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Osmoregulatory ability of *Penaeus indicus* H Milne Edwards in relation to varying salinities

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Abstract. Juvenile *Penaeus indicus* osmoregulated well between 3–26‰ S for 24 and 48 h duration with isosmotic points ≈S 18‰ and ≈S 14‰ respectively. Adults also showed good osmoregulatory capability between 5–30‰ S with isosmotic points ≈S 21‰ and ≈S 17‰ for 24 and 48 h duration respectively. A duration of 48 h is essential for prawns to adjust to the new medium. Influence of various neuroendocrine centres on the osmolal concentration of haemolymph was studied. In eyestalk ablated prawns osmolal concentration decreased with time and reached a lowest after 48 h. Eyestalk ablated prawns injected with extracts of eyestalk, brain and thoracic ganglia did not show any decrease in the values with time but remained on par with the values of normal ones.

Keywords. Osmoregulatory ability; neuroendocrine control; *Penaeus indicus*.

1. Introduction

Most marine animals either actively regulate the osmolality of their body fluids or passively conform to the salinity fluctuations of the external medium (Bursey and Lane 1971). Members of the genus *Penaeus*, are distributed over a wide range of salinities and are being cultured under variety of conditions in many tropical and sub-tropical parts of the world. During their life cycle many penaeid shrimps are found to have a common migratory behaviour of returning to more saline conditions for maturation and spawning (George and Vedavyasa Rao 1968; Castille and Lawrence 1981). The juveniles of many penaeids have also been found to enter the estuaries and move to shallow brackish nursery grounds to continue their growth (Panniker 1968). Such migratory patterns would reflect the osmoregulatory capabilities of the species in question. In recent years, certain aspects of osmoregulatory capabilities of some penaeid prawns have been documented (Castille and Lawrence 1981; Dall 1981; Howe *et al* 1982; Ferraris *et al* 1986).

Evidence of neuroendocrine control in hydromineral regulation in decapod crustaceans has been proved from time to time (Bliss *et al* 1966; Charmantier *et al* 1981). The role of eyestalk hormones, brain and thoracic ganglion in the ionic regulation and water balance has also been demonstrated (Tullis and Kamemoto 1971; Heit and Fingerman 1975; Kiron and Diwan 1984a, b). Schreiner *et al* (1969) pointed out the importance of abdominal ganglion in water balance of *Homarus* sp. However, there is a paucity of information on osmolal concentration and effects of neuroendocrine factors in penaeid shrimps. The osmoregulatory behaviour of *Penaeus indicus* is not thoroughly understood. Therefore, attempts were made to compare the responses of osmolal concentrations of the haemolymph of juvenile and adult prawns over a range of salinities varying from 3–40‰ and the influence of eyestalk, brain and thoracic ganglion in the regulation of haemolymph osmolality was determined.

2. Materials and methods

Juvenile and adults of *P. indicus* were collected from the grownout ponds of the Marine Prawn Hatchery Laboratory, Narakkal, Kerala. The size range of juveniles varied between 75–85 mm TL and for adults between 140–170 mm TL. Prawns were maintained in the laboratory for 48 h. Only intermoult stage prawns were segregated for the experimental purpose. The required experimental salinities were prepared by dilution of seawater with freshwater and the salinity measured as a direct reading of the salinometer. Higher salinities were prepared by partial freezing of seawater and removal of the ice formed thereby. The range of salinities for experimental media varied from 3–40‰. Six juveniles and adult prawns were released into each of the media. Three juvenile and three adult prawns were sampled from each of the media for haemolymph extract after 24 and 48 h duration. Haemolymph sample from individual prawns was collected from the pericardial cavity using chilled 1 ml hypodermic syringe previously rinsed with a anticoagulant (10% trisodium citrate). The haemolymph was delivered into small glass vials and kept in an ice water bath until further use. From each glass vial, 50 μl of haemolymph was pipetted with the help of an automatic micropipette and immediately transferred to the osmometer cuvett. The cuvett was further transferred to osmometer (Gonotech-Osmomat-030) where the value of osmolality (freezing point depression) was directly determined. Osmolal concentration of the water of each medium was also measured simultaneously and checked with chloride determination on salinometer.

