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SODIUM, CHLORIDE AND WATER BALANCE OF THE INTERTIDAL TELEOST, *XIPHISTER ATROPURPUREUS*

I. REGULATION OF PLASMA CONCENTRATION AND BODY WATER CONTENT

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INTRODUCTION

Because of physiological restrictions the vast majority of teleosts are able to tolerate only a relatively narrow salinity range near either sea water or fresh water. A few, however, are able to survive in salinities that are either hyperosmotic or hypo-osmotic to the concentration of the internal medium; eels (Sharratt, Chester Jones & Bellamy, 1964; Butler, 1966), salmon and trout (Gordon, 1959; Parry, 1961; Holmes & Stanier, 1966), sticklebacks (Lange & Fugelli, 1965), killifish (Clemens & Jones, 1954; Pickford & Slicher, 1965) and flounders (Lange & Fugelli, 1965; Motais, 1967), are the best studied examples.

Intertidal teleosts, which might be expected to be euryhaline because of their environment, have remained virtually unstudied. Raffy (1949) and Gordon *et al.* (1965) have presented the only published data on determinations of the degree of euryhalinity of an intertidal species; in both cases a member of the order Blennioidea was chosen. *Xiphister atropurpureus* is the most abundant blennioid teleost on the central California coast. Studies were undertaken to compare this species with the other two blennioids that have been studied and with other euryhaline teleosts.

MATERIALS AND METHODS

Adult *Xiphister atropurpureus* (hereafter referred to as *Xiphister*) were collected at low tides by hand from under rocks on a moist sand or pebble substrate. Most collections were made near Pigeon Point (Santa Cruz County, 37° 11′ N.; 122° 22′ W.) but a few individuals were collected near Yankee Point (Carmel County, 36° 33′ N.; 121° 56′ W.). Collections were made from December 1965 to November 1966. Individuals weighed from 7 to 40 g. and were held in a 100 l. Perspex aquarium at a temperature (13 ± 1° C.), similar to that of their environment. The animals were not fed after capture; they were usually used in experiments within 2 weeks. Individuals were allowed at least 2 days for salinity acclimation in smaller (5 l.) glass aquaria. 100% sea water contained 480 mM-Na/kg. and 560 mM-Cl/kg.

Before any procedure involving manipulation the animal was immobilized in a

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solution of MS-222 (Sandoz) diluted with a solution of the experimental salinity to make a 0·02 % solution of the anaesthetic. At the termination of most experiments the animal was weighed by drying it with paper towelling, weighing it wrapped in tissue paper and then weighing the tissue paper separately. The accuracy of this method, measured by repeated weighings of a single specimen was ± 2 %. The precision of the Mettler balance used was taken as 0·01 g. The sex of the animal was determined by inspection of the gonad.

Ancillary studies on this species (Evans, unpublished observations) showed that *Xiphister* is reproductively active from February to June. Duplicate experiments were performed on reproductive and non-reproductive individuals and any differences found will be discussed.

Blood was collected by exposing the heart, cutting the bulbus arteriosus and drawing a sample into a heparinized capillary tube. The ends of the tube were sealed with plasticine and the plasma was separated from the cells by centrifugation. The capillary tube was cut at the interface between the cells and the plasma, the cells were discarded and the portion of the tube containing the plasma was resealed and stored at − 10° C until sodium and chloride determinations could be made. Six months storage at this temperature had no measurable effect on sodium or chloride concentrations of the plasma.

The sodium concentration of a 5 μl. sample of plasma, diluted 1 : 1000 with distilled water, was determined using a Beckman model DU flame spectrophotometer with a photomultiplier attachment. Accuracy, determined by repeated measurements of a standard, was within ± 1 %. The precision of the spectrophotometer was of the order of 4 μM/kg.

The concentration of chloride was determined by mecuric nitrate titration of a solution containing 5 μl. of plasma, 50 μl. of distilled water, 50 μl. of 0·03 N-NHO_3 and 50 μl. of s-diphenylcarbazone. Titration was performed with a Beckman Spinco microtitrator with an accuracy of ± 2 % and a precision of 1 μM/kg.

