful conclusions. Despite this, the moisture (79.5%), ash (19.4%), carbohydrate (3.3%) and lipid (23.3%) contents were observed to be high in these groups of prawns with comparatively very low protein content (51%).

The RNA content of prawns (Fig. 5) was also significantly ($P < 0.05$) affected by the protein levels in diets. The prawns fed the protein-free diet and those fed with 10 and 20% protein levels in the diets had significantly ($P < 0.05$) less RNA than those fed diets with higher protein concentrations. While the maximum RNA content was recorded in prawns fed diets with 40% and 50% protein contents (2.19 μg/mg), the minimum was found in prawns fed a protein-free diet (1.13 μg/mg).
Fig. 5. Biochemical composition of prawns fed with different levels of protein in the diets (0-60%).
other treatments, the RNA content varied between 1.39 and 1.96 
μg/mg. However, with increasing protein levels in the diet of 
prawns, the RNA content increased up to 50% protein level, and 
further increase in the protein content of the diet resulted in 
decrease of the RNA content.

The DNA content of prawns (Fig. 5) showed a similar 
trend as that of RNA content, increasing with the protein concen­
tration in the diet of prawns. However, the DNA content did not 
show any decline beyond 50% level, as observed for RNA, but showed 
increase up to 60% level. It was also observed that the DNA 
content of prawns fed the protein-free diet was significantly 
(P < 0.05) less than that of prawns fed diets with different 
levels of protein. The highest DNA content was recorded in 
prawns fed on the diet with 60% protein level (2.13 μg/mg) 
and the lowest in prawns fed the protein-free diet (1.31 μg/mg). 
In all other treatment groups, the DNA content ranged between 
1.5 and 2.05 μg/mg.

Significant (P < 0.05) variations were also observed in 
the dry weight/total RNA ratio in prawns (Fig. 5) fed diets 
containing different levels of protein. However, prawns fed 
the protein-free diet and those fed diets with 10% protein had 
significantly (P < 0.05) higher ratios compared to prawns from 
other treatments. The dry weight/RNA ratio showed a decreasing 
trend with increasing protein content of the diet up to 50%, and 
thereafter a slight increase was observed at 60% protein diet.
Similarly, the dry weight/total DNA ratio (Fig. 5) decreased with increase in protein content of the diet of prawns and the highest was recorded in prawns fed on protein-free diet (0.79) and lowest in prawns fed with 60% protein (0.47) in the diet. For all other treatment groups, the ratio ranged between 0.44 and 0.67. The ratio was significantly (P < 0.05) higher for prawns fed the protein-free diet as well as those fed with 10% protein level that for other treatments.

The RNA/DNA ratio increased with the protein concentration in the diet up to 40% and thereafter it showed a decline. Though the RNA/DNA ratio was significantly (P < 0.05) influenced by the protein level in the diets, only prawns fed diets with 30 and 40% protein levels had significantly higher RNA/DNA ratios than prawns from other treatments.

The dietary protein concentrations also had highly significant (P < 0.01) effect on the calcium content (Fig. 5) of prawns. The prawns fed on the protein-free diet had significantly (P < 0.05) lower calcium content (1.87%) than that of prawns from other dietary protein treatments. The highest calcium content was recorded in prawns fed 40% protein diet (3.13%) followed by those fed 50% protein in the diet (3.05%).

The magnesium and phosphorus contents (Fig. 5) of prawns were not significantly affected by the levels of protein in the diet. While the magnesium content ranged from 0.44 to 0.55%, the phosphorus content varied between 0.95 and 1.02% in prawns from various dietary treatments.
Ammonia Concentration in Water:

The ammonia concentration in water was significantly (P < 0.05) influenced by the dietary protein levels (Fig. 6). With increasing protein level in the diet, the prawns showed an increasing trend in ammonia concentration in water. The highest ammonia concentration in water was recorded at 60% protein (0.47 mg/l) and lowest in prawns fed on the protein free diet (0.43 mg/l). However prawns fed between 20% and 40% protein in the diet showed almost the same ammonia concentration in the water.