To find out the possible role of different neuroendocrine centres, in the regulation of osmolality of haemolymph, the following experimental set up was designed. Prawns in the size range of 138–148 mm were divided into 6 batches (A–F), each batch consisting of 30 animals. Each batch was further divided into 10 groups each with 3 animals. All the prawns were maintained in plastic pools having a salinity nearest to the isosmotic points (672 mOsm/kg) of the haemolymph which was determined earlier.

Bilateral eyestalk surgery was performed by using electric cauterizer for prawns belonging to all batches except A and B. The prawns of batch A treated as the initial control and batch B sham operated. The extract of different neuroendocrine organs viz. eyestalk, brain and thoracic ganglion were prepared by homogenising the tissues separately in isosaline filtered seawater and subjected them for centrifugation at 8000 *g* for 10 min. Supernatant of each tissue was used as the injecting material to the test animals. Eyestalk ablated prawns of batches D, E and F were injected with eyestalks, brain and thoracic ganglion extracts respectively in the ratios of 2 eyestalks/0·2 ml/prawn, 0·2 ml/brain/prawn and 0·2 ml/thoracic ganglion/prawn. Ablated prawns of batch C were injected with 0·2 ml/prawn isosaline filtered seawater. After initiation of the experiment first haemolymph sample was collected immediately from the first group of prawns of all the batches. The next sampling was after 1 h and then at intervals of 2, 4, 8, 12, 18, 24, 48 and 72 h. The haemolymph was then delivered into the prechilled glass vials and osmolality was determined as per the method described earlier.

3. Results and discussion

The osmolal concentrations of the haemolymph of juveniles and adult *P. indicus*

maintained in different salinities for 24 and 48 h duration are indicated in figures 1 and 2. Duration of 48 h was essential for prawns to adjust to the new medium and regulate normally. Both juveniles and adults showed hyperosmotic behaviour in lower salinities (up to 15‰ S) and hyposmotic behaviour in higher salinities. In higher saline media, there was corresponding increase in the osmolal concentration of haemolymph of both juveniles and adult prawns. It was evident that juvenile prawns osmoregulated well in salinities 3–26‰ S for 24 and 48 h, with isosmotic points ≈S 18‰ and ≈S 14‰ respectively (figure 1). Adults also showed good osmoregulatory capability between 5–30‰ with isosmotic points ≈S 21‰ and ≈S 17‰ for 24 and 48 h duration respectively (figure 2). The results of the present investigations indicated hyperosmotic regulation of this species at low salinities and hyposmotic regulation at high salinities for both juvenile and adult and are in agreement with earlier reports (Williams 1960; McFarland and Lee 1963; Bursey and Lane 1971). The differences in the haemolymph concentrations at low and high salinities between juveniles and adults showed that while adults are better hyperosmotic regulators, juveniles are better hyposmotic regulators. Most of the penaeid species as reported earlier, are able to adopt extremely well to very low salinities during their early juvenile life but this ability appears to be reduced in adults (Dall 1981). Unlike the juveniles of other penaeids, the hyperosmotic behaviour of juveniles of *P. indicus* at low salinities was not pronounced in the present study. This is probably because the juveniles selected were in the late phase of their growth (size range between 75–85 mm). However, when isosmotic points are compared, juveniles showed lower isosmotic points than that of adults clearly indicating that the animals prefer lower saline media in the early phase of their growth. In adult prawns the capacity to osmoregulate both at low and high salinities was found to be extremely good. There is great diversity in osmoregulatory ability among penaeid species and other decapod crustaceans. Some have feeble powers of

