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# STUDIES ON THE IONIC AND ALKALINE PHOSPHATASE CHANGES ASSOCIATED WITH THE MOULT CYCLE AND EYESTALK ABLATION OF PENAEUS JAPONICUS (BATE)

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## ABSTRACT

This study establishes a relationship between the moult stages of prawn Penaeus japonicus (Bate) and calcium, magnesium and phosphorus levels in the exoskeleton, hepatopancreas and abdominal muscles at these stages. Both calcium and magnesium contents in the exoskeleton increased during the time between early postmoult to intermoult and decreased at premoult, demonstrating deposition and reabsorption of these minerals during the moult cycle. The low amounts of calcium and magnesium in the hepatopancreas suggest a limited role of the organ in the storage of these minerals in premoult. The role of the hepatopancreas in phosphorus mobilization is still unknown.

Extracts of the hepatopancreas from eyestalkless and control animals do not show any significant difference in their alkaline phosphatase activity. In both groups the alkaline phosphates activity increased over a period of captivity of 21 days.

## INTRODUCTION

Moulting is the most important process dominating the biology of crustaceans. Significant metabolic changes mediated by neuroendocine control occur throughout the moult cycle (Passano, 1960 and Skinner, 1985). In order to characterize these physiological and biochemical changes, it is essential to identify the moult stages of the animal. In addition, practical applications of moult staging techniques in crustacean culture, such as the timing of eyestalk ablation, hormone treatment and harvest, have been noted (Freeman et al., 1987 and Robertson et al., 1987).

Moult stages have been documented in many groups of Crustacea including penaeids (Robertson et al., 1987). A scheme to describe the moult cycle of natantians was devised by Drach and Techernigovtzeff (1967) and Chu et al., (1988). The five major stages in this scheme are A (early postmoult), B (late postmoult) C (intermoult), D (premoult) and E (ecdysis).

From the previous physiological studies on Crustacea, it is known that eyestalk hormones influence the metabolism process of the animal. Most data on the hormonal effects of the sinus gland on metabolism seem to indicate to an inhibition of oxygen consumption and a saving of glucose, (Passano, 1960).

The central role of hepatopancreas in storage and in digestive enzyme production and secretion is also beyond doubt (Von Buddenbrock, 1956; Vonk, 1960 and Vanherp, 1970).

The present work is aimed to study the changes of calcium, magnesium, and phosphorus levels in each of exoskeleton, hepatopancreas and abdominal muscles of prawn Penaeus japonicus at different moult stages. In addition, alkaline phosphatase in the hepatopancreas was also studied to look for the possible regulatory mechanisms in the sinus gland which might affect synthesis and/or secretion of alkaline phosphatases in the hepatopancreas.

Calcium is the major inorganic component of the exoskeleton and it is used to calcify the new exoskeleton. Calcium metabolism in crustaceans is complex, and is associated with the moulting cycle. In freshwater decapods (Procrambus clarkii), calcium from the eroding exoskeleton is transferred to the hemolymph during premoult, then stored in the hepatopancreas (Fieber and Lutz, 1985) for use during the next moult. In marine water decapods, replacement of body calcium lost during ecdysis is achieved mainly by calcium absorption from sea water (Parado-Estepa et al., 1989).

Serum and tissue enzyme analyses are becoming increasingly important for detection of the moult stages (Abd El-Hamid, 1988). Acid phosphatase is involved in mineral metabolism. Phosphate, borate and oxalate are known to inhibit all types of enzymes (Teitz, 1976). Some divalent ions, such as $Mg^{2+}$, $Ca^{2+}$ and $Mn^{2+}$, activate alkaline phosphatase (Teitz, 1976), whereas beryllium and silver inhibit it (Jackim et al., 1970).

Acid phosphatase participates in mineral metabolism and alkaline phosphatase is a metal-requiring enzyme (Jackim et al., 1970). Trace amounts of added cations might displace or otherwise affect the metal containing moiety and thus modify enzyme activity.

## MATERIALS AND METHODS

Adult specimens of both sexes, each weighing about 40-50 g were caught during the winter (from November to February) from Abou-Kir Bay (east of Alexandria). They were very carefully transported to the laboratory avoiding rough handling and injuries.

The specimens are placed in glass tanks (each measuring 30 X 40 X 40 Cm volume, with a small amount of circulating sea water created by pumps) each tank containing about 10 animals maximum.

