Osmotic Regulation in a Brackish Water Oligochaete, *Pontodrilus bermudensis* Beddard*

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*P. bermudensis* shows hyperosmotic body fluid concentrations in lower salinities (15% and below), and more or less isosmotic concentrations in higher salinities (15% and above). Body fluid concentrations of *P. bermudensis* are higher in the terrestrial oligochaete species indicating its brackish water inhabitance at the local harbour. The trend of hyperosmotic regulation in *P. bermudensis* is similar to the reported pattern in several brackish water and estuarine polychaete annelid worms.

Although osmoregulatory capacities have been extensively investigated in the semi-terrestrial oligochaetes, *Lumbricus terrestris* and *Phereclina posthuma*, investigations on the osmoregulatory adaptation of aquatic oligochaetes are lacking. Since *Pontodrilus bermudensis*, a brackish water oligochaete, shows tolerance of wide ranges of salinity and exhibits capacities for volume/weight regulation under heterosmotic stresses, the present investigation has been undertaken to understand its osmoregulatory capacities.

Method for determining the freezing point depression was the comparative melting point method of Jones as modified by Freeman and Rigler. Refinements like the use of polaroids and a binocular microscope as suggested by Gross were also adopted. Procedures of collection of worms and their maintenance were same as described earlier. Samples of body fluid were collected from worms after 3 days of exposure to various concentrations of experimental media at room temperature (28±2°C). Preliminary experiments had shown that the worms attained a steady state equilibrium at the end of 3 days. Osmotic concentrations of experimental media ranged from 0-5 to 17% NaCl (salinities from 5 to 30% respectively). Osmotic concentrations of body fluids have been given both in terms of per cent NaCl and in mM NaCl/litre. Also, the experimental media concentrations are shown in salinity and per cent NaCl units.

Data (average of 8-18 observations) are presented in Fig. 1. Mean osmotic concentrations of body fluid ranged from a minimum value of 1-31 to a maximum value of 3-04% NaCl in worms exposed to salinities from 15 to 30% respectively. The body fluid concentrations increased with increasing salinity concentrations of the media. The slope in the regression line (1) is higher than that of the regression line (2) indicating that the slope of the regression line (2) is steep and runs closer to the isosmotic line showing that the body fluid osmotic concentrations are more or less equal to those of the experimental media (15% and above). The slope of the regression line (1) is less steep and the body fluid osmotic concentration values (1-31 and 1-39% NaCl) are significantly higher than those of experimental media concentrations (5 and 10%). In other words, *P. bermudensis* showed hyperosmotic regulation in lower salinities and become more or less isosmotic in higher salinities. The pattern of osmoregulation in *P. bermudensis* resembles that of the hyperregulators which show hyperosmoticity in lower salinities followed by an osmotic conformity in higher salinities.

In the present investigation, body fluid of *P. bermudensis* showed increasing concentrations with increasing salinity concentrations of the media. While the osmoregulatory behaviour of an essentially brackish water oligochaete like *P. bermudensis* and that of terrestrial forms may be widely different, it is interesting to observe that the body fluid concentrations of 224-520 mM NaCl/litre in *P. bermudensis* are much higher than that of any semi-terrestrial oligochaete so far investigated. It is, however, significant that this body fluid concentration closely match with those recorded in some brackish water polychaetes, such as *Nereis diversicolor*, *Nereis limnicola*, *Nereis succinea* and *Leonereis culveri*. It is well known that the osmotic concentrations of the body fluid in brackish water organisms are higher than those of fresh water organisms but less than those of marine organisms.

Higher osmotic concentration of body fluid in *P. bermudensis* compared with that of the semi-terrestrial oligochaetes may be a result of its brackish water habitat. In the absence of investigations on the osmotic concentrations of estuarine/marine oligochaetes it is rather difficult to say as to how the osmotic concentrations of *P. bermudensis* would compare with those of its purely marine relatives.

Pattern of osmotic regulation was thoroughly investigated only in a semi-terrestrial oligochaete, *Lumbricus terrestris* which showed that *L. terrestris* held its body fluid osmotic concentrations always hyperosmotic to all experimental media (3 to 150 mM NaCl/litre). Potts and Parry reviewing the osmotic regulation in fresh water animals were of the view that terrestrial oligochaetes limited to damp habitats, generally in contact with a film of soil water, osmoregulate in some respect like aquatic animals. In the above context, the present investigations on *P. bermudensis*, yielded a few interesting results. In salinities below 15%, it was hyper-regulating. Whether or not this mechanism would break down in salinities below 5% as it did in a brackish water polychaete, *Nereis diversicolor* is presently not known, since salinities lower than 5% were not tested. In higher salinities of 15% and above, although the body fluid concentrations were slightly hypoosmotic except at a salinity of 30% (3-04% NaCl) they were not significantly different from isosmotic values. In other words, like hyperregulating polychaetes, *Nereis diversicolor*, *N. limnicola*, *N. succinea* and *Leonereis culveri*, *P. bermudensis* also behaved like an osmoconformer in higher salinities.
SHORT COMMUNICATIONS