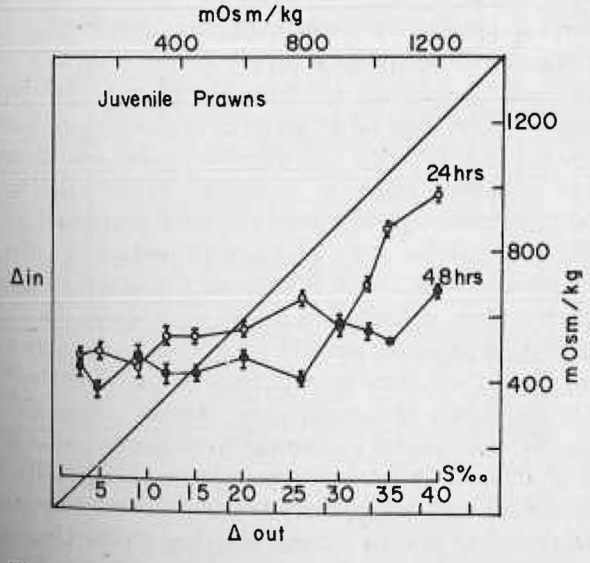

Figure 1. Osmolality of the haemolymph of juvenile *P. indicus* acclimated to different salinities.

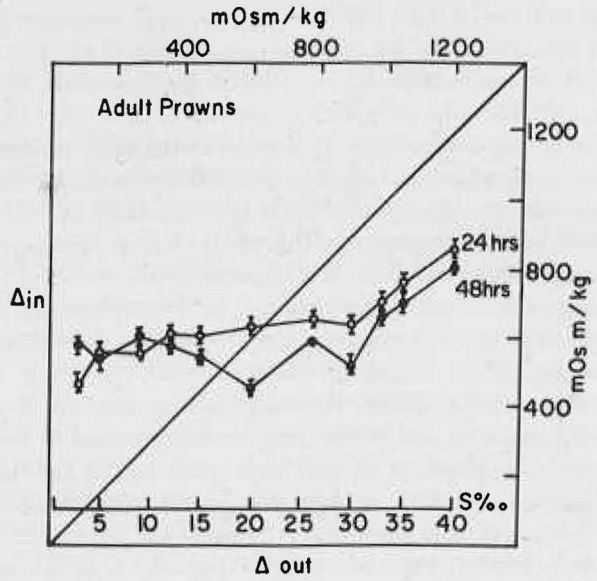

Figure 2. Osmolality of the haemolymph of adult *P. indicus* acclimated to different salinities.

osmoregulations, in some the larvae can osmoregulate but adults are osmoconformers, in others both larvae and adults have similar osmoregulatory patterns (Kalber 1970; Forskett 1977). Therefore, the osmoregulatory ability appears to be a purely adaptative feature and may change markedly during development and according to the environmental situation.

Both juvenile and adult *P. indicus* require at least 48 h for exhibiting stability in the haemolymph. Generally, when the prawns are acutely transferred to different salinities, there is a rapid change in the osmolal concentration of haemolymph and to reach a steady state equilibrium the animals requires time. Bursey and Lane (1971) have reported that for *P. duorarum* a period of about 24 h is required to establish a new steady state equilibrium for haemolymph concentration. Castille and Lawrence (1981) reported 3–4 days for *P. satiferus* to stabilize the haemolymph.

