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VARIATION OF pH, OSMOLALITY, SODIUM AND POTASSIUM CONCENTRATIONS IN THE HAEMOLYMPH OF SUB-ADULT BLUE SHRIMP (*PENAEUS STYLIROSTRIS*) ACCORDING TO SIZE

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Abstract—1. Haemolymph pH, osmolality, and potassium and sodium concentrations were determined in sub-adult blue shrimps (*Penaeus stylirostris*).

2. The pH and concentration of sodium are not modified according to the size of sub-adult *P. stylirostris*. The mean for pH was 7.3, and for sodium concentration was 450 mEq/l.

3. The haemolymph osmolality and the potassium concentration showed significant differences according to size.

4. The smaller shrimps are better osmoregulators.

5. Potassium has an important role in osmolality maintenance.

INTRODUCTION

Osmotic regulation in the shrimp is a physiological property which determines their distribution under different salinities. This osmoregulatory ability has been studied in many species, including: *P. aztecus* (Williams, 1960; McFarland and Lee, 1963; Bishop *et al.*, 1980; Castille and Lawrence, 1981a), *P. duorarum* (Williams, 1960; Bursey and Lane, 1971; Castille and Lawrence, 1981a), *P. esculentus* (Dall, 1981), *P. japonicus* (Thuet *et al.*, 1985), *P. merguiensis* (Dall, 1981), *P. monodon* (Cawthorne *et al.*, 1983; Ferraris *et al.*, 1986), *P. penicillatus* (Cheng and Liao, 1986), *P. setiferus* (McFarland and Lee, 1963; Castille and Lawrence, 1981a), *P. stylirostris* and *P. vannamei* (Castille and Lawrence, 1981a). All of these species are euryhaline penaeids which inhabit brackish water as postlarvae and juveniles, but migrate as adults to a marine habitat. Nevertheless, shrimps at all stages of development are able to hyperregulate osmolality in diluted media and to hyporegulate in concentrated media with isosmotic concentrations at 20–30 parts per thousand. The juveniles are more euryhaline and stronger osmoregulators than the adults, although differences have been found between species (Charmantier, 1987).

The control of internal pH is necessary for the proper functioning of animals since the pH of body fluids governs the ionization state of proteins, which in turn governs their physiological function (Reeves and Rahn, 1979). In crustaceans, including penaeids, some importance has been ascribed to the role of ion fluxes in pH regulation (Cameron, 1978; Truchot, 1979); however, the connection between the difference in ion concentration and pH is not yet clear.

Previous studies on *Penaeus* osmoregulation have relied on the physical transfer of the organism to different salinities and temperatures in order to determine the influence of medium changes on the haemolymph composition (Dall, 1981; Dall and Smith, 1981; Castille and Lawrence, 1981a,b,c; Cawthorne *et al.*, 1983; Charmantier, 1987). In contrast, shrimps for this study were obtained from only one marine location and without further manipulations. While previous studies compared shrimps at different stages of development, this study focused on three sizes of blue shrimp (*Penaeus stylirostris*) from 11 to 16 cm. Shrimps of these sizes are all considered sub-adult (McFarland and Lee, 1963; Bursey and Lane, 1971).

Although sodium concentration and changes in osmolality have been reported for juveniles and adults (Castille and Lawrence, 1981b), potassium concentration and pH values have not been ascertained. Thus, the main objectives of this research were to determine the Na^+ and K^+ concentration, pH and osmolality of the haemolymph from three sizes of sub-adult *Penaeus stylirostris* for comparison with previous reports on *Penaeus* osmoregulation.

MATERIALS AND METHODS

Shrimp and haemolymph collection

Male and female shrimps (*Penaeus stylirostris*) were collected from San Carlos Bay, B.C.S., México, in October of 1989. The water temperature was 21°C with a salinity of 36 parts per thousand (ppt). The haemolymph (50–200 μl) was obtained by inserting a sterile 25 gauge needle into the pleopod base of the first abdominal segment. Each sample was put into a 1.5 ml Eppendorf tube and kept at 10°C for transporting. In the laboratory, the cell-free haemolymph, or serum, was recovered after centrifugation for 10 min in an Eppendorf microfuge and removal of the cell clot. Only the haemolymph from intermoult shrimps was used.

