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OSMOREGULATORY ABILITY OF *PENAEUS MONODON* (FABRICIUS) IN RELATION TO VARYING SALINITIES

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ABSTRACT

Adult *Penaeus monodon* osmoregulated well between salinities 3 and 45‰ for 24 and 48 hour duration with isosmotic points around ≈ S 18.5‰ and ≈ S 23.5‰ respectively. A duration of 48 hr is essential for prawns to adjust to the new medium. Influence of eyestalk removal on osmolal concentration of haemolymph was studied. There was a significant decline in osmolal concentration in destalked prawns from 4 to 18hr after eyestalk surgery performance, but later recouping effect was seen. In destalked prawns when eyestalk extract was administered, the level of osmolal concentration did not decrease but always remained high throughout the experimental period. Probable reasons for such changes are discussed.

INTRODUCTION

Members of genus *Penaeus* live over a wide range of salinities and are cultured under varying conditions in many tropical and sub-tropical parts of the world. Many penaeid shrimps of euryhaline habitat have been found to have a common migratory behaviour of returning to high saline conditions for maturation and spawning (George and Vedavyasa Rao, 1968; Castille and Lawrence, 1981) and *Penaeus monodon* is no exception to this. This migratory pattern to higher salinities during maturation process possibly can be well correlated with osmoregulatory capabilities. Certain aspects of osmoregulatory capabilities of some penaeid species have been well characterized in recent times (Dall, 1981; Howe *et al.*, 1982, Ferraris *et al.*, 1986; Diwan and Laxminarayana, 1989).

The 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 factors in ionic regultion and water balance has been studied in many crustaceans (Tullis and Kamemoto, 1971; Heit and Fingerman, 1975; Kiron and Diwan, 1984 a,b). But still there is a paucity of information on these lines in penaeid groups.

In the present investigation, attempts were made to study changes occurring in osmolal concetnrations of haemolymph in adult *P. monodon* over a range of salinities varying from 3 to 45‰ and also the influence of eyestalk removal in the regulation of haemolymph osmolality.

MATERIAL AND METHODS

Adult *P. monodon* (size range 160-180 mm TL) required for the study were collected from the growout ponds in and around Cochin. Prawns after collection were main-

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tained in the laboratory for 48hr. Only intermoult staged prawns were segregated for the experimental purpose. Experimental salinities requried below S 35‰ 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 to 45‰. When salinity media were ready, six prawns were released into each of the media (totally 11 media). Initially three prawns from each of the media were sampled for haemolymph extract after 24 hr and subsequently the remaining three prawns from each of the media after 48 hr duration. Haemolymph sample from individual prawns was collected from pericardial cavity using chilled 1 ml hypodermic syringe previously rinsed with an anticoagulant (3% triosodium citrate). The haemolymph was delivered into small glass vials and kept in an ice water bath until further use. From each glass vial 50µ l of haemolymph was pipetted with the help of an automatic micropipette and immediately transferred to osmometer cuvette. The cuvette 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 of salinometer.

To find out the possible role of eyestalk in regulation of osmolal concentrations of haemolymph the following experimental set up was designed. Prawns numbering 132 in the size range of 160-180 mm TL were divided into 4 batches viz. A,B,C and D each batch consisting of 33 animals. Each batch was further divided into 11 groups each with 3 animals. All the prawns were maintained in plastic pools having a salinity nearest to the isosmotic point (574 mOsm/kg) of haemolymph which was determined earlier.

Bilateral eyestalk surgery was performed by using electric cauterizer for the prawns belonging to groups C and D. The prawns of the batch A were treated as the initial control and batch B sham operated. The extract of freshly cauterized eyestalks was prepared by homogenising the tissue in isosaline filtered seawater and subjected it for centrifugation at 8000 g for 10 min. The supernatent of the tissue was used as the injecting material to the test animals. Eyestalk ablated prawns of batch D were injected with the supernatent of eyestalks in ratio of 2 eyestalks/0.2ml/prawn. Ablated prawns of the 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 hr and then at intervals of 2, 4, 6, 8, 12, 18, 24, 48 and 72 hr. The haemolymph was then delivered into the prechilled glass vials and osmolality was determined as per the method described earlier.

Results and Discussion

The osmolal concentrations of the haemolymph of *P. monodon* maintained in different salinities for 24 and 48 hr duration are indicated in the Fig. 1. Duration of 48 hr was essential for prawns to adjust to the new medium and regulate normally. In the lower salinities (below S 19‰) prawns showed hyperosmotic behaviour and in higher salinities they behaved hyposmotically. The isosmotic points for 24 and 48 hr duration are found to be ≈ S 18.5‰ and ≈ S 23.5‰ respectively (Fig.1). The findings of hyperosmotic regulations of this species at low salinities

and hyposmotic behaviour at high salinities are in agreement with the earlier reports on other crustaceans (Williams , 1960; Mac Farland and Lee, 1963; Bursey and Lane 1971). Most of the penaeid species as reported earlier, are able to adapt extremely well to very low salinities during their early juvenile life but this ability appears to be reduced in adults (Dall, 1981). But it is found from the present investigation that adult prawns have extremely good capacity to osmoregulate both at low and high salinities. There is great diversity in osmoregulatory ability among penaeids and other decapod crustaceans (Kalberg, 1970; Foreskett, 1977). Therefore, the osmoregulatory ability appears to be purely an adaptive feature and may change according to the environmental situations.