The degree of dilution of an inert tracer (^{14}C-inulin) was used as a measure of the extracellular space (E.C.S.). The fish was injected and returned to the aquarium for 1 hr., after which it was removed and anaesthetized and blood was drawn. Samples were taken after 1 hr. because a series of experiments showed that complete mixing of the injected inulin took place within 1 hr. After 6 hr. the apparent E.C.S. had increased by approximately 75 %, presumably because inulin had been lost from the body. The volume of the solution injected (100 μl.) was never more than 2 % of the total body water of the fish.

The injected solution contained approximately 90 % non-radioactive inulin as carrier and approximately 0·5 μC of ^{14}C-inulin. The radioactivity of a 5 μl. sample of plasma or of a 5 μl. sample of a standard prepared by injecting 100 μl. of the ^{14}C-inulin solution into 3 ml. of saline (0·6 % NaCl) was determined by counting the sample in Bray's solution (Bray, 1960) to 1000 counts in a Nuclear Chicago Mark II Liquid Scintillation System. The volume of the ECS of the fish was calculated from the following relationship:

$$\frac{\text{E.C.S. in ml.}}{\text{volume of standard (3 ml.)}} = \frac{\text{C.P.M./}\mu\text{l. of standard solution}}{\text{C.P.M./}\mu\text{l. of fish plasma}},$$

$$\text{E.C.S. in ml./kg. fish} = (\text{E.C.S. in ml./wet weight of fish})\ (1000).$$

The total body water (T.B.W.) was determined by drying weighed fish at 100° C to constant weight. The T.B.W. was then calculated from the relationship:

$$\text{T.B.W. in g./kg. fish} = \frac{\text{wet weight of fish} - \text{dry weight of fish}}{\text{wet weight of fish}} \times 1000.$$

The volume of the intracellular space (I.C.S.) was estimated by changing the total body water directly to ml./kg. fish and subtracting the E.C.S. This substitution of ml./kg. fish for g./kg. fish introduced a small error dependent on the specific gravity of the body fluids (which was not measured). However, Thorson (1961) and Lange & Fugelli (1965) have shown that the specific gravity of tissues changes little with salinity variations. Thus the error introduced in these calculations would be relatively constant.

Experiments were undertaken in three salinities; 100% sea water, 31% sea water (approximately iso-osmotic) and 10% sea water (the low end of the range of salinity tolerance). In most cases the individuals used were the animals used in urine flow experiments to be described subsequently (Evans, 1967).

RESULTS AND DISCUSSIONS

The regulation of the plasma concentrations of sodium and chloride in the three salinities is shown in Fig. 1. It is obvious that *Xiphister* is an excellent regulator of

