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Fluctuations in Ca, Mg and P Levels in the Hemolymph, Muscle, Midgut Gland and Exoskeleton During the Moult Cycle of the Indian White Prawn, *Penaeus indicus* (Decapoda: Penaeidae)

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ABSTRACT. 1. Fluctuations of Ca, Mg and P in the hemolymph, midgut gland, muscle and exoskeleton of the penaeid prawn *Penaeus indicus* during different stages of the moulting cycle have been investigated. 2. Haemolymph, midgut gland and muscle showed a high content of Ca during late premoult stages and low content in late postmoult and intermoult stages. In exoskeletal tissue the Ca level was high in intermoult and early premoult stages and the lowest level was recorded in the early postmoult stage. Magnesium showed an almost similar trend to that of Ca. Phosphorus content did not show noticeable changes in haemolymph and muscle during moult cycle; in exoskeleton, higher levels were recorded in last premoult and early postmoult stages. 3. The quantitative distribution of Ca, Mg and P in different parts of the exoskeleton was mapped. COMP BIOCHEM PHYSIOL 114A;1:91–97, 1996.

KEY WORDS. Calcium, magnesium, phosphorus, moult cycle, *Penaeus indicus*

INTRODUCTION

The crustacean exoskeleton is extensively mineralized with calcium carbonate as the principal inorganic component, and small amounts of magnesium and phosphate salts (13). The minerals in the exoskeleton are in a constant state of flux, as these animals have to mineralize the newly formed exoskeleton after moulting and again demineralize the old exoskeleton in preparation for the next moult. The general pattern of Ca, Mg and P variation during the moult stages of highly calcified Decapoda is well known (7). However, detailed information on poorly calcified prawns, penaeids in particular, are scant, with the exception of Dall (5), Bursey and Lane (2), Weilender (18), Huner *et al.* (9,10), Wickins (19) and Sarda *et al.* (15).

The extent to which hardening of the cuticle takes place with mineralization showed greater variation not only among different crustaceans, but also between different regions of the same animal. The topographic variation of minerals in the exoskeleton has been studied in crabs (6) and crayfish (12). However, there are no reports available on topographic mineral distribution of panaeids.

The present investigation was carried out to understand the variation of Ca, Mg, and P in different body tissues (exoskeleton, muscle, midgut gland, and hemolymph) during the different moult stages of the Indian white prawn, *Penaeus indicus*. The distribution levels of Ca, Mg, and P in different regions of the exoskeleton were also mapped. Information on such physiological characteristics of the candidate species is important for the management of the ecosystem in which prawns are cultured, understanding of nutritional needs and pathological conditions of the prawn. The prawn selected for the present study is widely used in the shrimp culture fields of tropical regions along with *Penaeus monodon*.

MATERIALS AND METHODS

Live specimens of *P. indicus* were collected from the prawn culture fields of Vypeen island near Cochin, India, and were transported to the laboratory. They were maintained in fibreglass tanks containing filtered and aerated seawater (salinity: 32 ppt; pH: 8.0; and temperature: 30°C). Healthy immature prawns in the size range of 90–120 mm (TL) were selected, and housed individually in floating plastic cages. Moult staging was done using the uropod setogenesis, a method described by Vijayan (17). Prawns for mineral analysis were selected from seven moult stages, viz. stage A (early postmoult), stage B (late postmoult), stage C (intermoult), stage D0, D1′ (early premoult) and D1′′′, D2–3 (late premoult).

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Hemolymph (HL) samples from the individual prawns were collected directly from the pericardial cavity. The glass syringe and needle used were rinsed in an anticoagulant (10% trisodium citrate) prior to each collection. The hemolymph samples after collection were preserved in glass vials in frozen condition at −20°C until use.

After the extraction of the hemolymph, the prawns were sacrificed quickly and the tissues like midgut gland (MD), body muscle (MS) and exoskeleton (ES) were excised. Epidermis was scraped off from the exoskeleton samples. The tissues were cleaned, dried at 60°C till constant weight was obtained. After cooling, the samples were stored in desiccators with silica gel until further use.

