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OSMOTIC REGULATION IN *METAPENAEUS ENSIS*

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Abstract

Experiments were conducted to determine salinity tolerance and some aspects of osmotic regulation in the prawn *Metapenaeus ensis*. 100% survival was observed in the full range of sea water concentrations (0-100% s. w.) at the end of the test period of 4 days. In distilled water survival was relatively short. In full sea water the haemolymph was virtually isotonic to the medium. This osmotic concentration was maintained as the medium was diluted to 30% sea water. In media more dilute than this haemolymph osmotic concentration fell rapidly but was markedly hypertonic to the medium. About 50% of the haemolymph osmotic concentration was accounted for by Na salts. The changes in haemolymph osmotic concentrations were partly due to the changes in sodium concentrations. The animals were found to be permeable to a certain degree and it was evident that regulatory mechanisms that actively adjust the composition of the haemolymph were present.

Key word : Salinity tolerance., Osmotic regulation

1. Introduction

The prawn *Metapenaeus ensis* (greasyback shrimp) occurs in large numbers in brackish water lagoons and estuaries along the coast of Sri Lanka. It is believed that *M. ensis* spend most of their adult life in brackish waters and return to the sea several times during their lives to breed. Most estuarine species of animals exhibit varying degrees of euryhalinity, being able to cope with short term salinity fluctuations. But very few migrate between the extremes of sea water and fresh water. Vertebrates which do spawning migrations, such as salmon some lampreys and eels have attracted much attention and their osmoregulatory mechanisms have been studied in some detail. Few invertebrates can migrate between sea water and fresh water, and only one has been studied in detail; the chinese mitten crab, *Eriocheir sinensis* (Potts and Parry, 1964; Rankin and Davenport, 1981).

Metapenaeus ensis contribute significantly to the commercial fishery of Sri Lanka. A few studies carried out on this species provide information on aspects such as life cycle and larval development (Vanichkul, 1970), ovarian maturation and spawning (Yano, 1985), feeding habits (Jinadasa, 1990) and effects of chemical toxicity (Tseng and Chen, 1982). While osmotic regulation in other prawn species such as *Palaemon serratus, P. elegans, P. Longirostris, Palaemonetes Varians, P antennarius and Metapenaeus monocerus* (Potts and Parry, 1964; Rankin and Davenport, 1981) have been studied, no investigations have been carried out on *Metapenaeus ensis* on this subject. The present study reports on salinity tolerance and some aspects of osmotic regulation in *Metapenaeus ensis.*

2. Materials and methods

Animals for this study were obtained from fishermen operating in the Bolgoda lagoon, situated in the west coast of Sri Lanka. Experiments were carried out on *M. ensis* adults of 3-4cm carapace length. Salinity of the lagoon water where the prawns were collected was 9ppt. They were maintained in aquarium tanks containing 30% sea water (10.2 ppt salinity) of 100% air saturation.

For the determination of salinity tolerance, a range of sea water concentrations from 0-100% with gradual increments of 10% were made up using tap water. These concentrations corresponded to salinities of 0.10, 3.4, 6.9, 10.2, 13.6, 17.0, 20.3, 23.8, 27.2, 30.6 and 34ppt. respectively. Batches of 6 animals were taransferred to the various sea water concentrations and to distilled water. Survival times of the animals in the various media were recorded. Haemolymph samples were collected from these animals after 4 days of acclimatization in the various media. In the case of animals put into distilled water, samples were taken soon after their death. Haemolymph was obtained from the heart using a syringe fitted with a 23G hypodermic needle. Osmotic concentratins of haemolymph and the external medium were measured by the method of depression of freezing point (Hoar and Hickaman, 1975). Na^{+} concentrations of haemolymph were measured using a flame photometer (Corning 400). The degree of permeability of the animals was studied by determination of weight change of the animals (n=6) when transferred from the maintenance tanks (10.2ppt) into the different salinities. An animal was initially weighed after blotting out the water on its body and gill chambers, and was introduced into a given salinity. It was then weighed again after 30 min, 1h, 4h, 8h and after 24 hours. Energy expenditure during osmoregulation was found by determination of O_2 consumption of the animals (n=6) acclimated to the various salinities using a flow-through respirometer (Hoar and Hickman; 1975). Before an experiment an animal was allowed to remain in the respirometer for a conditioning period of about an hour. Metabolic rates were expressed as O_2 consumption per unit weight of animal. Statistical comparisons were made using the students' t-test at 5% level of significance.

