FLUCTUATION IN CALCIUM LEVELS IN THE EXOSKELETON, MUSCLE AND HAEMOLYMPH OF *PENAEUS INDICUS*, CULTIVATED IN A BRACKISHWATER POND

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ABSTRACT

The calcium content in the exoskeleton of *Penaeus indicus* cultivated in a brackishwater pond was found between 4 and 15%, which was low compared to other crustaceans. The haemolymph calcium was observed always at a higher level than the calcium in the pond water. Calcium content of pond water and haemolymph showed a direct relationship with the salinity. Relatively high levels of calcium in muscle and low levels in exoskeleton were observed in June, the probable reasons for this abrupt fall in the values are discussed.

INTRODUCTION

The role of calcium in crustacean physiology and metabolism is well documented (Travis 1955a and b, Haefner 1964, Dall 1965a and b, Sitaramaiah and Krishnan 1966, Sather 1967 and Graf 1978). Most of these works, however, relate to crabs and lobsters. Less mineralised natantians, particularly the penaeid prawns, have received only a little attention.

Dall (1965) studied the serum calcium in *Metapenaeus mastersii*, while Bursey and Lane (1971) and Huner et al (1979a and b) accounted for the variations in calcium levels in different moulting stages of *Penaeus duorarum* and *P. californiensis* respectively. The latter authors subsequently studied the concentration of whole body calcium, magnesium and phosphorous levels in different parts of the exoskeleton of post larvae, juveniles and adults of *P. californiensis*. Serum constituent including calcium in the freshly captured pink shrimp, *P. marginatus* from off Oahu in the Hawaiin Islands and those maintained under laboratory conditions, were investigated by Balazas et al (1974).

Information on calcium levels in the cutivable penaeid prawns of India is scanty. As the data on such physiological parameters are essential prerequisites for the management of the ecosystem in which prawns are cultured and for the understanding of the nutritional imbalances or pathological changes due to stress, the present investigation was undertaken to study the fluctuations of calcium in the haemolymph, exoskeleton and muscle of *P. indicus*, one of the important species farmed in the coastal waters of the country, and in the environment in which it is cultivated

Materials and Methods

Monthly samples of water, soil and prawns (*P. indicus*) for the present study were collected from a brackishwater culture field in the farm complex of the Prawn Culture Laboratory, located at Narakkal in the Vypeen Island near Cochin, from August 1981 to June 1982. Before sacrificing the animals for haemolymph and tissue calcium analysis, total length from the tip of rostrum to the tip of telson (83-139 mm M, 70-155 mm F), sex and moulting stage of each of the prawns were recorded. Only the specimens in the intermoult stage were selected for the study. Stages were determined by following the criteria suggested by Drach (1939). Water and soil samples were collected simultaneously from the field while collecting the prawn samples and they were analysed for physico-chemical parameters such as temperature, salinity, pH, and calcium concentrations. pH was measured with EIL battery-operated pH meter. Salinity was determined by silver-nitrate method of Knudsen. Haemolymph from each individual prawn was collected sex-wise in separate glass vials by using hypodermic syringe, previously treated with anti-coagulant (Sodium citrate 1%) and stored in ice. Haemolymph from 4-5 prawns of the same sex was pooled together for analysis. After collecting the haemolymph, heads of all prawns were peeled off. Remaining body exoskeleton and muscles were detached and segregated according to sex. These tissues were then dried in a hot-air oven at 80°C. The dried samples were charred at 200°C and ashed at 550°C in a muffle furnace. Ash samples were later subjected to calcium analysis. Calcium content of both the pond water and the haemolymph was estmated by Clark and Collip's modified titrimetric method (1925), while the tissue calcium was analysed according to the methods described in AOAC (1980). Soil calcium was determined based on the method described by Jackson (1973).