It is seen from the results that prawn *P. monodon* requires at least 48 hr for stabilising osmolal concentration of 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 equilibrium the animal requires time. Bursey and Lane (1971) have reported that for *P. duorarum* a period of about 24 hr is required to establish a new steady state of equilibrium for haemolymph concentration. Castille and Lawrence (1981) reported 3-4 days for *P. setiferus* to stabilise the haemolymph. A steady state of achievement within a short period of 1-2 hr has been reported for Krill and *Macrobrachium* sp. (Forward and Fyhn, 1983; Read, 1984). In an earlier study on *P. indicus* it was found that this species also requires minimum period of 48 hr to stabilise the haemolymph (Diwan and Laxminarayana, 1989).

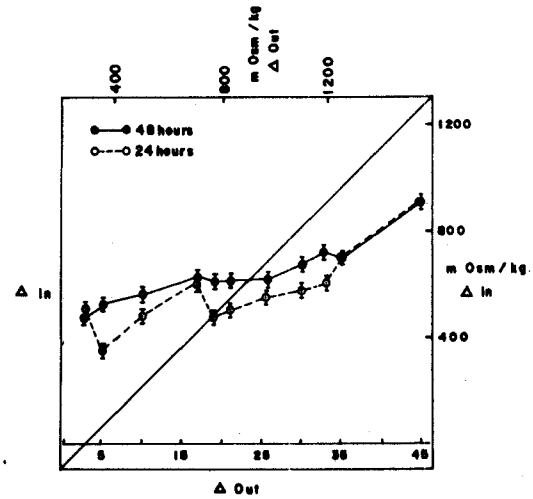

FIG. 1. Osmolality of the haemolymph of *P. monodon* acclimated to different salinities.

The influence of eyestalks on the regulation of osmolal concentration of haemolymph in *P. monodon* is summarised in Table 1. Initial rise was noticed in osmolal concentration of haemolymph in eyestalk ablated prawns and eyestalk ablated after administration of eyestalk extract injection. Subsequently in ablated prawns with isosaline seawater injection, the level of osmolal concentration decreased and was low from 4 to 18hr after eyestalk surgery performance but later there was recouping effect. In the destalked prawns where eyestalk extract was administered, the level of osmolal concentration did not decrease but remained on par with levels of normal ones. This probably indicates that eyestalk factors control the osmolal concentration of the haemolymph. Recouping effect of retaining normal values after 18 hr could be due to the release of osmolal stimulating factor from other neuroendocrine centres like brain and thoracic ganglion. There is an evidence for neuroendocrine control of salt and water concentrations of intermoult crustaceans. Kamemoto *et al.* (1966) using intermoult crayfish *Procambarus clarkii* demonstrated that eyestalk

TABLE 1. *Eyestalk control of osmolal concentration of Haemolymph in th prawn* P. Monodon *(mOsm/kg)*

Hrs after operation	Normal prawns	Sham operated	Bilateral eyestalk surgery + seawater injection	Bilateral eyestalk surgery + injection of eyestalk extract
0	625 ± 6	631 ± 6	615 ± 9	627 ± 7
1	628 ± 14	641 ± 5	679 ± 5	713 ± 9
2	638 ± 11	648 ± 7	634 ± 7	647 ± 11
4	646 ± 8	645 ± 9	566 ± 9	665 ± 8
5	624 ± 15	638 ± 7	559 ± 8	657 ± 13
8	630 ± 11	623 ± 9	543 ± 7	637 ± 9
12	633 ± 9	637 ± 8	580 ± 6	643 ± 7
18	630 ± 8	642 ± 7	594 ± 8	612 ± 5
24	620 ± 15	635 ± 6	638 ± 9	662 ± 9
48	633 ± 7	638 ± 7	621 ± 8	641 ± 7
72	644 ± 5	642 ± 8	668 ± 5	647 ± 7

Analysis of Variance showed that the means of different treatments and different time intervals differed significantly at 5% level (4 to 18 hrs). Each value represents a mean of 3 determinations. $\bar{X} \pm SD$ Temp. of medium = 26° C. Size range of the prawns = 160-180 mm T L. mOsm of medium = 574. Intramuscular injection = 0.2 ml/two eyestalks/prawn.

ablation resulted in lowering of the total blood salt concentration of specimens kept in tap water and injection of eyestalk extract into intact crayfish produced a net increase. Further Kato and Kamemoto (1969) found that in the crab *Metapograpsus messor* injection of eyestalk extract could partially prevent the lowering of total osmotic concentration of blood when eyestalk ligated crabs were maintained in media hyposmotic to the blood. Haemolymph chloride elevating factor has also been reported by Nagabhushanam and Jyothi (1977) in *Caridina weberi* and venkatachari *et al.* (1979) in *Barytelphusa guerini* in which removal of this factor through eyestalk ablation had resulted in a drop in haemolymph chloride, and replenishment of factor through extract injection had elevated it to normal levels. In our earlier observations on *P. indicus* we found similar results of decrease in osmolal concentrations of haemolymph in eyestalkless prawns. (Diwan and Laxminarayana, 1989).

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