Known weight (50–100 mg) of dried and powdered tissue and known volume (0.2–0.5 ml) of hemolymph were wet-digested using nitric acid and perchloric acid. Mineral (Ca and Mg) concentrations were determined by atomic absorption spectrophotometry on Perkin-Elmer model 2380 (1). Tissue samples were analysed for P using the method of Lowery *et al.* (11) reading the absorbance at 882 nm on an ECIL senior spectrophotometer.

Prawns in intermoult stage C were used for the mapping of minerals in the exoskeleton. For this purpose, different regions of the exoskeleton were selected as illustrated in Fig. 1 and mineral analysis was done as described above.

Statistical Analysis

Mean ($\bar{X}$) and standard deviation (SD) were calculated for all estimations. The significant differences in mineral values with respect to Ca, Mg and P during different moult stages were tested using one-way analysis of variance (ANOVA).

RESULTS

Calcium

Variations in calcium levels in hemolymph, muscle, midgut gland and exoskeleton during different stages of the moult cycle ($P < 0.01$) are given in Fig. 2.

Hemolymph Ca content showed a well defined pattern with a noticeable rise from the early premoult stage (0.826 mg · ml^{-1}) to its peak value at D2–3 (1.884 mg · ml^{-1}), followed by a decline through the postmoult stages to reach the minimum values during the intermoult (0.834 mg · ml^{-1}) and early premoult stages (0.826 mg · ml^{-1}). Muscle tissue showed the minimum Ca levels (1.745 mg · g^{-1}) in early postmoult stage A and increased gradually to attain maximum value (4.737 mg · g^{-1}) during the late premoult stage (D2–3). A gradual increase of midgut gland Ca from intermoult (7.506 mg · g^{-1}) to late premoult (12.7 mg · g^{-1}) and maximum in early postmoult (14.202 mg · g^{-1}) was noted during the study. In the exoskeleton, lowest Ca value (51.167 mg · g^{-1}) was detected in early postmoult stage A. From stage A onwards exoskeletal Ca content showed a linear increase up to early premoult stage D1′ (168.984 mg · g^{-1}). Thereafter, the level declined in the late premoult stage reaching the lowest level immediately after moulting.

Ca content in the exoskeleton varied from a minimum

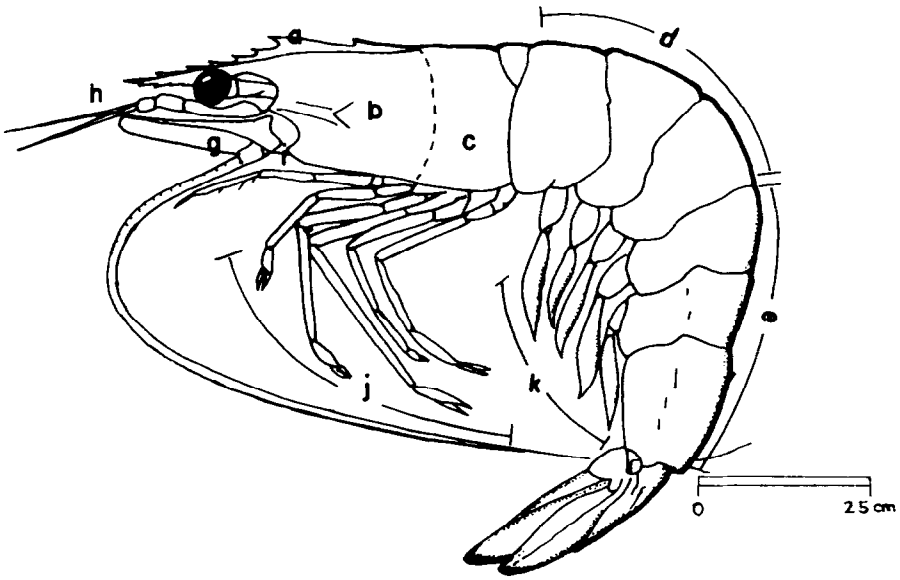

FIG. 1. Exoskeletal areas of *P. indicus* selected for mineral mapping

a) Rostrum
b) Upper region of carapace
c) Lower region of carapace
d) Upper abdomen
e) Lower abdomen
f) Telson
g) Antenna
h) Antennule
i) Mouth parts and maxillipeds
j) Walking legs
k) Swimmerets
l) Telson