Results

The monthly distribution of the environmental parameters are given in Table 1. The temperature of the surface water during the period of study was found varying between 28.2°C and 34.2°C, the lower temperature of less than 30° C being recorded in January and June. The salinity of the pond water during August to November was between 1.78 ppt and 2.5 ppt, due to dilution caused by southwest and northeast monsoon rains. With the cessation of the rains, the salinity increased to about 10 ppt in December and further rised, gradually, to 25.39 ppt in May. In June, however, the salinity of the pond water decreased (16.32 ppt) with the onset of the southwest monsoon. The distribution pattern of these parameters showed a trend similar to that of the Cochin backwaters, described by the earlier workers (Ramamirtham and Jayaraman 1963).

The pH values of the pond water and soil ranged from 6.8 to 7.8 and 7.5 to 8.5 respectively. It is interesting to note that pH of the pond water was less than 7 only in September (Table 1).

TABLE 1. *Distribution of environmental parameters of the pond from where samples of* Penaeus indicus *were collected.*

Month and year	Temperature of surface water (°C)	Salinity of surface water ‰	pH of water	pH of soil
1981				
Aug	30.5	2.10	7.6	7.8
Sept	30.2	2.50	6.8	8.5
Oct	33.0	1.96	7.4	8.3
Nov	30.4	1.78	7.5	7.9
Dec	31.0	10.10	7.7	8.1
1982				
Jan	29.8	14.15	7.8	7.6
Feb	31.8	17.48	7.7	8.0
Mar	34.2	21.95	7.6	8.3
Apr	33.5	23.48	7.8	8.4
May	31.0	25.39	7.8	8.5
Jun	28.2	16.32	7.1	7.5

During August to November calcium content of the pond water was low (0.045-0.09 mg|ml), when the salinity of pond water was also low (1.78-2.50 ppt). During December-May, concomitant with the increased salinity, the calcium content was increasing with the advance of the season, the highest value of 0.55 mg|ml being recorded in March and May. Subsequently, it dropped to the level of 0.15 mg|ml in June, when the salinity of the pond water also decreased. It is thus observed that, generally, the calcium content of the pond water shows a direct relationship with salinity.

The total calcium content of the bottom soil of the pond was always higher than in the overlying water (Table 2). In most of the months it wes varying between 5.1 and 7.1 mg|g except during January-March, when it was relatively low (4.9 and 3.8 mg|g).

The calcium level in the haemolymph of male *P. indicus* during August-November and June was found between 0.28 and 0.38 mg|ml, while in other months, it was relatively higher (0.54-0.80 mg|ml), the maximum being recorded in May. Between the sexes, no appreciable difference was discernible and the distribution pattern in different months was more or less similar (Table 2).

The calcium in the muscle of male *P. indicus* was around 4 mg|g in August-September and October. In the following months, November-April, it

TABLE 2. *Calcium levels in the pond-water, soil and haemolymph, muscle and exoskeleton of* P. indicus (*intermoult stage*).