Chemical analysis

The serum osmolality (mOsm/kg) was measured on a Wescor 5120B Vapor Pressure Osmometer which had been calibrated with standard solutions of 100, 290 and

Table 1. Parameters determined in sub-adult *Penaeus stylirostris* haemolymph. No significant difference ($P > 0.05$) between sexes was obtained by t-test

	Male ($N = 13$)	Female ($N = 20$)
pH	7.25 ± 0.14	7.33 ± 0.14
Sodium (mEq/l)	460.46 ± 18.38	452.30 ± 16.09
Potassium (mEq/l)	10.19 ± 0.62	10.18 ± 0.87
Osmolality (mOsm/kg)	826.74 ± 39.44	827.41 ± 46.46

Mean ± standard deviation.

1000 mOsm/kg. The serum pH was determined using a surface electrode. Haemolymph sodium and potassium concentrations (mEq/l) were determined on a Radiometer, FLM 3 flame photometer.

Experimental design

To determine the influence of animal size of serum K^+ and Na^+ concentration, as well as the haemolymph pH and osmolality, the whole shrimp population and each sex was separated into three groups of different sizes: group A was comprised of animals ranging in size from 11.0 to 12.6 cm; group B from 12.7 to 14.3 cm and group C from 14.4 to 16 cm.

The data obtained were analysed by ANOVA-1W (Analysis of variance one way), Student t-test or linear regression, using Lotus 1-2-3™ and Statgraphics™ programs.

RESULTS

The haemolymph pH, osmolality, sodium and potassium concentrations of the shrimps, ranging from 11 to 16 cm, and weighing between 11.8 and 27.2 g, are shown in Table 1. The t-test analysis of these parameters did not indicate significant differences ($P > 0.10$) between male and female values.

As expected, a good correlation between size and weight was observed in both male ($r = 0.8953$), and female ($r = 0.9632$), as well as in mixed populations ($r = 0.9393$). For this reason, further comparisons are made referring to size only and assuming that similar results could be obtained with reference to weight.

A poor correlation between size (or weight) and pH, osmolality or ion concentration was found when the data were analysed separately for female, male or mixed shrimp populations (Table 2). Nevertheless, despite the lack of good correlation between the size and potassium ($r = 0.5312$) or osmolality ($r = 0.5265$), some other variations were noticed according to the animal size. For potassium ion serum level and osmolality, a positive tendency was observed, indicating a size dependency. When these data were evaluated according to sex, it was observed that the contribution by the male and the female was different. For potassium, the female showed a better correlation ($r = 0.5560$) than the male ($r = 0.4712$). In a similar manner, osmolality exhibited a superior correlation ($r = 0.6228$) for female than for male shrimps ($r = 0.3149$).

Table 2. Linear correlation factors between evalued haemolymph parameters and size of *P. stylirostris* male, female and mixed population

	Male	Female	Male and female
pH	0.0439	0.0672	0.0480
Sodium	0.4745	0.2445	0.3163
Potassium	0.4771	0.5560	0.5312
Osmolality	0.3149	0.6229	0.5265

Table 3. Values (SE) of the parameters studied in the *Penaeus stylirostris* haemolymph. The shrimps were separated into groups according to size

	Group A 11.0–12.6 cm ($N = 10$)	Group B 12.7–14.3 cm ($N = 16$)	Group C 14.4–16.0 cm ($N = 7$)
pH	7.29 (0.18)	7.32 (0.13)	7.28 (0.16)
Sodium	448.20 (18.94)	456.12 (16.02)	464.57 (17.80)
Potassium	9.72 (0.71)	10.22 (0.75)	10.77 (0.62)*
Osmolality	794.79 (28.42)	835.00 (45.02)	855.42 (38.03)*

*Significantly different ($P < 0.05$) by ANOVA-1W.