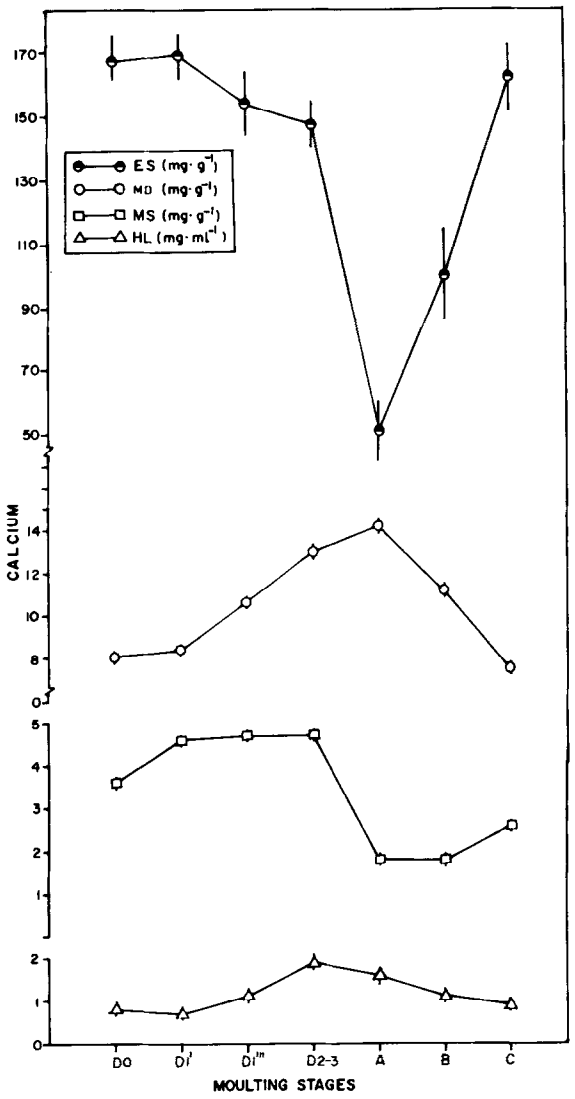

FIG. 2. Calcium fluctuations in the ES, MD, MS and HL during the different moult stages of *P. indicus*. (Each value is the average of eight replicate estimations: error bars indicate standard deviation.)

of 29.32 mg · g^{-1} in the swimmerets to a maximum of 393.75 mg · g^{-1} in the rostrum. The rostrum, carapace, abdomen, and telson showed higher levels of Ca with an average of 182.72 mg · g^{-1}, while average Ca content of the appendages was comparatively low with 136.85 mg · g^{-1}. Differences in the levels of Ca content were also noted between the adjacent areas of the same exoskeletal regions such as carapace and abdomen (Fig. 3). The anterior region of the carapace was found to contain more Ca (176.32 mg · g^{-1}) compared to the posterior region where it was comparatively low (82.81 mg · g^{-1}). Similarly, the Ca in anterior and posterior regions of the abdominal cuticle varied, with 136.35 mg · g^{-1} and 165.09 mg · g^{-1}, respectively. The mean Ca content of the exoskeleton in *P. indicus* was 159.52 mg · g^{-1}.

Magnesium

Changes in Mg levels in hemolymph, muscle, midgut gland and exoskeleton during different stages of moult ($P < 0.01$) are presented in Fig. 4. Moult-linked variations of Mg in the tissues followed a similar pattern to that of Ca, but the Mg levels of the tissues were considerably lower than those of Ca.

The Mg distribution pattern in the exoskeletal areas was similar to that of calcium distribution (Fig. 5). Magnesium content of the exoskeletal regions ranged from 3.99 mg · g^{-1} (swimmerets) to 28.91 mg · g^{-1} (rostrum). The mean Mg concentration of the exoskeleton was 11.94 mg · g^{-1}.

