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EFFECT OF SALINITY VARIATION ON HEMOLYMPH CALCIUM CONCENTRATION DURING THE MOULT CYCLE OF THE PRAWN PENAEUS JAPONICUS (BATE)

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ABSTRACT

Prawns Penaeus japonicus were obtained from Abu-Kir Bay (east of Alexandria) and maintained in glass aquaria in laboratory at different salinities ranging from 10 to 40‰. Total hemolymph calcium levels were measured during the moult cycle. Results showed that these levels were largely affected by moult stage and to a lesser degree by salinity. A sharp, transient increase in hemolymph calcium occurred 3 to 6 h postmoult, followed by an equally rapid decrease from 6h postmoult to intermoult. This biphasic response was limited to prawns in 10, 20 and 30‰ salinity; while in 40‰ salinity, hemolymph calcium remained the same throughout the experimental period. Peak concentrations of total calcium observed for low salinities, (10 & 20‰) were still higher than those recorded for the higher salinity (30 & 40‰). Salinity had no effect either on the duration of moult cycle or on time of moult occurrence.

INTRODUCTION

Moulting is essential to crustaceans and other arthropods for it enables the animal to grow as well as to recover from injury. Some penaeid prawns inhabit estuaries and are subjected to large fluctuations in environmental factors, mainly salinity and temperature. some reports have described faster growth in isosomatic salinities, Parado-Estepa et al. (1989), but these studies monitored growth of prawns in laboratory where salinity can influence not only the physiology of the organism but also the nature of the foodweb and quantity of food.

Calcium is the major inorganic component of the exoskeleton and it is used to calcify the new exoskeleton. Calcium metabolism in crustaceans is a complex process and is associated with the moulting cycle, Parado-Estepa, et al, (1989). In freshwater Decapoda (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. Other *freshwater decapods e.g.* (*Austropotamobius pallipes* & Gammarus pulex) have transport mechanisms with high affinity for calcium ions (Wright, 1979), and are able to absorb calcium from very dilute media. Regardless of absorptive or storage mechanisms involved, hemolymph calcium increases during premoult, and decreases during postmoult (Fieber and Lutz, 1985). In marine and brackishwater decapods, replacement of body calcium lost during ecdysis is achieved mainly by calcium absorption from seawater (Graf, 1978 and Greenaway, 1983). Differences in salinity and external calcium concentration could alter the availability of calcium ions to the organisms. Hence, salinity can influence calcium metabolism in penaeids, and affect moulting frequency. Haefner (1964) found a correlation between environmental salinity and postmoult body weight of crab Callinectes sapidus, and proposed that crabs in low salinities possess lighter exoskeletons than those in high-salinity waters.

The present study was initiated in order to examine the total calcium concentration in the hemolymph of Penaeus japonicus (Bate), as a function of moult stage and salinity variation. It is also aimed to monitor the duration of the moult cycle as a function of salinity changes and to note the moult process as a function of time of the day as well.

A higher salinity and temperature are usually more favorable for inducing maturity. Alikunhi, et al., (1975) achieved maturation of Penaeus monodon at a salinity of 24-31 per mill and at a temperature of 27.7-30.5 °C, while Primavera (1977) found a salinity of 30-34 per mill to be more conductive to maturation and spawning of the same species. With the Indian prawn Penaeus monodon, however, full maturation and spawning were achieved at a salinity of 15-25 per mill and a water temperature of 24.4-26.4 °C (Halder, 1978). Hashem (1991) observed that salinity of 32.5-37.5 per mill and a temperature of 27-32 °C were more suitable for maturation and spawning of the prown Penaeus japonicus (Bate).

MATERIALS AND METHODS

Juvenile prawn Penaeus japonicus (Bate) of mean weight 20 (± 5 g) were collected alive from Abu-Kir Bay (east of Alexandria) and were maintained in laboratory for at least two weeks in glass tanks with aerated and filtered sea water (with 30‰ Salinity and average temperature of 25°C).