3. Results

It was observed that the prawn *Metapenaeus ensis* survive the full range of sea water concentrations (0-100% s. w.) for the test period of 4 days. But survival in distilled water was relatively short, all animals being dead about 12-18 hours after introduction. Fig. 1 shows the relationship between osmotic concentrations of the haemolymph and the medium. In full sea water the haemolymph osmotic concentration was virtually isotonic to the

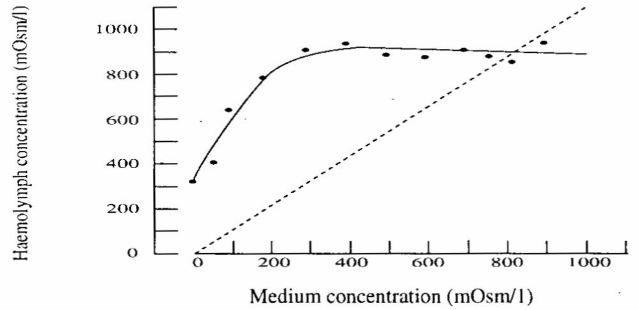

Fig 1 Relationship between the osmotic concentration of the haemolymph and that of the medium for animals acclimatized for 4 days in the range of 0-100% sea water concentrations (mean, n=6). Broken line is the line of iso-osmocity.

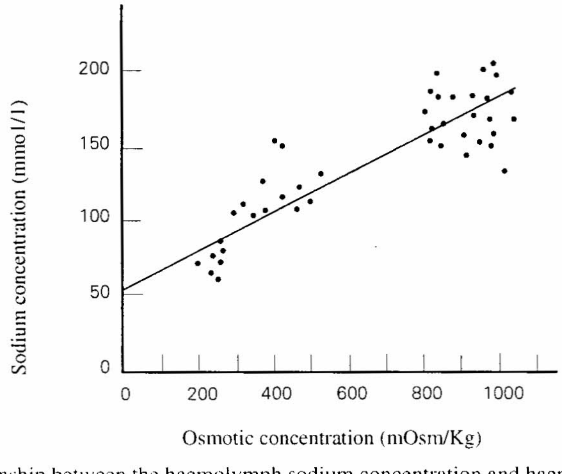

Fig. 2 Relationship between the haemolymph sodium concentration and haemolymph osmotic concentration. (Regression equation, Y=55.75 + 0.128x, r = 0.92).

external medium. This concentration was maintained when the external medium was diluted to 30% sea water. This demonstrates the relative constancy of the haemolymph osmotic concentration and its independance from the medium at high sea water concentrations (30-100%). At all concentrations below this level there was a gradual reduction in osmotic concentration of the haemolymph although it always remained hypertonic to the medium. Fig. 2 shows the relationship between sodium concentrations and osmotic concentrations of the haemolymph. The linear relationship between these two factors shows that the changes in the haemolymph osmotic concentrations are partly due to changes in sodium concentrations of the haemolymph. sodium accounts for about 25% of the total haemolymph osmotic concentration. Therefore sodium salts will account for approximately 50% of the osmotic concentration which indicates the presence of other important osmotic constituents which make up the balance 50%. This is typical of marine species and indicates a marine ancestry for this animal.

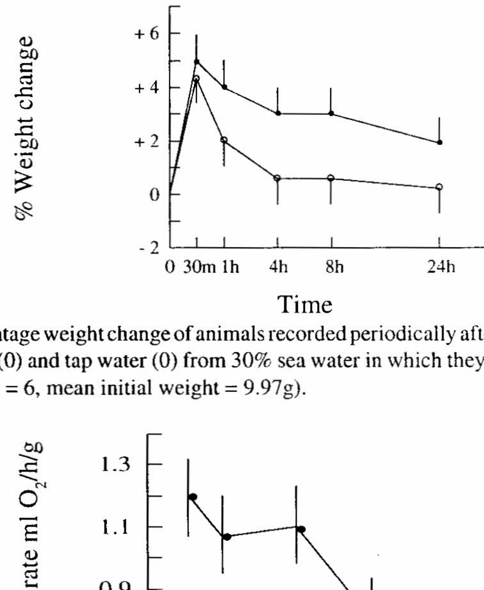

Fig 3. Percentage weight change of animals recorded periodically after introduction into 10% sea water (0) and tap water (0) from 30% sea water in which they were maintained. (mean ± s. e., n = 6, mean initial weight = 9.97g).

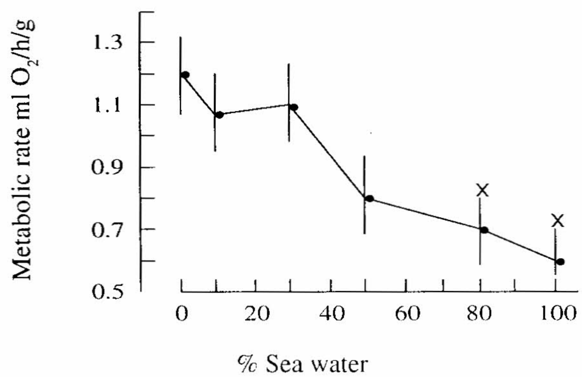

Fig 4. Metabolic rate of *M. ensis* measured in terms of oxygen consumpition in various concentrations of sea water (mean ± s. e., n = 6). x indicates mean values sinificantly different ($p<0.05$) from the tap water values.