Phosphorus

Phosphorus content of hemolymph, muscle, midgut gland and exoskeleton during different stages of moult are given in Fig. 6. Hemolymph and muscle did not show significant ($P > 0.05$) variation, while variation in exoskeleton and midgut gland were significant ($P < 0.01$). Minimum P levels in hemolymph were at stage DI′ (0.042 mg · g^{-1}) and maximum at D2–3 (0.108 mg · g^{-1}). Unlike Ca and Mg, higher P levels were noted in postmoult stages A and B. In muscle, the variation of P was similar to hemolymph, with an early premoult minima and late premoult maxima. Midgut gland P showed a gradual increase from stage A (1.46 mg · g^{-1}) to DI′ (2.077 mg · g^{-1}) and a fall thereafter with the onset of late premoult. In the exoskeleton, highest P levels (26.319 mg · g^{-1}) were detected in late premoult stage D2–3, just before moulting. After ecdysis the concentration showed a declining trend in postmoult stages A and B, and minimum value (12.475 mg · g^{-1}) was reached in the intermoult stage C.

In contrast to Ca and Mg, P did not exhibit any marked regional differences in its distribution in the penaeid exoskeleton (Fig. 7). Notable differences in the P levels were not detected between the different regions of the exoskeleton. The mean P concentration recorded in the exoskeleton was 12.07 mg · g^{-1}.

DISCUSSION

Studies on the distribution of Ca, Mg and P in hemolymph, muscle, midgut gland and exoskeleton of *P. indicus* showed a significant mobilization and accumulation of these minerals in the tissues during the moult cycle of the prawn. The most notable changes were exhibited by calcium, which is the principal inorganic material of the exoskeleton. The demand for calcium is particularly high as the exoskeleton is shed regularly with each moult to allow an increase in body size (7).

The Ca content of the midgut gland of intermoult animals increased from 0.75% to 1.3% in the late premoult stage D2–3 just before moulting and reached the highest level of 1.42% immediately after moulting in the A stage