Table 1 summarises the influence of various neuroendocrine centres on the osmolal concentrations of haemolymph in *P. indicus*. Initial rise was seen in osmolal concentrations of haemolymph in eyestalk ablated prawns as also in other groups where ablation and injection of various neuroendocrine elements were conducted. In ablated prawns with isosaline seawater injection osmolal concentration decreased with the time and reached lowest after 48 h. Recouping effect could be seen only after 72 h. Ablated prawns injected with extracts of eyestalk, brain and thoracic ganglia did not show any decrease in the values with time but remained on par with the values of normal ones. Results presented in table 1 provide a firm evidence that the osmolal concentration of haemolymph in *P. indicus* is under the control of different neuroendocrine elements. Eyestalk, brain and thoracic ganglion have similar controlling factor (osmolal stimulating factor) which upon injection into the destalked prawns showed identical effects. Decreasing trend of osmolal concentration in bilaterally ablated prawns might be due to loss of osmolal controlling factor as a result of eyestalks removal. Recouping effect of

Table 1. Neuroendocrine control of osmolal concentration of haemolymph in the prawn *P. indicus* (mOsm/kg).

Hours after operation	Normal prawn	Sham operated control	Bilateral eyestalk surgery + sea water injection	Bilateral eyestalk surgery + injection of eyestalk extract	Bilateral eyestalk surgery + injection of cerebral ganglion extract	Bilateral eyestalk surgery + injection of thoracic ganglion extract
0	975 ± 3	1132 ± 6	905 ± 7	1307 ± 10	925 ± 13	1075 ± 8
1	889 ± 4	934 ± 3	895 ± 8	878 ± 2	979 ± 12	904 ± 7
2	889 ± 5	882 ± 5	846 ± 5	927 ± 10	876 ± 4	856 ± 16
4	872 ± 18	874 ± 8	836 ± 3	852 ± 4	876 ± 4	846 ± 7
8	859 ± 5	864 ± 10	801 ± 3	877 ± 3	881 ± 9	877 ± 4
12	855 ± 4	870 ± 8	826 ± 7	868 ± 2	837 ± 12	874 ± 38
18	838 ± 17	873 ± 7	823 ± 10	878 ± 33	869 ± 5	856 ± 6
24	847 ± 27	861 ± 6	780 ± 4	908 ± 25	939 ± 53	895 ± 46
48	883 ± 7	883 ± 11	708 ± 2	858 ± 6	876 ± 4	899 ± 9
72	879 ± 36	875 ± 15	820 ± 5	838 ± 5	854 ± 19	896 ± 5

Analysis of variance showed that the means of different treatments and at different time intervals differed significantly at 5% level.
Each value represents a mean of 3 determinations.
$\bar{X} \pm SD$.
Intramuscular injection: 0·2 ml/two eyestalks/prawn; 0·2 ml/brain/prawn; 0·2 ml/thoracic/prawn ganglion. Temperature of the medium = 26°C; mOsm of the medium = 672; Size range of the prawns = 138–148 mm.

retaining normal values of osmolality in the ablated prawns after 48 h could be due to the release of osmolal stimulating factor from brain and thoracic ganglia. But the response of haemolymph osmolality to various neuroendocrine elements generally depends on ionic concentration of the surrounding medium. Heit and Fingerman (1975) have reported that eyestalkless crabs did not respond to blood sodium in hyperosmotic seawater but there was a drop in the levels either in isosmotic or hyposmotic media. Similar observations were earlier made by Kamemoto *et al* (1966) while testing the extracts of eyestalk, brain and thoracic ganglia on *Procambarus clarkii* and *Metopograpsus messor*. Haemolymph chloride elevating factor has also been reported by Nagabhushanam and Jyoti (1977) in *Caridina weberi* and Venkatachari *et al* (1979) in *Barytelphusa guerini* in which removal of this factor through extract injections had elevated it to normal level. In our earlier observations we found that there was an increase in sodium and chloride ion concentration of haemolymph in eyestalkless *P. indicus* (Kiron and Diwan 1984a, b) and the present investigation indicated decrease in osmolal concentration of haemolymph in eyestalkless prawns. The reason for this difference is due to the difference in the experimental ambient medium one being hyperosmotic (Kiron and Diwan 1984a, b) and the present one isosmotic. Therefore, the osmolal controlling factor which is present in the neuroendocrine centres reacts, according to the animal's need depending on the surrounding medium.

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