After being acclimatized, prawns then placed in 30-liter indoor tanks with four compartments, one prawn per each compartment. Four experimental 30-liter tanks of varied salinity (10, 20, 30 & 40‰) respectively used. A total of 20 prawns were placed per each salinity variant. Water (15 liters) in the tanks was changed daily. Prawns were allowed to moult once in the experimental tanks. It is considered that this first moult is a stress-induced, and all prawns were incorporated into the experiment only after this initial moult in the experimental tank has happened.

The time (in days) elapsed from the second to third moult was recorded. Moult duration was compared using the one-way analysis of variance.

Prawns were sampled as a function of time after the second moult. This method allows frequent sampling at well-defined intervals during early postmoult when more dynamic changes in ion and osmotic concentrations occur in the hemolymph. This is not possible when anatomically-defined moult stages are used. Exuviation (time 0) was defined as the time when the prawn had fully emerged from the old exoskeleton, before the new carapace and chelae had tangible rigidity. Hemolymph from four individuals was pooled together to constitute one sample, and three replicates were collected per moulting period at one salinity variant. Total calcium concentrations in the hemolymph were determined using Colorimetry (Sigma No 586 Diagnostic Kit for determination of calcium in serum and plasma). Changes in the concentration of ionized calcium in the hemolymph were highly correlated with changes in total calcium concentration (Correlation coefficient = 0.96; according to Greenaway, 1983, and our calculation). The equation used by Ferraris et al., (1987) to analyze the data for hemolymph osmolality and chloride concentration was modified slightly because changes in calcium concentrations in the hemolymph were not hyperbolic, but instead increased slightly from moult to about 3h after moult.

RESULTS

Results of the present experiment, showed, large fluctuations in hemolymph calcium in Penaeus japonicus (Bate) that occurred only in the first day of moulting. A sharp but transient increase (10 to 22%, $P < 0.05$) in total hemolymph calcium concentration occurred 3 to 6h postmoult in prawns maintained at 10, 20 and 30‰ salinity. After this peak, a rapid decrease followed until a constant low level has reached throughout intermoult (1 to 10 day after moulting). The parameter estimates for prawns in 30‰ salinity were high but statistically insignificant, while those for prawns in salinities of 10 and 20‰ were also high but statistically significant.

Hemolymph calcium concentration of prawns in 40 ‰ salinity was independent of moult stage; the values remained the same ($P > 0.05$) throughout the experimental period. The data did not fit into the hyperbolic model of Ferraris, et al., (1987) and showed non-linear regression yielded a weak correlation and insignificant parameter estimates Fig. (1) and Table (1).

The hemolymph calcium of newly-moulted (3,6,12 h) prawns showed a statistically significant ($P < 0.05$) dependency of salinity. Hemolymph calcium of these newly-moulted prawns was reduced by 0.072 mM for every unit increase in salinity. Hemolymph calcium of prawns during moult and 1 to 10 d postmoult was statistically independent of salinity Fig. (2).