	Sex and size (mm)		Haemolymph (mg|ml)	%	Exoskeleton (mg|g)	%	Muscle (mg|g)	%	Pond water (mg|ml)	%	Pond soil (mg|g)	%
1981												
Aug	83-112	M	0.38	38	141.7	14.17	3.93	0.393	0.09	9	5.9	0.59
	70-140	F	0.45	45	135.7	13.59	2.40	0.240				
Sept	102-120	M	0.28	28	131.9	13.19	4.26	0.426	0.045	4.5	5.1	0.51
	76-116	F	0.35	35	128.8	12.88	3.41	0.341				
Oct	85-115	M	0.37	37	101.3	10.13	3.76	0.376	0.06	6	7.1	0.71
	77-119	F	0.28	28	90.6	9.06	2.31	0.231				
Nov	97-137	M	0.37	37	134.5	13.43	2.26	0.226	0.07	7	5.4	0.54
	93-136	F	0.22	22	120.6	12.06	2.22	0.222				
Dec	88-130	M	0.54	54	122.8	12.28	2.23	0.223	0.15	15	5.4	0.54
	107-155	F	0.48	48	110.7	11.07	2.43	0.243				
1982												
Jan	91-118	M	0.52	52	151.2	15.12	2.62	0.262	0.20	20	4.9	0.49
	95-121	F	0.63	63	143.0	14.30	2.55	0.255				
Feb	111-119	M	0.50	50	123.7	12.37	3.84	0.384	0.43	43	4.5	0.45
	98-134	F	0.63	63	113.1	11.31	5.36	0.536				
Mar	109-126	M	0.65	65	115.5	11.55	2.80	0.280	0.55	55	3.8	0.38
	108-132	F	0.65	65	106.8	10.68	2.64	0.264				
Apr	112-128	M	0.65	65	117.8	11.78	3.36	0.336	0.50	50	6.0	0.60
	113-134	F	0.65	65	108.2	10.82	3.64	0.364				
May	121-129	M	0.80	80	111.7	11.17	4.16	0.416	0.55	55	5.6	0.56
	113-134	F	0.68	68	111.5	11.15	2.72	0.272				
Jun	96-139	M	0.30	30	43.1	4.31	10.25	1.025	0.15	15	5.3	0.53
	108-140	F	0.40	40	37.6	3.76	12.36	1.236				

decreased and was seen fluctuating between 2.23-3.84 mg|g. In May, the calcium concentration in the muscle increased again to 4.16 mg|g and to the highest value of 10.25 mg|g in June. While the calcium in the muscle of female *P. indicus* was generally lower than in the males the seasonal distribution pattern was similar to males (Table 2).

In the exoskeleton the calcium was fluctuating between 101.3 mg|g and 151.2 mg|g in males and between 90.6 mg|g and 143.0 mg|g in females during

TABLE 3. *Haemolymph of* P. indicus|*Pond-water calcium ratio in different months.*

Month and year	H.|P.W. ratio	
	Male	Female
1981		
August	4.22	5.00
September	6.22	7.78
October	6.16	4.67
November	5.29	3.14
December	3.60	3.20
1982		
January	2.60	3.15
February	1.16	1.47
March	1.18	1.18
April	1.30	1.30
May	1.45	1.24
June	2.00	2.67

August-May. In both sexes the maximum values of calcium was recorded in January. In June when there were environmental changes in the pond ecosystem consequent on the onset of monsoon, the calcium content in the muscle was found drastically reduced to 43.1 mg|g in males and 37.6 mg|g in females (Table 2).

Haemolymph|pond-water calcium ratio (Table 3) indicated a notable difference in different months. It was relatively high during August-November when the salinity of the pond water was comparatively low. In the increasing salinity condition of the pond water during February-May, it was lower (1.16|1-1.45|1) and when the salinity of the water decreased in June, the H|P.W. ratio showed an increase (2.0|1).

Discussion

In decapod crustaceans wide variation, ranging from 8.3 to 40%, of calcium concentration in the exoskeleton of the intermoult stage has been reported (Mills and Lake 1976). Dall (1965) recorded 30% calcium content in the exoskeleton of *M. mastersii,* while it was found to be 30-40% in *Homarus americanus* (Clarke and Wheeler 1922) and 26% for *Panulirus argus* (Travis 1955). The calcium content in the exoskeleton of *P. indicus* in the present investigation, which was found between 4 and 15%, is low as compared to that in these crustaceans. This difference might be due to the difference in the ecosystem.

The haemolymph calcium in *P. indicus* cultured in the brackishwater environment was found between 28-80 mg|100 ml in males and 22-68 mg|100 ml in females. While this range is comparable to that in the marine species *P. marginatus* (53-73 mg|100 ml), reported by Balazas et al (1974), it is lower than the freshwater *Macrobrachium rosenbergii* (66-112 mg|100 ml) and higher than the freshwater *Astacus* (42-47 mg|100 ml) and *Cambarus* (40-49 mg| 100 ml) (Schlatter 1941). The haemolymph calcium in *P. indicus* was found at a higher level than in the pond water, which is in agreement with the observation of the previous workers (Dall 1965 a and b, Bursey and Lane 1971, Balazas et al 1974), who opined that the serum calcium is generally maintained at a higher level than in the water of the natural habitat.