Ammonia Excretion Rates:

The ammonia excretion rates showed significant (P < 0.05) influence of dietary levels of protein (Fig. 6) with increasing concentration of protein in the diet, the prawns excreted higher concentration of ammonia. The highest ammonia excretion rate was recorded in prawns fed on 60% protein (0.28 mg/g prawn/day) and lowest in prawns fed on protein free diet (0.19 mg/g prawn/day). Prawns fed on 30% and 40% protein did not show much variation in ammonia excretion rates.
Fig. 6. Ammonia concentration in seawater and ammonia excretion rate in prawns fed on different levels of protein in the diets (0-60%)
FIG. 6

AMMONIA CONCENTRATION IN WATER

AMMONIA EXCRETION RATE

mean NH₃-N mg/l./treatment/day

NH₃-N mg/l./g prawn/day

PROTEIN (% IN DRY DIET)
Observations made on the basis of exuviae collected (Table 6), indicate that molting in prawns is affected by the protein levels in the diet. Greater numbers of exuviae were collected from treatments with 30%, 40% and 50% protein levels (32 nos) compared to that of other treatments. The number of exuviae recorded were not absolute figures since, molting occurs during night and the exuviae at times were eaten by cohabiting prawns. Post-molt deaths were relatively more in the treatment fed protein-free diet, after 15 days from start of experiment, suggesting that post-molt deaths occur due to the deficiency of dietary protein in the diet. Similar, observations were also made in the case of prawn groups fed diets containing 10% and 20% protein after the third week, indicating inadequacy of the protein in the diet for normal physiological processes.

Food Intake:

Food intake in prawns was not affected during the first two weeks in all the treatment groups. However, from the second week onwards, food intake was reduced in the prawns fed on the protein free diet. From the end of the third week, food intake was greatly reduced and the prawns responded passively when the feed was introduced in the aquaria. There was not much variation
<table>
<thead>
<tr>
<th>Protein in the diet</th>
<th>Mean nos. of molts recovered</th>
<th>Mean nos. of post-molt deaths</th>
<th>Texture of the body</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19</td>
<td>25</td>
<td>SO</td>
</tr>
<tr>
<td>10</td>
<td>21</td>
<td>16</td>
<td>SO</td>
</tr>
<tr>
<td>20</td>
<td>25</td>
<td>16</td>
<td>SO</td>
</tr>
<tr>
<td>30</td>
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<td>8</td>
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<td>40</td>
<td>32</td>
<td>8</td>
<td>H</td>
</tr>
<tr>
<td>50</td>
<td>32</td>
<td>13</td>
<td>SO</td>
</tr>
<tr>
<td>60</td>
<td>31</td>
<td>16</td>
<td>SO</td>
</tr>
</tbody>
</table>

H - Hard, SO - soft
in the food intake of prawns from various treatments, though minor variations were observed during molting.

**Behaviour Towards Light:**

When table lamp light \((1625 \times 10^2 \text{ lux})\) was shown, prawns from the various dietary treatments, responded differently. Normally prawns preferred low intensity lights and evaded brightly lighted regions. During the first two weeks, no striking differences in response to photostimuli occurred in prawns from various treatments; but in subsequent weeks at 0%, 10% and 60% protein levels, languid response of prawns was observed. However, in other treatments, the response was spontaneous and the prawns moved away to shaded regions in the experimental tanks.

**External Morphology:**

The prawns subjected to different levels of protein in the diet, showed distinct variability in their activity. While the prawns fed diets with 0%, 10% and 60% protein levels showed hypoactivity, those fed diets containing 20%, 30% and 40% protein in the diet showed normal activity and prawns fed with 50% protein in the diet showed hyper-activity. The hepatopancreas of prawns fed with diets containing 10%, 20% and 50% protein levels showed diffused appearance and brownish color. However, in all other treatments, the hepatopancreas of prawns appeared compact, brownish in color underlined by a whitish mass. No other significant changes in the morphology was observed in the different treatment groups.
EXPERIMENT II

The second experiment was conducted based on the results of the first experiment in which the diet containing a protein level of 40% produced maximum growth. In this experiment, protein levels ranging from 32.5 to 47.5%, with an interval of 2.5%, were selected with a view to determining near optimum levels of protein, which produce maximum growth.