SALINITY CONCENTRATION OF THE EXPERIMENTAL MEDIA, %

Fig. 1 — Relationship of osmotic concentration in the body fluid of *P. bermudensis* to that of experimental medium after adaptation to heterosmotic media over 3 days [Temp. 28°C ±2°C. Each point is the mean of 8-18 estimations]

Yet another aspect in which *P. bermudensis* resembles the osmoregulating brackish water polychaetes such as *Nereis diversicolor, N. limnicola, N. succinea* and *Leucosynecis culveri* is the level at which the mechanism for hyperregulation is triggered into operation. In all the osmoregulating polychaetes cited, the phase for hyperregulation started at a medium concentration level of about 200 mM NaCl/litre. In *P. bermudensis* also, the hyperregulatory phase commenced at a medium concentration level ranging from 171 to 255 mM NaCl/litre. In the upper level of commencement of hyperregulatory phase, it further resembled another brackish water onuphid polychaete, *Onuphis magna*, which has a higher level (256-300 mM NaCl/litre) at which hyperregulation began. Perhaps, the level of hyperregulation in most brackish water organisms commences at about the medium concentration level of 200 mM NaCl/litre. At least it does so in brackish water annelid worms as already shown by previous investigations on polychaetes and the present work on the brackish water oligochaete, *P. bermudensis*.

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Adaptations to Osmotic Stress in the Marine-Euryhaline Teleost *Periophthalmus dipes*:
Tissue Water & Mineral Content

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Water, sodium, potassium, calcium and phosphorus contents were estimated in different tissues of *P. dipes* after adaptation to various concentrations of sea water. Water content did not vary significantly in any tissue on adaptation to various salinities. Sodium, potassium and phosphorus levels of different tissues showed decreasing trend in most of the salinities and absolute fresh water. The results revealed that this fish has ability to adjust the mineral status of its tissues according to the nature of salinity and hence it becomes gradually acclimatized to different salinities without becoming waterlogged.

Salinity and temperature have been categorized as ecological master factors for estuarine species. Acclimation to different salinities not only affects the rate of metabolism but also its efficiency. In general, structural consequences in rates of metabolism affect development, differentiation, and relative growth of cells and organs. Salinity tolerance in fishes has been investigated from many angles. Studies of changes in the levels of various inorganic substances in relation to marine or fresh water were carried out. The mudskipper *Periophthalmus dipes*, living in intertidal zone, is a euryhaline teleost capable of withstanding wide changes in salinity (47-24%). In view of the scanty information on the effects of salinity adaptation on metabolism in marine euryhaline fishes, the present investigation has been undertaken.

*P. dipes*, collected from muddy sea coast of Sikha (about 28 km from Jamnagar) during ebb-tide, was maintained in the laboratory aquaria containing sea water for a couple of days. They were fed with frog muscles and dried prawn powder on alternate days. After adaptation to laboratory conditions for 5 days, the fishes were separately acclimated to absolute sea water (SW), and 90, 70, 50, 30, 10% SW and 100% fresh water (FW) for 5 days.

The fishes, 12-16 cm in total length group and weighing 20-24 g, were used for experiments and analysis. After acclimation of the fish to the respective salinities for 10 days, they were starved for a day and killed. Tissues such as gill, heart, liver, kidney, red muscle and white muscle were isolated and analysed. Water content was determined gravimetrically by calculating the difference between the fresh weight and dry weight of tissues concerned. For mineral analysis, air-dried material was subjected to wet digestion using concentrated sulphuric acid, 20 volumes hydrogen peroxide and catalysts like copper sulphate and selenium to enhance the digestion. The clear solution obtained was diluted to 10 ml. Sodium, potassium and calcium were analysed as described by Jackson employing a flamephotometer using indane gas at 10 atm. Phosphorus was analysed as described by Oser using EEL colorimeter with red filter.

Charges observed in water content and mineral components in different tissues of fish exposed to different salinities of sea water are given in Table 1.

The experiments on salinity tolerance in *P. dipes* reveal that this fish can tolerate very wide range of salinity. Water and inorganic constituents from the major components of body tissues are readily affected by change in the salinity of the medium. Changes in water content in relation to different salinity adaptations have been examined in few fishes. Love stated that the water content of body muscle increases slightly when euryhaline fishes are transferred from sea to fresh water. In the present investigation, water content of different tissues shows little variation on acclimation to different concentrations of sea water (Table 1), except in heart and gill which show -33% (in 70% SW) and -21.9% (in 10% SW) variation over control respectively. The fact that water content does not vary significantly on transfer to different salinities may contribute to the adaptability of fish to fresh water without making its tissues waterlogged.

Variations in the inorganic components on salinity acclimation have been examined in a variety of fish tissues. Sharratt *et al.* and Chan *et al.* have demonstrated a decrease in sodium, potassium and magnesium level of parietal muscle in a euryhaline fish *Anguilla anguilla* after transfer from marine to fresh water. Lange and Fugelli have also observed depletion in the level of sodium and potassium in body muscle of fish *Platichthyes flessus* on acclimation to fresh water. Similar results have been reported by Pequignot and Serfaty in

References