The prawns were fed daily on minced fresh prawns at a rate of 10 % of their body weight. Prawns were maintained in these tanks for at least 2 weeks acclimatization period before being taken for observation and analysis. During this study, the water temperature ranged from 22°C to 26°C and salinity ranged from 30 to 33 ‰. The duration of moult cycle for the animals under these environmental conditions was about 3 to 10 days.

For the determinations of mineral levels in body tissues, carapace length of the prawn after identifying its moult stage was measured with a pair of calipers. Exoskeleton (excluding the cuticle of the appendages), hepatopancreas and abdominal muscles were then carefully isolated. Firstly, the tissues were separately dried in a an oven at 60°C to constant weight and reweighed. They were then ashed at 550°C in Muffle Furnace for 24h and dissolved in concentrated hydrochloric acid. Calcium and magnesium concentrations were determined with flame photometry. Whereas concentration of total phosphorus was determined according to Fiske and Subbarow (1925) method.

To study the influence of sinus gland extirpation on the alkaline phosphatase in the hepatopancreas, in the course of time, the following experimental protocol was adopted. After a period of adaptation to laboratory conditions, twenty five prawns were used in each experiment. From which 15 sinus glands were removed following the method of Liao and Huang (1972). While the remaining 10 animals were used as controls. The ablated and intact animals were analyzed after 1 , 14 and 21 days respectively.

After rinsing the hepatopancreas for one minute in streaming water, its wet weight was determined and then homogenized for one minute with its double volume of extraction solution (0.9 % Na Cl, containing 4 % N, butanol, (Denuce, 1967). After one hour, the homogenate was centrifuged at 9000 rpm/min under refrigeration. Alkaline phosphatase activity in the supernatant was assayed by the method of Bessey et al (1946), using p-nitrophenylphosphate (sodium salt) as a substrate. The concentrations are measured by a Beckman DU spectrophotometer at a wave length of 405 U, using one cm wide glass cuvettes.

The results are treated statistically as suggested by Bergmeyer (1962). The phosphatase activity was expressed in m-mole units/g hepatopancreas tissue.

## RESULTS

Dry weight of exoskeleton, hepatopancreas and abdominal muscles for animals of 17 mm carapace length are calculated for each moult stage and are shown in Table (1). It is evident that dry weights of the exoskeleton, hepatopancreas

Table (1): Mean values of dry weights of exoskeleton, hepatopancreas and abdominal muscles (mg) during the moult stages of peneus japonicus (Bate)

| Dry wt of tissues (mg) | Moult stages A | | | B | | | C | | | D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. | Mn. | Wt. | No. | Mn. | Wt. | No. | Mn. | Wt. | No. | Mn. | Wt. |
| Exoskeleton | 6 | 120 | ± 0.13 | 9 | 161 | ± 0.21 | 9 | 173 | ± 0.15 | 10 | 155 | ± 0.21 |
| Hepatopancreas | 6 | 19 | ± 0.01 | 10 | 27 | ± 0.16 | 10 | 26 | ± 0.10 | 10 | 25 | ± 0.32 |
| Abdominal muscles | 6 | 215 | ± 0.12 | 10 | 270 | ± 0.11 | 9 | 256 | ± 1.31 | 10 | 250 | ± 0.41 |

No. indicates numbers of prawn that regression analysis is based on.
Mn. Wt. indicates Mean Weight in mg. (Carapace length in 16 mm) at different moult stages.

and abdominal muscles respectively in animals at stage A (early postmoult) were lower than those at the later moult stages. Dry weight of exoskeleton was the highest at stage C (intermoult).

At each of these weight values, the corresponding three mineral concentrations were determined. Variations of calcium, magnesium, and phosphorus levels in the hepatopancreas, muscles and exoskeleton during each of the five moult stages are shown in Fig. (1). The data were analyzed by one-way analysis of variance. Variation in calcium with moult stages was significant ($0.01 < P > 0.05$) for the exoskeleton and muscles. No variation in phosphorus for the muscles was significant in all the moult stages. Calcium concentrations in the exoskeleton of animals at stages A and D appear to be lower than those at stage B and C, whereas the phosphorus concentration showed a reverse trend. Calcium amounts in the muscles was high at stage A, while phosphorus concentration in the hepatopancreas was low at stage C. Variations in minerals concentration at other stages were statistically insignificant ($P > 0.05$).