Survival:

The survival rate of prawns (Fig. 7) showed an increasing trend up to 37.5% protein level in the diet, and thereafter showed a decreasing trend as the concentration of protein in the diet increased further. Analysis of variance performed on the survival rate data failed to give any significant differences between treatments. While, the highest percent survival (70%) was recorded in groups of prawns fed diet with 37.5% protein level, the lowest (52%) was recorded in the prawn groups fed diet with 32.5% protein level. In all other treatments, the percent survival varied from 55.5% to 67.5%. There was also no significant treatment to treatment difference in the weekly survival of prawns.

Growth:

Significant ($P < 0.05$) differences in the mean percent gain in length of prawns were observed (Fig. 8) between the
WeeKly percemt survival of prawns fed with different levels of protein (32.5-47.5%) in the diets.
treatments. The mean percent gain in length of prawns increased with the protein level in the diet up to 40% and thereafter declined sharply with further increase in the dietary protein level. The maximum mean percent gain in length was recorded in the prawns fed with diet containing 40% protein (80%); whereas the minimum was recorded at 45% protein (61%) in the diet. The mean percent gain in length of prawns recorded in diets with 35 and 37.5% protein level were almost equal (72%).

The mean percent gains in wet weight and dry weight of prawns recorded from the various treatments are shown in Fig. 8. The protein levels in diets had highly significant (P < 0.01) influence on the mean percent gains in wet weight and dry weight of prawns. The mean percent gain in wet weight increased with the protein level in the diet up to 37.5% and thereafter it declined as the protein level in the diets increased to 42.5% and beyond this protein level, it remained more or less steady. The mean percent gain in wet weight obtained at 35% protein level (480%) and 40% protein level (445%) were not significantly different from the maximum recorded at 37.5% protein level (494%).

The maximum mean dry weight gain of prawns was recorded at 40% protein level (405%) and minimum at 47.5% (251%) protein level and these results are different from that observed for mean percent wet weight gains. The mean dry weight of prawns also increased with the protein level in the diet up to 40% and thereafter a steady decline was observed with further increase
Fig. 8. Percent gain in length and weight, and total biomass (g) of prawns fed with different levels of protein (32.5-47.5%) in the diets.
in protein level in the diet. There was no significant difference between 37.5 and 40% protein in the percent gain in dry weight.

**Specific Food Consumption (SFC):**

Data on specific food consumption recorded from the treatments are shown in Fig. 9. Significant (P < 0.05) differences were observed in between the specific food consumption of prawns fed on diets with different protein levels. The SFC was highest in 42.5% protein level (7.26%) and lowest in 37.5% protein level (3.68%). However, the SFC recorded at 35% protein level (3.71%) was slightly higher than that recorded at 37.5% protein level. In all other treatments the SFC ranged between 4.8 and 6.2%.

**Food Conversion Ratio (FCR):**

Food conversion ratios obtained from the different treatments are shown in Fig. 9. There were no significant differences among the FCRs recorded from the different treatments, though the FCRs recorded from treatments with 32.5% and 35% protein levels were relatively less than that of other treatments. In all other treatments FCR ranged between 0.99 and 1.02.

**Protein Efficiency Ratio (PER):**

Protein efficiency ratio showed (Fig. 9) an inverse relationship with that of food conversion ratio. There were statistically no significant differences in between the PER...
Fig. SFC, FCR and PER for different levels of protein (32.5-47.5%) in the diets.
FIG. 9

PROTEIN EFFICIENCY RATIO

FOOD CONVERSION RATIO

SPECIFIC FOOD CONSUMPTION (%)

PROTEIN (% IN DRY DIET)
from the treatments. The maximum PER was recorded at 35% protein level (3.4) and minimum at 45% protein level (1.97). The PER did not vary markedly between 40 and 47.5% protein levels.

**Biochemical Composition:**

The moisture, ash, protein, lipids and carbohydrate contents of prawns from different dietary treatments are shown in Fig. 10. While the dietary protein level had significant \( P < 0.05 \) influence on the moisture, ash and lipid contents, the protein and carbohydrate contents were not significantly affected. The prawns fed on the diet containing a protein level of 47.5% had the maximum moisture (80.9%) and ash contents (21.8%). There was not much variation in the moisture content of prawns fed diets containing protein levels between 35% and 40%. There was also a decrease in the moisture content with the increase in protein level in the diets up to 40% and thereafter the moisture content showed a steady increase as the protein level in the diet increased to 47.5%. The ash content of prawns fed on diets containing 32.5% protein level (18.2%) did not differ significantly from the minimum (17.7%) recorded at 35% protein level. However, an increasing trend was observed in the ash content with increasing protein level in the diet above 35% protein.