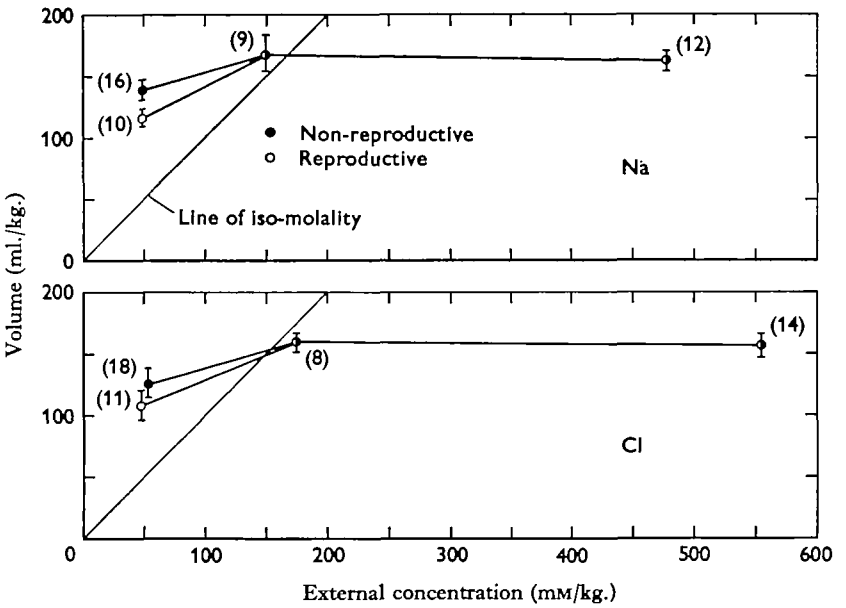

Fig. 1. Regulation of plasma concentrations of sodium and chloride in 100, 31 and 10% sea water. Each point is the mean ± standard deviation (number of samples).

sodium and chloride over this range. This degree of ionic regulation is comparable to that described for the European blenny, *Blennius pholis* (Raffy, 1949) and the tropical blennioid *Periophthalmus sobrinus* (Gordon *et al.* 1965) and, although the degree of

euryhalinity is less, it is also comparable with the degree of regulation demonstrated for the better known euryaline teleosts.

It is evident from the data that in 10% sea water the reproductive fish maintain a plasma concentration of sodium and chloride below that of the non-reproductive fish. Data from both sexes were combined in these experiments because variation was too great to establish a difference. Further, all experiments were performed on fish approximately within the same range of weight to avoid any systemic variation caused by size.

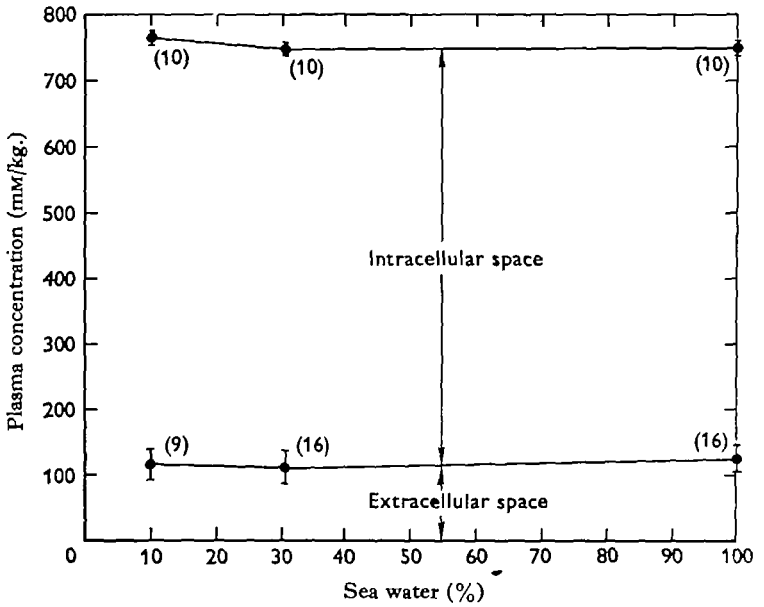

Fig. 2. Regulation of extracellular and intracellular spaces in 100, 31 and 10% sea water. Each point is mean ± standard deviation (number of samples).

The regulation of the E.C.S. and the I.C.S. in the three salinities is shown in Fig. 2. When these data are expressed graphically it appears that, while the E.C.S. remains virtually constant over the salinity range, the I.C.S. does increase slightly in 10% sea water. On the other hand, if the E.C.S. and the I.C.S. are expressed in ml./g. dry weight of fish then it becomes evident that both increase by approximately 6%. Nevertheless, it is evident that regulation of the water spaces is relatively good. Lange & Fugelli (1965) have shown that in *Pleuronectes flesus* and *Gasterosteus aculeatus* some of the intracellular regulation is due to control of organic solutes. Further, it is clear that the decline in plasma content of sodium and chloride is due mainly to ion loss, since it would require a 24% increase in the E.C.S. to account for the drop in blood concentration displayed by individuals acclimated to 10% sea water. An E.C.S. of approximately 125 ml./kg. fish is comparable to Thorson's (1961) data for marine teleosts. The data of Gordon *et. al.* (1965) are unclear but seem to show that the E.C.S. of *Periophthalmus sobrinus* increases slightly in 20% sea water. Their high E.C.S. value is probably due to delayed sampling of the blood. No other data on the regulation of the E.C.S. over a salinity range have been published (Parry, 1966). An I.C.S. of approximately 625 ml./

kg. fish is somewhat (13%) higher than that reported by Thorson (1961) for marine teleosts.