To determine the influence of animal size on serum K^+ and Na^+ concentration, as well as the haemolymph pH and osmolality, the whole shrimp population was separated into three groups according to size. Tested by ANOVA-1W, significant differences ($P < 0.05$) in both potassium levels and osmolality were observed (Table 3). On the other hand, serum sodium levels and haemolymph pH did not show significant differences ($P > 0.10$).

Similar results were obtained when the data were analysed according to sex (Table 4). For the male, a significant difference ($P < 0.05$) was found in potassium level and osmolality according to their size. However, the female shrimp only showed significant variations in osmolality ($P = 0.024$), not in potassium levels ($P > 0.05$). Using the t-test, each group was analysed for differences between male and female. As shown in Table 4, a significant difference was only observed in the osmolality values of group C (14.4–16 cm). Increases in potassium concentration and osmolality were observed according to animal size in males, females and both together (Fig. 1).

The relationship between the monovalent cation (Na^+ and K^+) concentration and osmolality was found using the ratio $[Na^+]/[K^+]$. As shown in Fig. 2, a good correlation is observed, indicating that osmolality is largely dependent on these cation concentrations.

DISCUSSION

The osmoregulatory abilities of euryhaline penaeids can be correlated with their distribution in environments of different salinity (McFarland and

Table 4. Values (SE) obtained in male and female shrimps separated according to animal size

	Group A 11.0–12.6 cm	Group B 12.7–14.3 cm	Group C 14.4–16.0 cm
Male			
pH	7.17 (0.23)	7.29 (0.14)	7.28 (0.07)
Sodium	445.33 (15.01)	464.29 (17.53)	466.67 (24.44)
Potassium	9.43 (0.35)	10.43 (0.64)	10.40 (0.01)*
Osmolality	776.00 (13.06)	850.05 (35.30)	823.11 (20.51)*a
Female			
pH	7.35 (0.13)	7.34 (0.13)	7.28 (0.24)
Sodium	449.43 (21.38)	449.78 (12.14)	463.00 (15.10)
Potassium	9.84 (0.81)	10.06 (0.83)	11.05 (0.72)
Osmolality	802.84 (30.04)	823.29 (50.13)	879.66 (28.01)*b

The groups are compared using ANOVA, and rows with a significant difference ($P < 0.05$) are marked with (*). Differences between sexes were established for each size group using t-test, and significant differences ($P < 0.05$) are indicated with superscript letters.