No significant differences were observed (Fig. 10) between the protein content of prawns fed diets containing different protein levels. The prawns fed the diet with 37.5%
Fig. 10. Biochemical composition of prawns fed with different levels of protein (32.5-47.5%) in the diet.
protein had the highest protein content (61.9%); whereas the lowest protein content was observed in prawns fed diets containing 32.5% and 42.5% protein (59.3%).

Significant (P < 0.05) differences were also observed between the total lipid content of prawns from the various treatments. The highest lipid content was recorded at 42.5% protein level (18.5%) and the lowest at 47.5% protein level (10.9%). However, no specific trend was observed between the lipid and moisture contents of prawns fed diets with different protein levels.

The carbohydrate content in prawns fed with various protein diet also did not show any specific trend, though the prawns fed diet with 47.5% protein level (3.5%) and 35% protein level (3.4%) had relatively higher carbohydrate contents.

No significant variations were observed in the RNA and in the DNA contents in between treatments (Fig. 10). The highest RNA and DNA contents were recorded in prawns fed diets containing 37.5% protein (2.67 μg/mg) and 47.5% protein (2.50 μg/mg), respectively. In all other treatment groups, the RNA content ranged between 2.16 and 2.56 μg/mg, the lowest RNA being at 32.5%.

The dry weight/total RNA ratio of prawns did not show any significant variation with reference to the levels of protein in the diet. However, prawns fed with lower protein levels (<35%) had relatively higher ratios compared to prawns fed with higher protein levels in the diet. Similarly,
Fig. 11. Biochemical composition of prawns fed with different levels of protein (32.5–47.5%) in the diet.
Dry weight/total DNA ratio was also not significantly influenced by the different dietary levels of protein, though the ratios varied between 0.39 and 0.45.

The RNA/DNA ratios of prawns were significantly (P<0.05) influenced by the dietary protein level. However, the prawns fed with the 32.5% protein diet had significantly (P<0.05) lower RNA/DNA ratio than that of prawns fed diets containing other dietary protein levels. The highest RNA/DNA ratio was observed in prawns fed diets with 37.5%, 40% and 45% protein (1.13) and lowest ratio in prawns fed the diet containing 32.5% protein (0.89). A declining trend was observed in the RNA/DNA ratio in prawns fed beyond 40% protein level, excepting an unexpected rise at 45% protein level in the diet.

The calcium, magnesium and phosphorus contents of prawns, expressed as percentages, from various dietary treatments are shown in Fig. 10. Analysis of variance of the data showed that the protein levels in the diet do not significantly influence these parameters. While the calcium contents varied between 2.13 and 2.7%, the magnesium contents varied between 0.41 and 0.57% and the phosphorus contents ranged from 1.62 to 1.83%.

**Ammonia Excretion Rates**

Ammonia excretion rates of prawns (Fig. 12) was also significantly (P<0.05) influenced by the dietary protein levels. Significant treatment differences were observed in the ammonia
Fig. 12. Ammonia concentration in seawater and ammonia excretion rate in prawns fed on 32.5–47.5%.
FIG. 12

AMMONIA CONCENTRATION IN WATER

mean NH₃-N·mg/l/treatment/day.

0.01
0.02
0.03

AMMONIA EXCRETION RATE

0.20
0.25
0.30

NH₃-N·mg/g prawn/day.

0 32.5 35.0 37.5 40.0 42.5 45.0 47.5

PROTEIN (% IN DRY DIET)

(0.32)
excretion rates between prawns fed on diets with less than 42.5% to that of prawns fed on diets more than 42.5% protein. The highest was recorded in prawns fed with 45% (0.32 mg/g prawn/d) and lowest in prawns fed with 32.5% (0.21 mg/g prawn/d).

**OBSERVATIONS**

**Molting**

The number of exuviae collected from the various treatments (Table 6b) did not show much variation. Relatively few numbers of exuviae were collected from treatments with diets containing 32.5, 35 and 47.5% protein, when compared to other treatments.