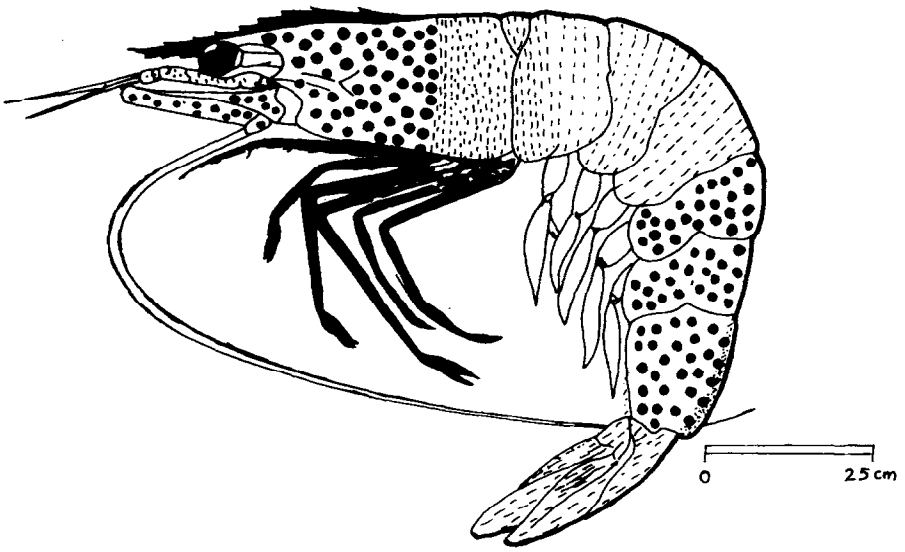

FIG. 3. The pattern of Ca distribution in the exoskeleton of *P. indicus*

- 0–50 mg · g^{-1}
- 50–150 mg · g^{-1}
- 15–250 mg · g^{-1}
- >250 mg · g^{-1}

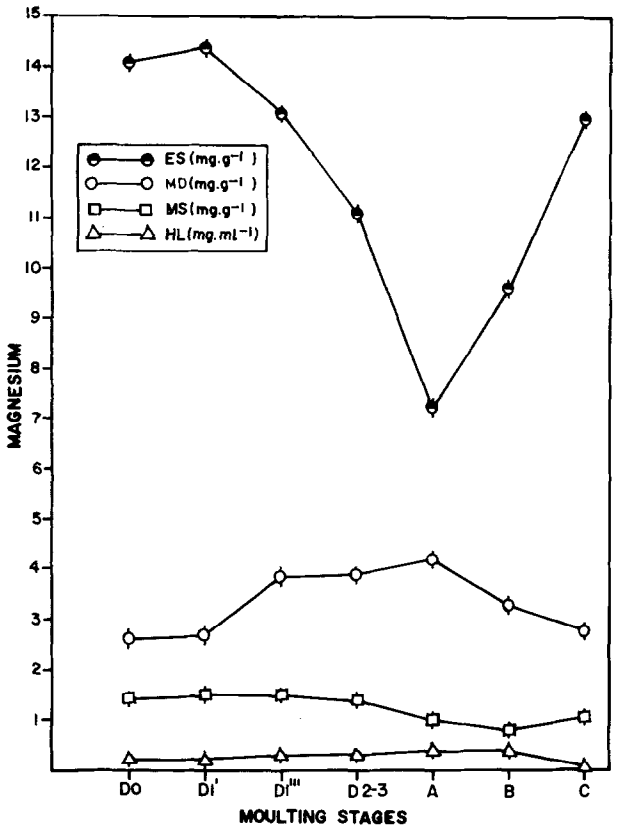

FIG. 4. Magnesium fluctuations in the ES, HP, MD and HL during the different moult stages of *P. indicus*. (Each value is the average of eight replicate estimations: vertical lines indicate standard deviation.)

(Fig. 2). These changes observed in the Ca levels of midgut gland before and after ecdysis probably show that the resorbed Ca from the old exoskeleton during late premoult is being transported to the MD for its subsequent use in postmoult mineralization.

Moderate Ca build-up noticed in the muscle tissue from postmoult to premoult can be due to the absorption from food and water and their deposition in the muscle until ecdysis. A notable reduction of Ca levels in muscle tissue of *P. indicus* from D2–3 (4.74 mg · g^{-1}) to stage A (1.75 mg · g^{-1}) in the present study probably indicates that the muscle tissue may also contribute Ca at the time of postmoult calcification.

Hemolymph being the transportation medium, Ca levels of the *P. indicus* showed marked changes during different stages of the moult cycle (Fig. 2). Just before ecdysis, the concentration of Ca in the hemolymph increased to about double the intermoult value and then decreased in postmoult stages. A premoult rise and postmoult fall in the hemolymph Ca can be cited as an evidence for Ca absorption from the ambient water, muscle, midgut gland and old exoskeleton during ecdysis, and its successive mobilization to the newly formed exoskeleton for calcification. Similar observation on calcium fluctuation during the moult cycle of the marine prawn *Aristeus anttennatus* was reported by Sarda *et al.* (15).

The levels of Ca variation in different tissues of *P. indicus* during the moult cycle suggest that the major source of Ca for cuticular mineralization comes from the ambient water by direct absorption. Tissue Ca of the prawn may probably play a supporting role by supplying Ca when there is a sudden postmoult demand. Contrary to penaeids, in freshwater crustaceans the main source for exoskeletal calcification is the stored Ca in tissues such as gastrolith, midgut gland and haemolymph (16).