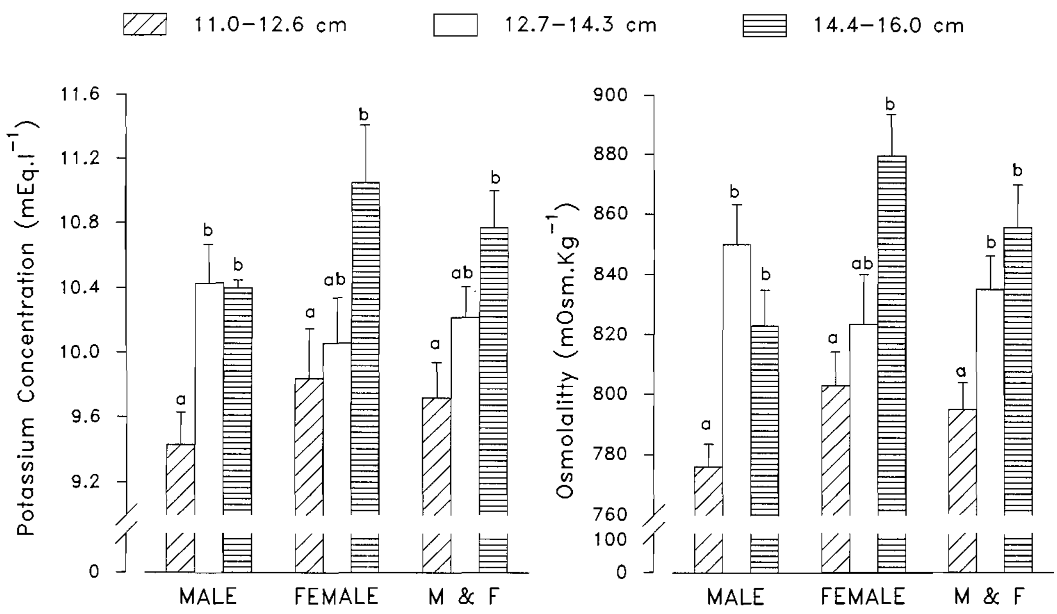

Fig. 1. The haemolymph osmolality and K^+ concentration for male, female and mixed sex blue shrimp (*Penaeus stylirostris*) according to size. Mean ± SE are given by the diagram. Significant differences are indicated by letters.

Lee, 1963). However, there are many factors other than salinity or osmoregulatory ability *per se* that might determine the distribution of the shrimp population (Dall, 1981). Castille and Lawrence (1981b) pointed out that this migratory behavior is not directly related to osmotic regulation capability since adult animals are still capable of hyperosmotic regulation at salinities below the isosmotic point. However, adult migrations to water of high salinity may be necessary for ovarian development (Chu and Hanaoka, 1975; Oshiro, 1984), spawning (Panikkar, 1968), and for embryonic and larval development (Lim, 1982).

It has been suggested that maximum growth of an organism occurs in isosmotic media, since the animal would be expending the least energy in osmotic regulation (Panikkar, 1968). This may be true in *P. vannamei* and *P. stylirostris* but not in *P. aztecus*, *P. dourarum* and *P. setiferus* (Zein-Eldin, 1963). Moreover, the salinity itself has little effect on the metabolic rate of euryhaline shrimps (Kutty *et al.*, 1971; Bishop *et al.*, 1980; Gaudy and Sloane, 1981), indicating that the energy required for osmoregulation may be relatively small.

The results using sub-adult *P. stylirostris* animals from one oceanic location (salinity = 36 ppt) show a variation in the haemolymph osmolality according to animal size. This contrasts with the findings of Castille and Lawrence (1981b) who reported that in *P. stylirostris*, the osmoregulatory capacity is the same for juvenile and adult animals at salinities of 36.2 ppt. This difference could be due, in part, to the shrimp populations used for the study. While Castille and Lawrence studied juveniles (68 mm) and adults

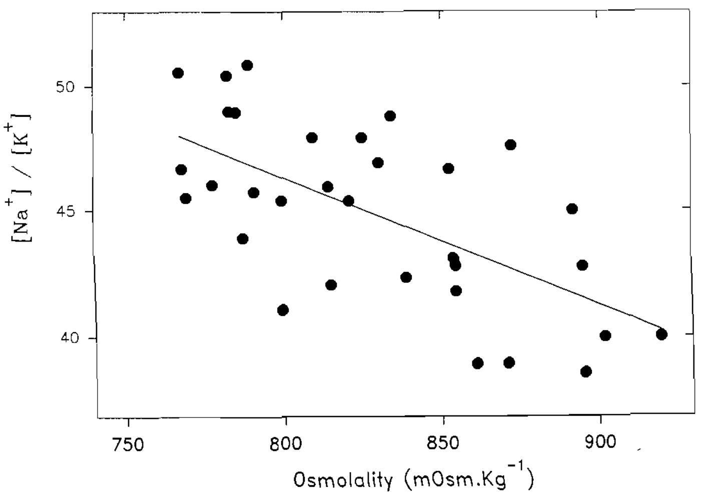

Fig. 2. Linear correlation between the osmolality and Na^+/K^+ ratio of the *Penaeus stylirostris* haemolymph. r = 0.6415; $N = 33$.

(174 mm) from different sources and transferred them to 36.2 ppt salinity, we used wild animals from one location without acute salinity transfer.