The number of post-molt deaths (Table 6b) recorded were invariably similar for all the treatments, excepting in the case of treatment with 32.5% protein level, where a slight increase in the number of post-molt deaths was observed.

**Food Intake**

Although not much variations were observed in the amount of left-over feed in different treatments, slightly reduced feed intake was observed in prawns fed diets containing protein levels greater than 45%, from the fourth week onwards.

**Behaviour Towards Light**

When a light source from a table lamp (1625 x 10^2 lux) was suddenly flashed into the experimental tanks, almost in all the treatment groups, similar active response was observed during the first two weeks. However from the third week onwards,
<table>
<thead>
<tr>
<th>Protein in the diet %</th>
<th>Mean nos. of molts recovered</th>
<th>Mean nos. of post molt deaths</th>
<th>Texture of the body</th>
</tr>
</thead>
<tbody>
<tr>
<td>32.5</td>
<td>22</td>
<td>24</td>
<td>SO</td>
</tr>
<tr>
<td>35.0</td>
<td>22</td>
<td>18</td>
<td>H</td>
</tr>
<tr>
<td>37.5</td>
<td>24</td>
<td>18</td>
<td>H</td>
</tr>
<tr>
<td>40.0</td>
<td>29</td>
<td>18</td>
<td>H</td>
</tr>
<tr>
<td>42.5</td>
<td>29</td>
<td>22</td>
<td>H</td>
</tr>
<tr>
<td>45.0</td>
<td>28</td>
<td>18</td>
<td>H</td>
</tr>
<tr>
<td>47.5</td>
<td>21</td>
<td>23</td>
<td>H</td>
</tr>
</tbody>
</table>

H - hard, SO - soft
the prawns fed diets containing 32.5, 42.5 and 47.5% protein showed quite passive response. In all other treatment groups, active response was observed throughout the experimental period and the prawns moved away from the lighted area in response to light.

External Morphology:

No specific changes in the external morphology were observed in prawns from different treatments, after 30 days of feeding with the test diets. However, few prawns fed diets containing 32.5 and 47.5% protein were observed to have few scattered brown spots in the abdominal region. These prawns, however, grew as well as those without these brown spots.

DISCUSSION

Dietary protein requirements of a number of crustaceans, especially the penaeid prawns, have been widely studied and optimal protein levels in diets for different developmental stages have been recommended (New, 1976; Biddle, 1977; Maquire, 1980; Milli^kin et al., 1980). In most of these studies, protein requirements have been reported based on experimental trials using semi-purified or compounded diets and very few studies have been conducted with
purified diets using casein as a protein source (Kanazawa et al., 1970, 1976; Deshimaru and Kuroki, 1974a, 1975a; Boghen and Castell, 1980; Bhaskar and Ali, 1984). Since casein is the only protein source available in highly purified form, its use as a protein source reduces considerably extraneous nutritional factors, which markedly alter the protein requirement of prawn species. Thus, purified diets allow precise nutritional studies, whereby relationships between particular dietary ingredients and physiological indices can be observed (D'Abbramo et al., 1982). In the present study, to define the protein requirement of juvenile P. indicus, a purified diet, with casein as the primary protein source, was used.

The present findings clearly show that protein level in the diet significantly influence the survival and growth of prawns, which is in accordance to earlier observations in crustaceans (New, 1976; Maguire, 1980). In the first experiment, using wide range of protein intervals, survival rate was found to increase with the protein level in the diet upto 40% and thereafter it declined. In the subsequent experimental study using narrower ranges of protein level, survival rates increased with increasing protein level upto 37.5% and thereafter a declining trend was observed. The survival rate of prawns at 40% protein level differed insignificantly between the two experiments. The variations may be due to minor changes in the experimental conditions as well as due to genetic variations in the broodstock, since the juvenile prawns used for the two
experiments came from different parentage.

The results also indicate that protein levels below 32.5% and above 45% have detrimental effect on survival and growth. In the case of prawns fed on the protein free-diet, almost the whole population was wiped off with very low survival, largely due to their cannibalistic behaviour, as well as due to devouring of freshly molted prawns by the cohabiters. This severe effect of protein deficiency in prawn's diet suggests that prawns have a minimum requirement for protein to meet their basal metabolic needs. However, the highest survival in prawns fed the 37.5% protein in the diet, signifies that the juvenile prawns may have a requirement around this level for normal metabolism. According to Colvin and Brand (1977), protein requirement decreases with the increase in size and the post-larvae of *P. indicus* have been reported to require about 40% protein in the diet (Bhaskar and Ali, 1984). All these observations indicate, the significant variations in the dietary protein requirement of various growth stages of prawns.