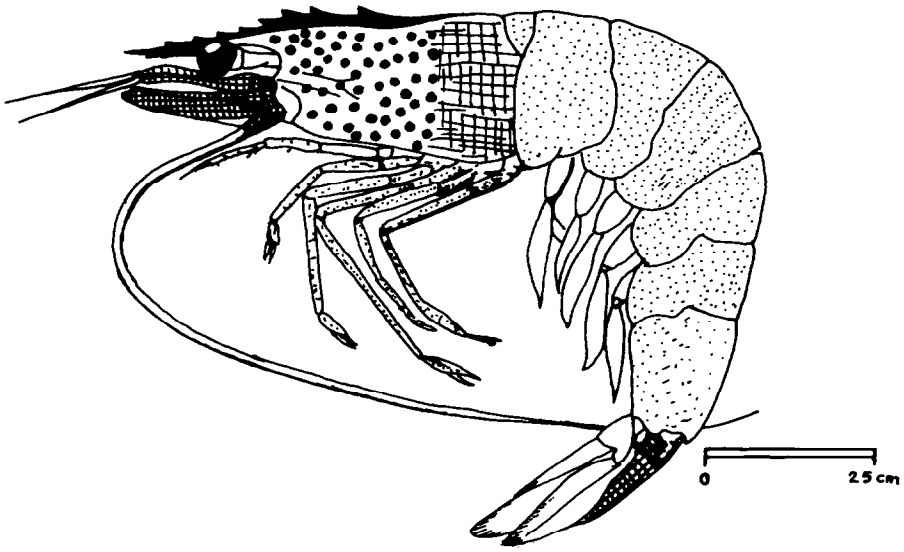

FIG. 5. The pattern of Mg distribution in the exoskeleton of *P. indicus*

- 0–5 mg · g^{-1}
- 5–10 mg · g^{-1}
- 10–15 mg · g^{-1}
- 15–25 mg · g^{-1}
- >25 mg · g^{-1}

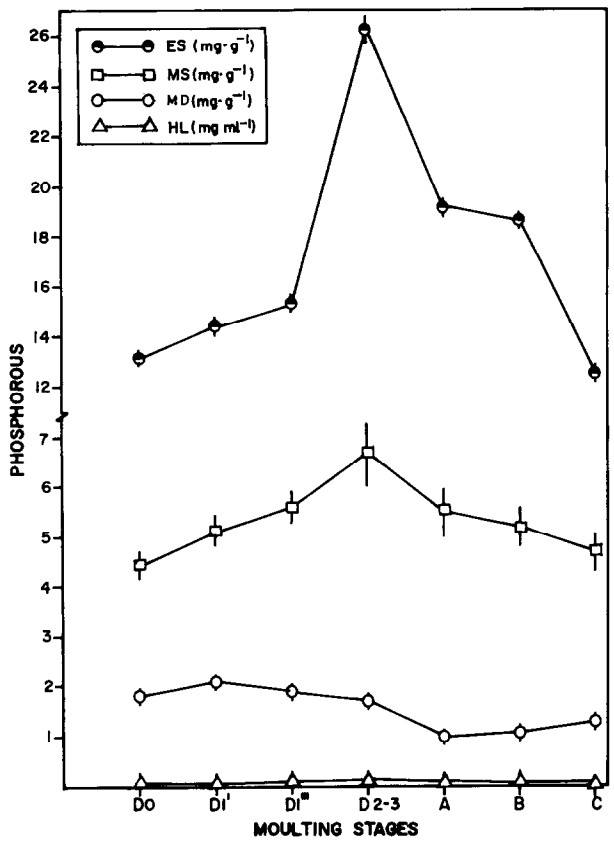

FIG. 6. Phosphorus fluctuations in the ES, MS, HP, and HL during the different moult stages of *P. indicus*. (Each value is the average of eight replicate estimations: error bars indicate standard deviation.)

The distribution of exoskeletal Ca observed in *P. indicus* (Fig. 3) showed a close resemblance with the basic pattern reported for heavily mineralized decapods (7), and this concurs with the observation of Huner et al. (9), who studied the postmoult mineralization of exoskeleton in the juvenile prawn *P. californiensis*. The concentration of Ca observed in the exoskeleton of *P. indicus* is low (16%), when compared with those of highly mineralized crustaceans with 20–29.1% (3,4). It is, however, comparable to the values obtained for other prawns, *P. monodon* and *P. occidentalis* (14–17%) (19), *P. duorarum* (16%) and *P. californiensis* (12.14%) (18), and *Metapenaeus* sp. (19%) (5). The differences in the concentration of exoskeletal Ca can be attributed to species differences (8).

