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Note

Haemolymph *p*H, clotting time and cation (Na, K, Ca & Mg) status of Indian white prawn, *Penaeus indicus* during mud bank formation

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Abstract

Haemolymph *p*H, clotting time and cation (Na, K, Ca & Mg) contents were measured in Indian white prawn, *Peneus indicus* H. Milne Edwards during mud bank formation and compared with prawns collected simultaneously from non-mud bank area. During mud bank formation, haemolymph recorded higher levels of cations (Na, K, Ca & Mg), lesser clotting time and constant *p*H values. Significantly higher concentration of cation sum was observed ($P < 0.05$) during mud bank formation. High sodium and low magnesium index was noticed in both the samples with the following sequence of cations - $Na^+ > K^+ > Ca^{2+} > Mg^{2+}$. Faster clotting rate of 6.02 min. was typical of stress condition created during mud bank formation. Significant change in cation and clotting time observed during mud bank indicated the physiological, biochemical and immunological changes undergone by prawns to cope with the surrounding environment. Alterations in cations can be used to study the adaptations of species to the environment and haemolymph-clotting time could be a useful indicator of stress.

The survival and successful establishment of an organism in a given habitat depends on the ability of each of its developing stages to adapt to the environment. Many fundamental features of the class Crustacea are reflected in the nature of the internal medium, the haemolymph. The haemolymph composition of organisms reflects the metabolism of the tissues and organs bathed by this fluid, which is a carrier of substrates, end products of catabolism and hormones. Some of the studies on Haemolymph in Arthropoda pertain to various biochemical parameters such as content of free amino acids (Faug *et al.*, 1992), proteins (Ferraris *et al.*, 1986), ions (Greenaway, 1993; Wheatly *et al.*, 1996) and other constituents. The mechanism of osmoregulation is based on efficient ionic regulation (Guy Charmantier, 1998). Despite considerable work on osmoregulatory ability of representative species, very few work has been done in the field of ionic concentration in penaeids. Related changes in the haemolymph electrolytes have been reported in *Procambarus clarkii* by Wheatly *et al.* (1996). Greenaway (1993) measured calcium and magnesium

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concentration in terrestrial crabs.

The Mud bank is a unique phenomenon noticed along the southwest coast of India (Regunathan *et al.*, 1984; Kurian and Sebastian, 1986). It is a calm and undisturbed area boardered by disturbing waves, formed during the wake of southwest monsoon, especially along the Kerala coast. The area of its formation is rich in organic load, clay, micro and macrofauna that form food of crustaceans, especially prawns. Mud bank area generally contributes to a good fishery for prawns and pelagic fishes (Nair, 1983; Regunathan *et al.*, 1984). Information regarding the haemolymph ionic status and clotting mechanisms during mud bank formation is scanty with reference to the Indian white prawn *Penaeus indicus*. This report presents how the stressful mud bank environment alters the ionic balance, *p*H and clotting mechanism in this prawn.

Material and methods

Samples of *P. indicus* of total length 120-130 mm (intermoult stage) were collected (n =50) from mud bank area off Vadanappilly, near Chettuva in Kerala during the southwest monsoon period of July - August 1997. Simultaneous collections of the same species (n =50) from non- mud bank areas were also made for a comparative study. Haemolymph was withdrawn from the pericardial sinus with No.24 hypodermic needle and immediately used for estimation of *p*H, clotting time and cations following standard procedures. Clotting time was measured by placing 0.5 ml of the haemolymph in a watch glass and examination of clot formation with a glass rod. Haemolymph *p*H was measured in a *p*H meter immediately after withdrawal. Sodium, potassium and calcium cations were assayed by Emission Flame Photometer (EFP). Magnesium ions were assayed by Atomic Absorption Spectrophotometer (AAS) using non- citrated haemolymph immediately after withdrawal. Results are given as mean values and standard deviations of triplicate analysis. Statistical significance of differences was calculated using ANOVA (Ross, 1987).