Like the survival rate, significant effect of protein levels was observed on growth. In the first experiment, growth of prawns was found to be relatively high between 30% and 40% protein. But the subsequent experimental study, significantly high growth was obtained with 35 to 40% protein in the diet. While *P. japonicus* (Kanazawa et al., 1970), *Palaemon serratus*
(Forster and Beard, 1973) and *Hormorbus americanus* (Castell and Budson, 1974) have been found to require less than 50% protein in the diet. *P. aztecus* (Shewbart et al., 1973), *P. setiferus* (Andrews et al., 1972) and *P. duorarum* (Sick and Andrews, 1973) seems to require relatively lower protein levels ranging from 20-30%, and in *Procambarus clarkii* (Huner and Heyers, 1979) and *P. meridionalis* (Sedgewick, 1979), the protein requirement is found to be about 34 to 42%. Thus, it is evident that the present experimental species is having a relatively lower protein requirement than many of the other species so far studied. However, Colvin (1976) and Ali (1982a) found that *P. indicus* require around 43% protein in the diet for optimal growth. These values are significantly higher than what has been observed in the present study and can be accounted for the type of protein source used, as the above researchers used compounded diets as against the purified diet in the present case. Probably, compounded feeds might have had growth promoters which could have influenced the growth. However, use of purified diet in the present study seems to have removed the effect of growth promoters (Kanazawa et al., 1970; New, 1976; Conklin et al., 1980).

Although, the growth and survival in prawns showed slight difference between 35%, 37.5% and 40% protein levels; the observed differences were not statistically significant. So it is evident that dietary protein levels ranging from 35 to 40% can be used for formulation of complete practical diets for
juvenile *P. indicus* without affecting survival and growth significantly. On the other hand, considerably reduced growth attained by prawns fed diets with 32.5% or less of protein or above 40% protein level, indicate that underfeeding and overfeeding of protein, significantly affect growth probably due to alterations in the metabolism. As in the protein deficient diet, poor growth and relatively poor survival were observed at 10% and 20% protein levels, which clearly indicate that at these protein levels and restricted feeding, the prawns are unable to meet their dietary protein requirements for proper growth.

The protein level in the diet also had significant effect on the specific food consumption (SFC), with the values increasing with levels of protein from 0 to 30% and above these levels, no significant variation was observed between the treatment groups. These results indicate that the prawns tend to reach a saturation level for protein requirement, resulting in optimum food consumption, maximum growth, and high survival rate, above 30% protein. In comparison, the food consumption was relatively poor in prawns fed with less than 30% protein in the diet and in prawns fed without protein, the food consumption was very low leading to poor growth and survival. Similarly, high values of SFC against poor growth in prawns fed with high protein (> 50%) diets, indicate the interference of excess dietary protein on the growth of the prawns. Following the second set of experimental results, it is evident that there
is no significant variation in SFC values between 35 and 37.5% protein levels and so the optimal protein level could be well within this range. The values recorded for SFC in these levels of protein fed prawns are almost same to that recorded in juvenile *H. americanus* which were fed on a natural diet (Bordner and Colvin, 1981).

The FCR and PER values are also significantly influenced by the protein levels in the diet. Protein levels less than 20% in the diet gave significantly higher FCR and PER values than higher levels of protein. However, the lowest FCR and highest PER at 35% protein level in the diet indicate that food as well as protein are efficiently utilized. But considering the growth and survival, 37.5% protein seems to be better than that of 35% protein. However, since the FCR and PER were not significantly different between 35% and 37.5% protein, it is apparent that the optimal protein requirement may fall within the range of 35% to 37.5%. On the other hand, high FCR and low PER values recorded at protein levels below 32.5% and above 40%, indicate that dietary protein inadequacy or excess affect food conversion and dietary protein utilization.