In *P. indicus* the trend of variation exhibited by the Mg in different tissues studied was similar to that of Ca (Fig. 4). It is logical that Mg, which is believed to be a substitute for Ca in the mineral matrix of the crustacean exoskeleton (14) showed the same trend as that of Ca. Although Dall (5) has reported the Mg levels in intermoult carapace of decapods to be below 0.5%, Huner *et al.* (9) have reported a Mg level of 1.25% in the carapace of *P. californiensis*, which is comparable to the present observation in *P. indicus*. The relatively low concentration of exoskeletal Mg suggests a less important role for this mineral in the overall structural integrity of crustacean exoskeleton.

Phosphorus levels in the haemolymph and muscle of *P. indicus* are so low that its role in the cuticular mineralization is seemingly less important in this prawn, as reported earlier by Passano (13). A slow but steady increase of midgut gland P from early postmoult to early premoult and its fall immediately before and after moult observed in the present study, probably indicates the build-up of P during the tissue growth and its subsequent use at the time of ecdysis as an energy source (Fig. 6). Exoskeletal P content

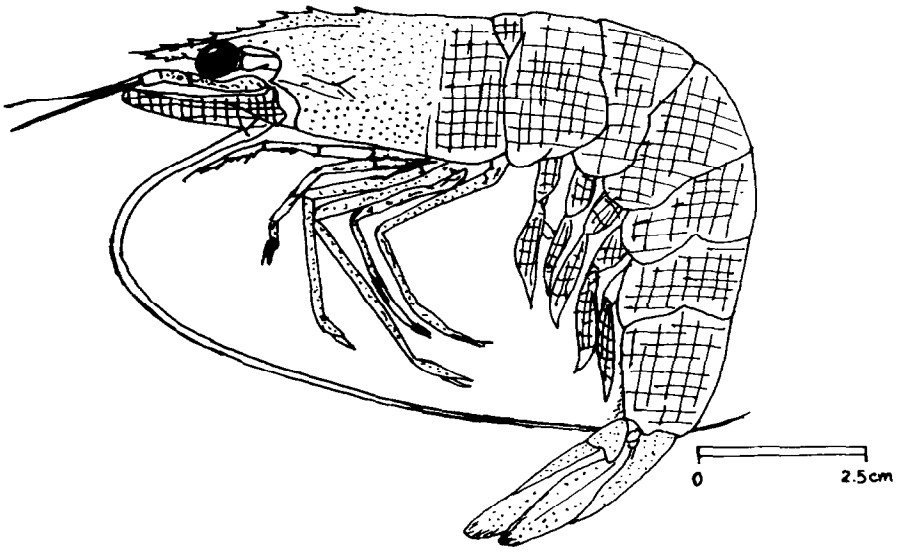

FIG. 7. The pattern of P distribution in the exoskeleton of *P. indicus*
0–12 mg · g^{-1}
>12 mg · g^{-1}

of *Metapenaeus* sp. and *P. californiensis* was studied by Dall (5) and Huner *et al.* (10), and their values, 0.5% and 0.7%, respectively, were comparatively lower than the values obtained (1.2%) in the present study. In contrast to the exoskeletal Ca and Mg, P values showed maximum concentration in late premoult and postmoult, showing its less important role in the exoskeletal mineralization.

The present investigation on the distribution of Ca in *P. indicus* has shown that the Ca levels in each region of the exoskeleton varied. Relative differences in Ca distribution were similar to those reported for crayfish (12), although *P. indicus* is considerably less calcified than the crayfish. Regional differences in the exoskeletal Ca content of *P. indicus* may be due to the restricting distribution for weight reduction, since penaeids are swimming animals.

The pattern of Mg distribution in the exoskeletal regions in *P. indicus* showed a close resemblance to that of Ca distribution (Fig. 5). Unlike Ca and Mg, P did not show much variation over the different regions of the exoskeleton (Fig. 7). An earlier report by Huner et al. (10) on the exoskeletal Mg and P of *P. californiensis* is in agreement with the present study.

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