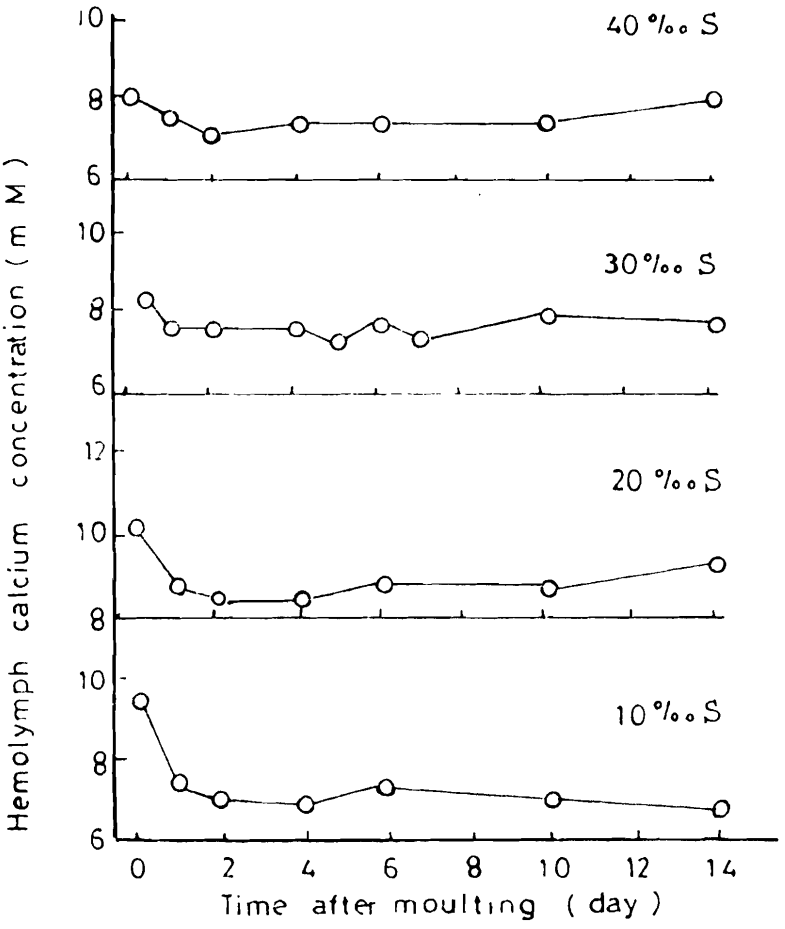

Figure 1: Variation of total calcium levels in hemolymph as a function of time after moulting, for Penaeus Japonicus at different salinities levels.

Table (1): Effect of different salinities on mean estimated hemolymph-calcium concentrarion in Penaeus Japonicus during moult and intermoult stages.

Salinity ‰	Mean estimated hemolymph calcium (mM) during:		Difference between moults	Survival rate between the 2nd and 3rd moults
	Postmoult	Intermoult		
10	9.4 ± 0.21	6.2 ± 0.42	20.5 ± 1.5	89
20	10.1 ± 0.24	8.4 ± 0.51	17.5 ± 1.3	94
30	7.8 ± 0.20	7.5 ± 0.42	24.0 ± 3.2	98
40	7.0 ± 0.16	7.3 ± 0.20	21.1 ± 2.4	97

Each point represents mean (+ standard error of the mean).

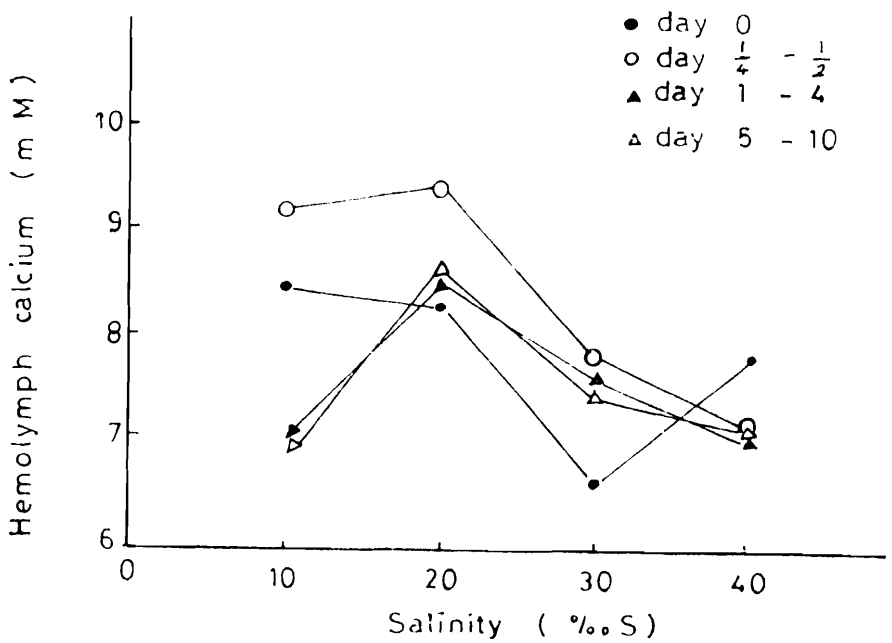

Figure 2: Variation of total calcium conceneration in hemolymph of Penaeus Japonicus under different external salinity.