The biochemical composition of carcass of prawns further provide evidences in support of the above suggestions relating to the optimal requirements in the prawns. Amongst, the various biochemical parameters determined moisture, protein, lipid, ash
and calcium are the most prominent to be affected by the dietary levels of protein. It is evident that prawns fed below 35% or above 40% protein levels, tend to have more moisture than prawns fed diets containing protein between 35% and 40%. This signifies that when prawns are fed with supra-optimal or sub-optimal protein levels, the organic matter accumulation is reduced considerably. However, it was observed that the moisture content in prawns fed with 35–40% protein was low, indicating that at near optimal protein requirement, maximum nutrient deposition occurs.

From the results it is evident that the ash content of prawns is significantly affected by the dietary protein level with the prawns fed below 35% protein in the diet having significantly lower ash content than those fed diets containing above 35% protein. However, in the first experiment, prawns fed diet containing 40% protein level recorded the lowest ash content for unknown reasons, but in the subsequent experiment, the ash content recorded at 40% protein level was almost as high as in other protein level fed prawns.

Ash from prawns fed with experimental diets when analysed for calcium, magnesium and phosphorus, showed significant variations only in the calcium content. The prawns fed with the protein deficient diet had significantly lower calcium content than those fed with more than 30% protein level; however, at higher protein levels (>50%), the calcium content showed a gradual decline. Prawns fed diets with protein
ranging between 32.5% and 47.5% showed an increasing trend in calcium content but plateauing beyond 37.5% protein level. This suggests that possibly calcium contents is not influenced by protein levels between 37.5 and 47.5%. No significant variations were observed in magnesium and phosphorus content of these prawns fed diets containing increasing protein levels though in higher vertebrates protein level in the diet has been shown to influence the degree of utilization of phosphorus and magnesium (Georgievskii et al., 1979). The variations in the present observation with that of the above workers may be due to the differences in the physiological processes taking place in these forms.

So it appears, from the results obtained on calcium content, that possibly the uptake, repletion and depletion of calcium in prawns is influenced by the protein levels, since calcium in crustaceans forms an important major inorganic constituent. On the other hand, magnesium and phosphorus are relatively minor components (Richards, 1951, Humer et al., 1978) and are probably not influenced by dietary protein levels, so insignificant variations were observed in the different treatment groups.

The protein content in prawns, helps interpret the effect of dietary protein level, as well as other metabolic changes associated with the dietary treatment. Protein content in prawns was significantly influenced by the protein level in
the diet. The protein content increased with the protein level up to 40% and thereafter declined. On the other hand, the prawns fed the protein deficient diet had significantly low protein content, indicating that under dietary protein deficiency, the tissue proteins are catabolised leading to depletion in tissue protein levels. The ammonia excretion rate was also found to be significantly low in prawns from this treatment on prolonged deprivation of protein. Further, Mendes and Waterlow (1958), demonstrated that protein malnutrition results in the loss of cellular protein fraction, and thus, the low protein content in these prawns are quite expected.

The prawns fed with more than 30% protein in the diet had higher protein content than prawns fed with less than 30% protein. This indicates that since dietary proteins availability was limited, both due to the low protein level in the diet and restricted feeding, protein deposition in the body is greatly affected. However, insignificant variations in protein content of prawns was observed with further increase in concentration of protein in the diet. Besides, the protein contents recorded in the first experiment are not exactly comparable to that of the second experiment. Probably, differences in brood stock from which prawn juveniles were obtained could have contributed to this variation in protein content. Even then, it appears that protein level in the diet does not significantly affect the protein content in prawns fed on diets having protein levels ranging from 32.5% to 47.5%. The experimental study, however,
shows that when threshold levels of protein are added in the diet to meet the minimum protein requirements of the animal, the protein deposition is not significantly affected with any further increase in the levels of dietary protein.

The RNA and DNA content in prawns are important parameters since the ratio of these explains the state of metabolic activity undergoing in the tissues (Hotchkiss, 1955; Buckley, 1979a) and in many cases, the RNA content in organisms has been related to their growth rates (Leick, 1968; Sutcliffe, 1970; Dagg and Littlepage, 1972). However, the changes in RNA–DNA ratios are primarily due to changes in RNA–P rather than DNA–P which remains constant (Bulow, 1970). According to Bulow (1971) and Buckley (1979a, b), RNA–DNA ratios are very sensitive to changes in feeding levels and could be used as indicators of growth. The present study also shows the significant influence of dietary protein levels on the RNA and DNA content in prawns.