Results

Table 1 presents data on two parameters, *p*H and clotting time of *P. indicus* haemolymph collected from mud bank and non- mud bank area. Haemolymph *p*H remained almost constant without much variability during mud bank formation ($P > 0.05$). Faster clotting rate of 6.02 min. was measured in the prawns from mud bank. Significant difference in clotting time was observed between samples ($P < 0.05$).

The changes in cation contents during mud bank formation are summarised in Table 2. Significant difference in cation

Table 1. *Comparison of haemolymph* pH *and clotting time between locality*

	Locality	
Parameter	Mud bank	Non- mud bank
*p*H ^{ns}	7.54 ± 0.17	7.45 ± 0.22
Clotting time *	6.02 ± 0.12	9.06 ± 0.08

* $P < 0.05$ ^{ns} - not significant

Table 2. *Comparison of haemolymph cation concentration (Na, K, Ca & Mg) vs. locality*

	Locality †	
Parameter	Mud bank	Non- mud bank
Na mmol/ l *	122.94 ± 1.35	97.08 ± 1.84
K mmol/ l *	49.0 ± 3.38	46.20 ± 1.48
Ca mmol/ l *	6.36 ± 0.27	4.04 ± 0.475
Mg mmol/ l *	1.23 ± 0.054	0.73 ± 0.05

† mean of triplicate analysis. * $P < 0.05$

concentration (Na, K, Ca & Mg) was observed in *P. indicus* collected from mud bank region ($P < 0.05$). Cation sum was found to be higher in mud bank sample. High sodium and low magnesium index was observed in the following order - $Na^+ > K^+ > Ca^{2+} > Mg^{2+}$.

Discussion

Significant difference in cation concentration and clotting time during mud bank formation indicated variability. Constancy in the *p*H was indicative of the well-organized internal medium for cellular activities, physiology and metabolism. Higher concentration of cation sum recorded in the mud bank sample showed the stressful environment prevailed during the formation of mud bank. Drilhon (1935) reported a number of metabolic changes leading to increase of Ca^{2+} and Mg^{2+} in the haemolymph of *Carcinus maenas* during infection. Increase of Ca^{2+} and endotoxins of microorganisms have been found to stimulate clotting mechanism in crustaceans (Durliat, 1985). Mud bank is an area teeming with microorganisms (Kurian and Sebastian, 1986). Faster clotting rate of mud bank haemolymph may be due to the stress generated during mud bank formation and increase of Ca^{2+} ions in the haemolymph. Changes in clotting time could be an early indicator of stress or disturbance (Jussila *et al.*, 2001). The *p*H of the haemolymph remained unchanged during formation. It appeared that constant internal *p*H should be maintained for the smooth functioning of the organism and euryhaline crustaceans achieve this through ionic balance (Wolcolt, 1991).

High sodium and low magnesium index explains the efficient ionic regulation met by the aquatic crustaceans. During mud bank formation osmoregulation speeds up. Occurrence of osmoregulation is based on efficient ionic regulation (mainly Na^+ and Cl^-) and increased levels of Na^+ and K^+ ATPase activity (Guy Charmantier, 1998; Lignot *et al.*, 2000). Higher Ca^{2+} and Mg^{2+} concentration was experienced by mud bank prawns. A change in the salinity of sea water normally results in an equivalent change in its calcium concentration. In *Carcinus maenas* and *Callinectus sapidus*, calcium levels in the haemolymph increased in dilute medium (Greenaway, 1976).

Significant increase of cations, faster clotting time and constant *p*H level in the haemolymph of prawns collected from mud bank establishes that the prawns internal mechanisms can cope up with the surrounding stressful conditions. This study signifies the euryhaline crustancean's adaptive capability to overcome the surrounding stressful environmental conditions. Change in cation concentration or clotting time can be taken into consideration to study how physiological and immunological mechanisms of prawns help in survival of species under stressful environment.

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