Significant changes in body weight were recorded only from animals introduced into 10% sea water and tap water from 30% sea water (Fig. 3). They gained approximately a 5% increase in body weight within the first 30 minutes. This initial increase was followed by a decrease in body weight, which indicated that compensations to correct this increase were brought about within an hour after the introduction. Animals transferred from 30% sea water into more concentrated media showed no change in body weight. Fig. 4 shows the variations in metabolic rate of *M. ensis* in different salinities. The highest rate was recorded in tap water while the lowest occurred in 100% sea water.

4. Discussion

Among the invertebrates all echinoderms and coelenterates are osmoconformers as are the majority of polychaetes bivalve molluscs, cirripede crustaceans and many malacostracan crustaceans (Spaargarten, 1978). These animals allow their extracellular fluid osmotic concentration to fall in line with that of the environment. From the preceding study the prawn *Metapenaeus ensis* can be described as a osmoregulator within the range of 30-100% sea water. Below 30% sea water it cannot be considered a perfect osmoconformer because although its osmotic concentration falls in line with that of the external medium it is nevertheless hypertonic to it.

The limited survival of *M. ensis* in distilled water suggest that it is unable to live in media devoid of any salts. But the fact that it survived in tap water for the duration of the test period demonstrates the ability of *M. ensis* to actively take up ions from very dilute media containing only minute quantities of salts. The ability of this animal to survive the duration of the test period in the full range of sea water concentrations supports the behavioral pattern of an animal living in fresh/brackish waters which has to return to the sea periodically to breed. The fact that *M. ensis* does not breed in the lagoon may be because its larvae cannot survive low salinities. In view of this it would be of interest to examine salinity tolerance of the larvae of *M. ensis*.

The fact that the changes in haemolymph osmotic concentrations are partly due to changes in Na^+ concentration of the haemolymph indicates permeability of the animals to Na^+ and /or water. Significant changes in body weight were recorded from animals transferred from 30% sea water into more dilute media. On introduction to a more dilute medium water tends to move into the animal while salts tend to move out across the more permeable parts of the body surface. Since water movement is faster than salt diffusion, it is expected that they gain a considerable amount of water before osmotic equilibrium is attained, causing them to increase in weight. This swelling is obviously disadvantageous to the animal as it impairs body activities, including locomotory and food collection mechanisms (Rankin and Davenport, 1981). From fig. 3 it is evident that the animal starts eliminating the excess fluid by the first hour after being transferred to low salinities. Werntz (1962; see Potts, 1968) has shown that the volume of urine produced in hyposmotic media is proportional to the osmotic gradient between the heamolymph and medium, a finding which suggests that the urine output may be equivalent to the osmotic inflow. similarly it is possible that the initial osmotic swelling observed in *M. ensis* in dilute media was reversed by an increase in urine output. When *M. ensis* were transferred from 30% sea water into more concentrated media, no change in body weight was recorded. In such a medium they would tend to lose water and gain salts. Since the body weight remained unchanged when introduced in to hypertonic media it appears that *M. ensis* is more permeable to water in dilute media than in concentrated ones. Kamemoto et al (1966; see potts, 1968) and Bliss et al (1966; see Potts, 1968) working on *Procambarus* and on *Gecarcinus* respectively have found evidence of a hormone released from the eyestalk which decreases the permeability of the gills and body wall to water. Bliss et al (1966) suggest that there is also another hormone favouring uptake and retention of water. It is possible that similar hormones control the permeability of *M. ensis* in different salinities.

Reduction in salinity of the medium resulted in increased O_2 consumption in *M. ensis.* Similar increases in O_2 consumption in hypotonic media have been recorded for *Metapenaeus monocerus* and *Artemia salina* (Rao, 1958). But Potts (1954) has shown that in *Eriocheir* only a very small fraction of the increased O_2 consumption represented osmotic work done.

This preliminary study shows that, *M. ensis* survive in 0-100% sea water. In full sea water the haemolymph was isotonic to the medium. This osmotic concentration was maintained as the medium was diluted to 30% sea water. In media more dilute than this, the haemolymph osmotic concentration fell rapidly but was markedly hypertonic to the medium. The changes in haemolymph osmotic concentrations were partly due to changes in sodium concentrations. The animals were found to be permeable to a certain degree and it was evident that regulatory mechanisms that actively adjusted the composition of the haemolymph were present.

5. Acknowledgements

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