The increase in the concentration of RNA upto 40% protein level in the diet shows the increased rate of protein synthesis with the increase in protein level in the diet. The highest RNA content at 40% protein level, indicate active efficient protein synthesis, which was also reflected in the highest growth achieved at this level. The RNA–DNA values recorded in the prawns during the present study are within the limits reported by Bulow (1970) and Buckley (1979b) in fishes and Sutcliffe (1970) in amphipods,
Since the RNA-DNA ratio as well as the growth of prawns was observed to be highest at 40% protein level, the suggestion by Bulow (1971) and Buckley (1979a, b) for fishes that RNA-DNA ratio can be used as growth indicator may also hold true for prawns. Similar observations were also made in the second set of experiment where the highest RNA-DNA ratio was recorded at 37.5% and 40% protein levels. These results indicate that the high growth attained at 37.5% protein level is justifiable, since the protein synthesis was perhaps most efficiently functioning, resulting in high protein deposition in the tissues for growth. This is in conformity with the observations of Leslie (1955) and Brachet (1955) who postulated that although DNA content of the fish tissue will vary little, RNA content will vary much more and be highest in those fishes undergoing fastest growth or protein synthesis.

Lipid content in prawns decreased with the increase in protein level in the diet and prawns fed with protein-free diet had significantly high lipid content compared to prawns fed with more than 45% protein which had significantly low lipid content. These results clearly indicate the effect of dietary protein on lipid metabolism. As observed in the present study, high lipid content was also obtained in the fish, sea bass, fed on a protein-deficient diet (Metaller et al., 1973). On the other hand, prawns fed protein diets ranging from 20 to 40%, did not show any significant variation in the lipid content, though there was variation in
growth. The relatively low lipid content in prawns fed with high protein diets indicate that probably energy derived from lipid is used for deamination associated with protein catabolism which requires energy. The process of active deamination of proteins in these prawns is evident from the high ammonia excretion rates. The carbohydrate content also shows similar trends like lipid content in these prawns decreasing with increasing protein level, though not prominent as the lipid content.

From the ammonia concentration in water, it is evident that prolonged protein deficiency leads to low ammonia excretion indicating reduced metabolic activity (Harper, 1971; Clifford and Bricks, 1978). The passive responses observed in these prawns when disturbed, further support the above observations of low metabolic rate.

In the case of prawns fed the high protein diets (more than 42.5%), the carbohydrate content was high and the ammonia excretion rates were significantly higher than that of prawns fed diets with lower (less than 42.5%) protein levels. This suggests that in this case, the excess proteins are catabolised and probably part of the energy liberated is converted into carbohydrate and lipid, releasing ammonia in the process.
CONCLUSIONS

The present study indicates that juvenile prawns, *P. indicus* require proteins for normal growth, survival and general maintenance of body functions. Deficiency of proteins in diets for longer duration results in near complete mortality of prawn population. From the second week onwards these prawns show increasing cannibalistic behaviour. Prolonged deficiency results in alterations in metabolic activities, which is evident from the carcass composition and general declining activity.

Sub-optimal levels of proteins in diets are unable to sustain growth for long, and to meet their energy and protein requirements, the cohabiting prawns resort to feeding on freshly molted prawns and thereby lower survival was recorded.

The preferable levels of protein in diets of juvenile *P. indicus* seems to be within the range 35 to 40%. These results are sufficiently supported by other parameters studied and are well within the protein levels suggested for this species using compounded diets (Colvin, 1976). However, it is difficult to point out optimal protein requirement specifically for a species as multivariate factors are involved in determining the nutritional requirements for a species which are difficult to control at a time (Goodwin and Hanson, 1977).
Also, Colvin and Brand (1977) observed that protein requirement decreases as the size increases and thus the developmental stage of prawn has significant influence on protein requirements.

Protein diets above 40% results in reduced growth, increased catabolism of protein, increased ammonia excretion rates and affects the deposition of inorganic and organic nutrients, finally resulting to lower survival rate. Thus, for proper maintenance of body metabolism which reflects on the growth and survival, prawns like any organism needs optimal protein concentration in their diets.