As the weight of body tissues changes with moult stage, expression of minerals composition by concentration cannot reflect actual changes in minerals content.

Absolute amounts of calcium, magnesium, and phosphorus in each prawn tissue were calculated and are shown in Fig. (2). Differences in amount of calcium or magnesium in the exoskeleton during the moult cycle are highly significant. The amounts of both minerals were low in prawns at stage A, increasing to high values at stages B and C and decreasing at stage D. From stage C to D calcium and magnesium decreased by 20 and 22 % respectively. The two minerals seemed to show a reverse trend in the hepatopancreas, but such a variation was not statistically significant ($P > 0.05$). No significant variation in calcium and magnesium was found in the abdominal muscles. The variation in phosphorus was, however, significant for the hepatopancreas ($0.01 < P > 0.05$), but neither in the exoskeleton nor in the abdominal muscles the variation was significant.

Table (2) shows the results of the experiment made in April, whereas Table (3) presents the results of the experiment performed in June of the same year (1993).

It is shown that the alkaline phosphatase activity rises continuously during the whole experiment in the intact as well as in the sinus glandless animals. A statistical analysis of the data shows that this rise is significantly different between the $7^{th}$, $14^{th}$ and $21^{st}$ day.

There is no significant variation in the alkaline phosphatase activity between the intact and $7^{th}$ operated animals. It is clear that the sinus gland extirpation has slight influence on the alkaline phosphatase activity in the hepatopancreas of the prawn Penaeus japonicus (Bate) at 21 days.

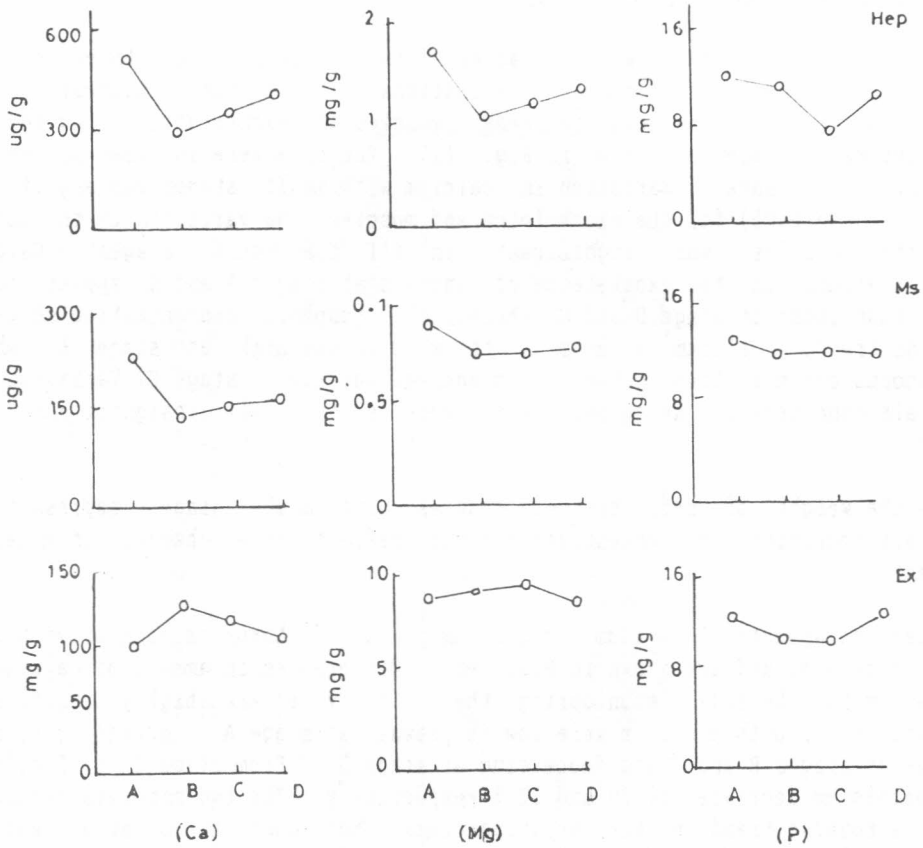


Figure 1: Calcium, magnesium and phosphorus concentrations (in ug or mg per dry weight) in the exoskeleton, hepatopancreas and abdominal muscles of Penaeus japonicus at different moult stages. Data are expressed as mean values of 10 individuals.