Other penaeid species like *P. aztecus* (Williams, 1960; McFarland and Lee, 1963; Castille and Lawrence, 1981a), *P. duorarum* (Williams, 1960; Bursey and Lane, 1971; Castille and Lawrence, 1981a), *P. esculentus* (Dall, 1981), *P. japonicus* (Thuet *et al.*, 1985), *P. merguiensis* (Dall, 1981), *P. monodon* (Cawthorne *et al.*, 1983; Ferraris *et al.*, 1986), *P. plebejus* (Dall, 1981), *P. penicillatus* (Cheng and Liao, 1986), *P. setiferus* (McFarland and Lee, 1963; Castille and Lawrence, 1981a) and *P. vannamei* (Castille and Lawrence, 1981a) display an important variation in osmoregulatory capacity according to the age of the animal. In all of these species, juvenile animals are better regulators than adults. For salinities above their isosmotic point, adult shrimps show a higher haemolymph osmolality than juveniles. Because the isosmotic point of *P. stylirostris* is near 24 ppt or 699 mOsm/kg (Castille and Lawrence, 1981a) at 36 ppt (1020 mOsm/kg), the animal is under osmotic stress. In this condition the haemolymph osmolality of the larger shrimp must be greater than that of the smaller shrimp. This suggests that smaller shrimps have a better osmoregulatory capacity.

Our results support this idea, because the osmolality of the bigger shrimp (855.42 ± 38.03 mOsm/kg) is significantly ($P < 0.01$) higher than that of the smaller shrimp (794.79 ± 28.42 mOsm/kg). This phenomena can be observed in the whole population, as well as in the males and females when the data are analysed separately. As shown in Table 4, no significant differences between males and females were observed in animals between 11.0 and 14.3 cm in length (group A and B); however, in group C (14.4–16 cm), a significant difference was found ($P < 0.05$). Furthermore, a clear difference between group A (11–12.6 cm) and group C (14.4–16 cm) was demonstrated for both sexes.

The sodium and/or potassium haemolymph concentrations were modified according to the concentration of these ions in the external medium. This has been demonstrated in some penaeids: *P. aztecus*, *P. duorarum*, *P. esculentus*, *P. merguiensis*, *P. plebejus*, *P. setiferus*, *P. stylirostris* and *P. vannamei* (Castille and Lawrence, 1981b; Dall and Smith, 1981), and variations between species can be noted. Despite these variations, the curves for anions are similar in shape to those for the osmotic concentrations, indicating that ions are the major contributors to osmotic maintenance (Dall, 1981).

In this work, no modification in the ionic composition of the environment was made. However, differences in the haemolymph ionic composition were observed which were similar to osmolality. While there was no difference in the sodium concentration, the potassium was significantly different ($P < 0.05$) in the three groups studied. This result is analogous to those obtained in other *Penaeus* (Dall and Smith, 1981; Castille and Lawrence, 1981b; Charmantier, 1987), with respect to the proportionate increases in ionic concentration and osmolality. Thus, small shrimps (11–12.6 cm), which are better osmoregulators, show less haemolymph potassium concentration (9.72 mEq/l) than shrimps of 14.4 cm or more (10.77 mEq/l). This difference is very significant ($P < 0.02$).

Castille and Lawrence (1981a) reported that sodium and chloride ions account for 76–94% of the haemolymph osmotic pressure in several penaeid species. However, only *P. aztecus* and *P. dourarum* of the genus Melicertus increased the sodium and chloride contribution to the haemolymph osmotic pressure when environmental salinity increased. Penaeids of the subgenus *Littopenaeus*, which are better osmoregulators, apparently rely on other methods to minimize fluctuations in haemolymph osmolality during variations in external salinity (Castille and Lawrence, 1981a). According to this idea, the potassium ions make an important contribution to the osmotic equilibrium of the sub-adult *P. stylirostris*. The combination of K^+ with other major ions, like Na^+, seems to be an adequate measure, and the $[Na^+]/[K^+]$ ratio confirms this hypothesis (Fig. 2).

In summary, osmoregulatory ability has been demonstrated in both juvenile and adult (Castille and Lawrence, 1981b), as well as pre-adult *P. stylirostris* (this work), suggesting that the migration to or from offshore waters is not directly necessitated by osmotic regulation. Although there are differences between species, this is a general characteristic of migratory penaeids, and the capability is greater in the early stages.

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