Peak hemolymph calcium concentration were still higher in prawns at 10 and 20‰ than in those at 30 and 40‰ salinity. ($P < 0.01$); Table (1), Fig. (2). *Intermoult concentration of hemolymph calcium were nearly the same ($P > 0.05$) in* all salinities. The high hemolymph calcium in prawns at 10 and 20‰ salinity. was due to a large salinity dependent difference in hemolymph calcium between intermoult and early postmoult Table (1).

The time interval between moults was 20.5 ± 1.5 for 10‰ salinity, 17.5 ± 1.3 for 20‰, 24.0 ± 3.2 for 30‰ and 21.1 ± 2.4 for 40‰. Moult interval was, therefore, independent of salinity ($P > 0.10$). The survival rates between the second and the third moults for 10,20,30 and 40‰ salinity. treatments were 89, 94, 98 and 97% respectively. Moulting was most frequent between 18.01-0.00 (40%) than between 0.01-6.00 h (34%) and 6.01 - 12 h (16%) and least between 12.01-18.0 h (6%). Most moults (78%) occurred at night.

DISCUSSION

Hemolymph calcium in Penaeus japonicus (Bate) is mainly affected by moult stage and, to a much lesser degree, by salinity. There is no relationship between hemolymph calcium and external salinity for prawns 3 to 6h postmoult. In contrast, Ferraris et al., (1987) found that hemolymph osmotic and chloride concentrations to be simultaneously and interactively affected by salinity and moult stage.

Hemolymph osmolality and chloride increased when moult occurs at 10%.S.; remain relatively constant at 20‰ salinity., a salinity close to isosomatic and isoionic (chloride) values (Ferraris et al., 1987), or decrease at 30 or 40%. salinity. In contrast, the response of hemolymph calcium to a change in moult stage is biphasic at 10,20 and 30‰ salinity., with peaks occurring 3 to 6 h postmoult. These pronounced differences in the patterns of hemolymph response to a change in moult stage indicate that the mechanisms controlling calcium concentration has not been affected by those controlling osmolality or chloride concentration. The present observations on hemolymph calcium in Penaeus japonicus (Bate) differ slightly from those on whole-body calcium in P. californiensis (Huner et al., 1979). In this latter species, the whole body calcium increased rapidly from soft moulting stage to early post moult stage and intermoult, then subsequently declined to late premoult stages.

Intermoult penaeids regulate calcium levels (Penaeus aztecus; Mc Farland and Lee, 1963), Metapenaeus benettae; (Dall, 1965, P. monodon; Ferraris et al., 1986) and Parado-Estepa et al., 1989). Other decapod crustaceans (about 60% of those studied) also regulate hemolymph calcium independent of external calcium concentrations (Mantel and Farmer 1983). In contrast, studies conducted by Greenaway 1983) and Charmantier et al., (1984) showed that hemolymph calcium levels in Callinectes sapidus and Homarus gammarus change in parallel with the medium, with slopes close to unity. Compared to the other crustaceans, intermoult penaeids are generally excellent iono-and osmoregulators (Ferraris et al., 1986) and can survive abrupt changes in environmental salinity (Parado-Estepa et al., 1987).

Salinity affects hemolymph osmolality and chloride concentrations not only in new moults but also to a lesser degree, in intermoults. Moreover, at 20‰ salinity or greater, the differences between peak and intermoult concentrations of hemolymph osmolality and chloride, and the times required for these concentrations to reach intermoult values increase with salinity (Ferraris et al., 1987). This difference between peak and intermoult concentrations for hemolymph calcium as well as the time required decrease with salinity. It is obvious that, effects of salinity on hemolymph calcium are different from its effects on hemolymph osmolality and chloride.

The rapid reduction in hemolymph calcium from peak to intermoult values within one day after moult coincides with the observations that the exoskeleton of Penaeus japonicus is already hard and rigid (rostrum can not be bent), and that feeding commences within 24h after moulting. Because animals moult every 10 d, a 24 h non-feeding period represents a large (10%) fraction of the moult cycle. A slower development of the exoskeleton (24 h) could be detrimental because of starvation and increased chances for predation. Even under laboratory conditions, the mortality increases for individuals whose exoskeletons have not hardened by 24 h.

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