Hep = hepatopancreas M = muscles Ex = Exoskeleton
Ca = calcium Mg = magnesium P = phosphorus.

Table (2): Mean values of alkaline phosphates activity in the hepatopancreas of sinus glandless and control prawn during April, 1993.

| Time of determination (in days) | State of Animal | Wet wt of Hep/ (g) | alkaline phosphatas m-moles/g hep. |
|---|---|---|---|
| 7 | operated | 1.5977 ± 0.143 | 4.92 ± 0.071 |
| | control | 1.3789 ± 0.128 | 4.416 ± 0.0967 |
| 14 | operated | 1.2776 ± 0.210 | 16.248 ± 0.120 |
| | control | 1.4447 ± 0.019 | 12.19 ± 0.110 |
| | operated | 0.85632 ± 0.094 | 25.25 ± 0.210 |
| 21 | control | 0.856 ± 0.086 | 24.60 ± 0.163 |

Hep = Hepatopancreas tissue ± = standard error of the mean.
Each data point represents mean (10 animals). -

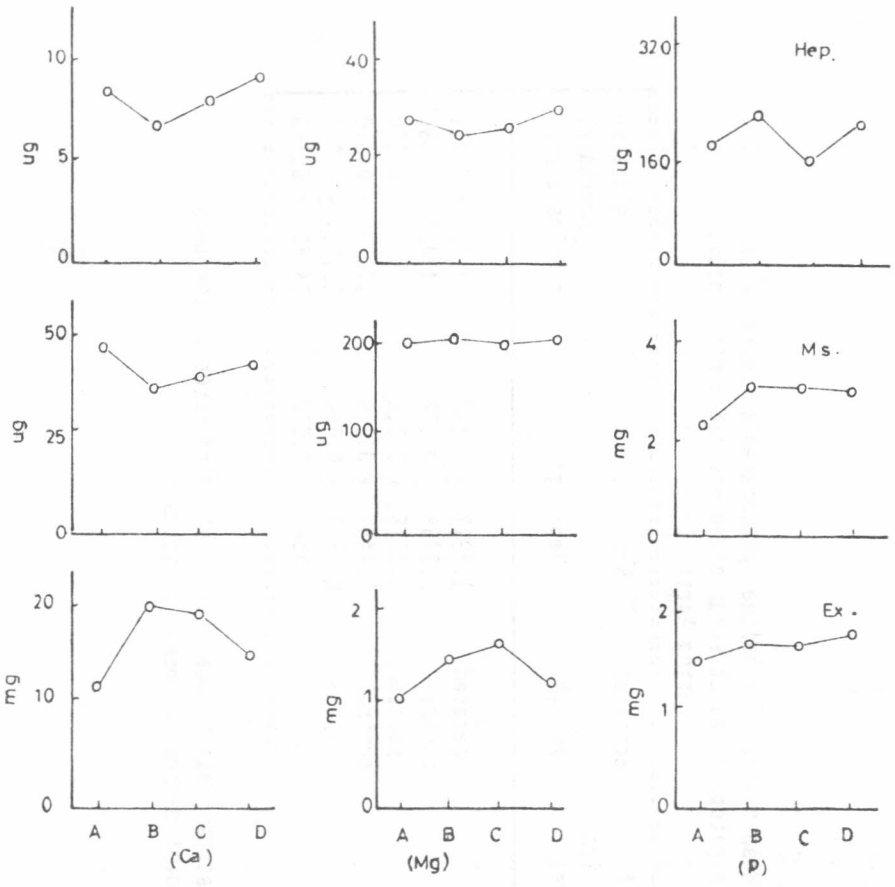


Figure 2: Absolute amounts of calcium, magnesium and phosphorus in the hepatopancreas, abdominal muscle and exoskeleton of a standard Penaeus japonicus (Bate) (Carapace length = 17 mm) at different moult stages. Data are expressed as mean values of 10 individuals.

Hep = hepatopancreas M = muscles Ex = Exoskeleton
Ca = calcium Mg = magnesium P = phosphorus.