Thus it seems that *Xiphister* is an excellent regulator in salinities that are both hyperosmotic and hypo-osmotic to its internal environment. More data are necessary before one can conclude that most intertidal teleosts are euryhaline but they certainly offer opportunities for investigation.

SUMMARY

1. Studies were undertaken to determine the degree of regulation of sodium, chloride and water displayed by the intertidal teleost, *Xiphister atropurpureus*, over a range of salinities.

2. The plasma concentrations of sodium and chloride declined by approximately 15% in 10% sea water (48 mM-Na/kg.) and the intracellular and extracellular spaces increased by approximately 6% in 10% sea water.

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REFERENCES

BRAY, G. A. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1**, 279–327.

BUTLER, D. G. (1966). Effect of hypophysectomy on osmoregulation in the European eel. (*Anguilla anguilla L.*) *Comp. Biochem. Physiol.* **18**, 773–81.

CLEMENS, H. P. & JONES, W. H. (1954). Toxicity of brine from oil wells. *Trans. Am. Fish. Soc.* **84**, 97–109.

EVANS, D. H. (1967). Sodium, chloride and water balance of the intertidal teleost, *Xiphister atropurpureus*. II. The role of the kidney and the gut. *J. exp. Biol.* **47**, 519–34.

GORDON, M. S. (1959). Ionic regulation in the brown trout (*Salmo trutta*). *J. exp. Biol.* **36**, 227–232.

GORDON, M. S., BOETIUS, J., BOETIUS, I., EVANS, D. H., MCCARTHY, R. & OGLESBY, L. C. (1965). Salinity adaptation in the mud-skipper fish, *Periophthalmus sobrinus*. *Hvalrådets skrifter.* **48**, 85–93.

HOLMES, W. N. & STANIER, I. M. (1966). Studies on the renal excretion of electrolytes by the trout (*Salmo gairdnerii*) *J. exp. Biol.* **44**, 33–46.

LANGE, R. & FUGELLI, K. (1965). The osmotic adjustment in the euryhaline teleosts, the flounder, *Pleuronectes flesus L.* and the three-spined stickleback, *Gasterosteus aculeatus* L. *Comp. Biochem. Physiol.* **15**, 283–92.

MOTAIS, R. (1967). Les méchanismes d'échanges ioniques branchiaux chez les téléostéens. *Ann. Inst Oceanogr.* **45**, 1–83.

PARRY, G. (1961). Osmotic and ionic changes in the blood and muscle of migrating salmonids. *J. exp. Biol.* **38**, 411–27.

PARRY, G. (1966). Osmotic adaptation in fishes. *Biol. Rev.* **41**, 392–444.

PICKFORD, G. E. & SLICHER, A. M. (1965). Plasma specific gravity and serum osmotic pressure in fresh water and salt water adapted killifish *Fundulus heteroclitus*. *Anat. Rec.* **151**, 471.

RAFFY, A. (1949). L'euryhalinité de *Blennius pholis* L. *C.r. Soc. Biol.* **143**, 1575–6.

SHARRATT, B. M., CHESTER JONES, I. & BELLAMY, D. (1964). Water and electrolyte composition of the body and renal function of the eel (*Anguilla anguilla* L.). *Comp. Biochem. Physiol.* **11**, 9–18.

THORSON, T. B. (1961). The partition of body water in osteichthys: phylogenetic and ecological implications in aquatic vertebrates. *Biol. Bull. mar. biol. Lab. Woods Hole.* **120**, 238–54.