Mills and Lake (1976), working on calcium concentration in the crayfish, suggested that exoskeleton calcium is related to the amount of available calcium in the water in which they inhabit. The calcium content of the pond water in the present study showed a gradual increase from December to May when the haemolymph calcium was also relatively high. In June when there was an appreciable fall in the salinity, temperature, pH and calcium of the pond water, due to the ecological changes consequent on the onset of monsoon, the calcium level in haemolymph and exoskeleton of the prawn also decreased, but not in the pond soil and in the muscle of the prawn.

The opinion is inconsistent on the hypothesis that there are differences in the body size of the crustaceans with greater size increase occuring in waters of lower salinity when haemolymph|water calcium ratio is high, and smaller increase occuring in waters of higher salinities when haemolymph|water calcium ratio is low (Haefner 1964). It is indicated that greater mineral deposition is observed in the crustaceans living in higher saline environment as compared to those living in lower saline waters (Haefner 1964). In the present study, although the haemolymph|pond-water calcium ratio showed difference in the low-saline (June-November) and in the higher saline (December-May) periods (Table 3), there was noteworthy difference in the calcium level in the exoskeleton of the prawn except in June.

An interesting observation made during the present investigation is that in June the calcium in the pondwater and in the haemolymph of the prawn was in a comparable level, but in the muscle it was the highest and in the exoskeleton the lowest recorded values. This difference in the calcium levels in the haemolymph, muscle and exoskeleton indicates some imbalance in the absorption and transportation of calcium from the external medium to the exoskeleton, particularly from the haemolymph to the integument via muscle. It is known that calcium is continually being withdrawn from the plasma by the epidermis and is deposited in the integument (Robertson 1937, Drach 1939), Travis 1955 b). This process is facilitated by the presence of organic acids, which increase solubility of calcium and act as a carriage mechanism (Paul and Sharpe 1916, Travis 1955 a). This in turn reduces the calcium content of the plasma, facilitating its further continuous absorption from the external medium (Robertson

1957, Haefner 1964). The present data indicate that calcium is absorbed from the pond water to the haemolymph. However, due to some imbalance in the physiological process, the carriage mechanism of calcium from the haemolymph to integument is impaired. It is interesting to note that, during June drastic changes in the pond environment occur, due to lowering of salinity, temperature and pH. The effect of such rapid ecological changes and the consequent impairment of the metabolic pathway of calcium in prawn is worthy of further investigation.

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REFERENCES

A.O.A.C. 1980. *Official Methods of the Association of Official Analytical Chemists*. 13th Edition. 1980. A.O.A.C. Washington D.C.

BALAZAS, G. H., S. E. OLBRICH AND M. E. TUMBLESON. 1974. Serum constituents of the Malayasian prawn (*Macrobrachium rosenbergii*) and pink shrimp (*Penaeus marginatus*) *Aquaculture*, 3(2): 147-157.

BURSEY, C. R. AND C. E. LANE. 1971. Ionic and protien concentration changes during the moult cycle of *Penaeus duorarum*. *Comp. Biochem. Physiol.*, 40A: 155-162.

CLARK, E. P. AND J. B. COLLIP. 1025. A study of Kramer-Tisdall method for determination of calcium with suggested modifications. *J. biol. Chem.*, 63: 461-464.

CLARK, F. W. AND W. C. WHEELER. 1922. The inorganic consituents of marine invertebrates. *U.S.Geol. Surv. Prof. Pap. No. 124*.