Table (3): Mean values of alkaline phosphatase activity in the hepatopancreas of sinus glandless and control : Penaeus japonicus (Bate) during June 1993.

| Time of determination (in days) | State of Animals | Wet wt of Hep/ (g) | alkaline phosphatase m-moles/ g hep |
|---|---|---|---|
| 7 | operated | 1.5977 ± 0.05 | 4.920 ± 0.022 |
| | control | 1.3789 ± 0.05 | 4.416 ± 0.091 |
| 14 | operated | 1.2776 ± 0.04 | 16.248 ± 0.132 |
| | control | 1.4447 ± 0.03 | 12.190 ± 0.183 |
| 21 | operated | 0.8563 ± 0.01 | 25.250 ± 0.220 |
| | control | 0.856 ± 0.01 | 24.606 ± 0.201 |

Hep = hepatopancreas
Each data point represents mean (10 animals).
± standard error of the mean.

## DISCUSSION

The study of three mineral level in body tissues indicates the moult stages of Penaeus japonicus (Bate). The pattern of variation in calcium and magnesium levels in the exoskeleton, representing mineral reabsorption and deposition at premoult and postmoult respectively, was found to be similar to other Decapoda (Passano, 1960 and Chu et al., 1988). From Fig (1) it was estimated that about one-fifth of the calcium content in the exoskeleton of prawn is removed at premoult. Although the possibility of rapid calcium reabsorption just before ecdysis cannot be excluded, this finding supports the view that much calcium is lost with the exuvia. Reabsorbed calcium is believed to be taken up into the haemolymph and then eliminated or stored (Passano 1960). Elevation of calcium concentration in haemolymph at premoult has been demonstrated in Penaeus duorarum (Bursey and Lane, 1971) and Penaeus chinensis (Chu et al., 1988).

It has been suggested that the hepatopancreas is responsible for calcium mobilization in the moult cycle, particularly for the storage of calcium during premoult (Glynn, 1968). The amount of calcium in the hepatopancreas of Penaeus japonicus, is however, much too low for the organ to play a significant role in this process. Similar evidence has been reported for Metapenaeus sp. (Dall, 1965). These findings suggest that most of the calcium removed from the exoskeleton during premoult is not stored in the hepatopancreas and may be eliminated. The reabsorption of calcium would facilitate the stretching and splitting of the old exoskeleton during ecdysis.

The present study shows that calcification of the new exoskeleton was essentially completed at stage B, with levels reaching values similar to those in intermoult (stage C). In penaeids, stage A usually lasts less than 24 hours (Smith and Dall, 1985, and Chu and Chow, 1992). Completion of mineral deposition within a short time after ecdysis has been reported for penaeids (Huner and Colvin, 1979) as well as for other decapods (Vigh and Dendinger, 1982). Calcium needed for calcification of the new integument can be supplied from the diet or by absorption from ambient water (Stevenson, 1985).

The importance of phosphorus in the moult cycle has been ignored in most studies. Phosphorus variation in Penaeus japonicus appears to be different in the three studied tissues. Glynn, (1968) suggested that erratic phosphorus variation in organs was due to intense competition for it from diverse metabolic demands. Variations in phosphorous levels in tissues evidently depends on changes in organic and inorganic constituents, both of which were shown to vary with moult stage (Yamaoka and Scheer, 1970). Further studies on changes in the levels of these metabolites during the moult cycle are necessary to elucidate phosphorus mobilization in penaeids.

The variability in enzyme activity between several individuals of a given series can be attributed to the presence of enzymes in the circulatory system of the hepatopancreas, and which was not washed out completely (Van Herp, 1970). Also it is possible that this variability reflects different stages in the secretion process as suggested by Denuce (1967) for Orconectes virilis and Van Herp (1970) for Astacus leptodactylus.

The phosphatase activity in the hepatopancreas is not influenced by the sinus gland, the question is left whether the changes in enzyme activity were due to environmental conditions or not. Mc Whinnie and Kirchenberg (1966) obtained two peaks of phosphatase activity in the hepatopancreas of Orconectes virilis and found that the activity, in connection with the peak at pH 8.0 - 8.5, rises in spring. Hirsch and Jacobs (1929) pointed out large seasonal changes in the amylase, peroxidase, and protease activities from Astacus leptodactylus. The possibility that the changes in phosphatase activity are correlated with temperature and seasons are not excluded. The rise could be in accordance with the results of Van Herp (1970) and could also be a part of the preparations for moulting, which are also reflected in the glycogen, nitrogen, protein, lipid and mineral (mainly calcium and phosphate) metabolism (Hashem and Abd El-Hamid, 1988). Restricted freedom of movement could be another explanation for the increasing phosphatase activity during captivity.

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