DALL, W. 1065a. Studies on the physiology of a shrimp *Metapenaeus* sp. (Crustaeea: Decapoda: Penaeidae) Composition and structure of integument. *Aust. J. mar. Freshwat. Res.*, 16: 13-23.

DALL, W. 1965b. Studies on the physiology of a shrimp *Metapenaeus* sp. (Crustacea: Decapoda: Penaeidae) Calcium metabolism. *Aust. J. mar. Freshwat. Res.*, 16: 181-203.

DRACH, P. 1939. Mue et cycle d'intermue chez les crustaces decapodes. *Ann. Inst. Oceanogr. Monaco, N.S.*, 19: 103-391.

GRAF, F. 1978. Les sources de Calcium pour les crustaces venant ae Muer. *Arch. Zool. exp. gen.*, 119; 143-161.

HAEFNER, P. A. 1964. Hemolymph calcium fluctuations as related to environmental salinity during ecdysis of the blue crab, *Callinectes sapidus* Rathbun. *Physiol. Zool.*, 37(3): 247-258.

HUNER, J. V., L. B. COLVIN AND B. L. REID. 1979a. Postmoult mineralisation of the exoskeleton of juuenile California brown shrimp, *Penaeus californiensis* (Decapoda: Penaeidae). *Comp. Biochem. Physiol.*, 62A: 889-893.

HUNER, J. V., L. B. COLVIN AND B. L. REID. 1979b. Whole-body calcium, Magnesium and Phosphorous levels of the California brown shrimp, *Penaeus californiensis* (Decapoda: Penaeidae) as functions of moult stage. *Comp Biochem Physiol.*, 64A: 33-36.

JACKSON, M. L. 1973. *Soil chemical analysis* Prentice-Hall of India Private Limited New Delhi.

MILLS, B. J. AND P. S. LAKE. 1976. The amount and distribution of calcium in the exoskeleton of the intermoult crayfish *Parastacoides tasmanicus* (Erichson) and *Astacopsis fluviatilis* (Gray). *Comp. Biochem. Physiol.*, 53A: 355-360.

PAUL, J. H. AND J. S. SHARPE. 1916. Studies in calcium metabolism. I. The deposition of lime salts in the integument of decapod crustacea. *Jour. Physiol.*, 50: 183-192.

RAMAMIRTHAM, C. P. AND R. JAYARAMAN. 1963. Some aspects of the hydrological conditions of the backwaters around Willington Island (Cochin). *J. mar biol. Ass. India*, 5(2): 159-169.

ROBSERTSON, J. D. 1957. Osmotic and ionic regulation in aquatic invertebrates, In *Recent Advances in Invertebrate Physiology*. Eugene: University of Oregon Press: 229-246.

ROBERTSON, J. D. 1960. Ionic regulation in the crab *Carcinus maenas* (L.) in relation to the moulting cycle. *Comp. Biochem. Physiol.*, 1: 183-212.

SATHER, B. T. 1967. Studies in the calcium and phosphorus metabolism of the crab *Podophthalmus vigil* (Fabricius). *Pacific Science*, 21: 193-209.

SCHLATTER, M. J. 1941. Analyses of the blood serum of *Cambarus clarckii*, *Pachygrapsus crassipes* and *Panulirus interruptus*. *J, Cell. Comp. Physiol.*, 17(2): 259-261.

SITARAMAIAH, P. AND G. KRISHNAN. 1966. Calcium metabolism in *Emerita, asiatica*. *Ind. J., exp, Biol.* 4: 34-36.

TRAVIS, D. F. 1955a. The moulting cycle of the spiny lobster, *Panulirus argus* Latreille. II. Preecdysial histological and histochemical changes in the hepatopancreas and integumental tissues *Biol. Bull.*, 108: 88-113.

TRAVIS, D. F. 1955b. The moulting cycle of the spiny lobster, *Panulirus argus* Latreille. III. Physiological changes which occur in the blood and urine during the normal molting cycle, *Biol. Bull.* 109: 484-503.