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SODIUM AND WATER BALANCE IN THE CICHLID TELEOST, *TILAPIA MOSSAMBICA*

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INTRODUCTION

The ability of some fishes to live in both sea water and fresh water has attracted a great deal of interest. Many euryhaline fishes are difficult to rear in laboratory conditions; some do not survive in completely fresh water, and migratory fishes are often euryhaline for only limited periods during their life-history. Species of the genus *Tilapia* have none of these disadvantages; they are easily reared, they tolerate salinities as low as 0·1 mM-Na/l. and can be transferred abruptly between fresh and sea water. Further, they are able to survive very high salinities (to 69 ‰, Spector, 1956, p. 456). The main features of sodium and water balance have been studied in *Tilapia mossambica*.

MATERIALS AND METHODS

The fishes were reared in the laboratory from a stock which had been inbred for several generations. Adults for breeding were kept together in a large tank of fresh water at 21–24° C. and fed daily on chopped heart and commercial dried fish food. This diet was supplemented once each week with fresh lettuce. The young were shaken from the mouth of the adult shortly after the eggs were hatched and subsequently kept in smaller tanks of fresh water at the same temperature as the breeding tanks. At this stage they were fed entirely on the dried fish food. Most of the experiments were performed on the young fishes from 5 to 10 weeks after hatching. At this time they weighed between 0·5 and 3·0 g.

The fresh water used in this experiment was either Birmingham tap water, containing *c.* 0·2 mM-Na/l., or an artificial medium containing 0·3 mM-Na/l. to which was added a little solid $CaCO_3$. Sea-water aquaria were filled with water obtained from the Plymouth Marine Laboratory and from Bangor and contained 420 mM-Na/l. 'Forty percent sea water' was prepared by the dilution of full-strength sea water with tap water. In some cases '200% sea water' was prepared by the addition of Analar NaCl to normal sea water to give a final concentration of 840 mM-Na/l.

Young fishes were adapted to 40% sea water by placing them directly in this medium at least 3 days before use. Fishes to be adapted to 100% sea water were placed in the 40% medium for at least 24 hr. and then transferred to the 100% medium for a minimum of 3 days before use. Fishes to be adapted to 200% sea water were placed in the 40% medium for at least 1 day and in 100% sea water for at least 2 days before being transferred to 200% sea water. They remained in this medium for at least one week before being used in these experiments.

Measurement of whole-body sodium concentrations

The sodium content of the fish was estimated by dissolving it in a small quantity of Analar concentrated nitric acid and diluting this solution with de-ionized water. The sodium concentration was measured by comparison with a standard solution containing 0·2 mM-Na/l. using an E.E.L. flame-photometer. The sodium concentrations of the various media were measured using the same method.

Measurement of sodium fluxes

The rate of uptake and loss of sodium from fishes in equilibrium with the medium in which they were bathed was measured by labelling with the radioactive isotope ^{24}Na as sodium chloride. In the various sea-water media the amount of sodium chloride added to label the solution was very small in comparison with the total amount already present, hence the changes in sodium level of these media were insignificant. When labelling the freshwater solutions, however, an entirely artificial fresh water had to be used since the addition of sufficient ^{24}NaCl to normal tap water would have significantly altered the sodium level of this medium. A small amount of calcium carbonate was added to the solution.

The animals, in batches of four, were placed for a period of either 1 or 2 hr. in 250 ml. of the labelled loading solution, after which they were washed for 5 min. in three changes of inactive medium to remove sodium on the surface of the skin and in the mouth. Animals in which the rate of influx of sodium was to be estimated were then killed, weighed and dissolved in 5 ml. of concentrated nitric acid. The activity of this solution was then measured using an EKCo sodium iodide crystal scintillation counter and an EKCo 610B scaler. The activity of a sample of the medium in which the fishes had been loaded was also measured. The rate of influx (K_i) was calculated using the formula

$$K_i = \frac{1}{t} \log_e \frac{A_\infty}{A_\infty - A_t},$$

where t is the time, in hours, of influx, A_∞ is the maximum number of counts in the animal if all the sodium had exchanged, calculated from the specific activity of the bathing medium and the total sodium content of the fish, and A_t is the activity in the fish after loading for t hours. The duration of the experiments was such that the specific activity of the medium was not significantly decreased.

Animals from which the rate of efflux of sodium ions was to be measured were loaded and washed as above and then placed in individual beakers containing 50 or 100 ml. of inactive medium. After a period of 1 hr. a 5 ml. aliquot of the bathing medium was removed and its activity was measured. At the same time the animal was removed, weighed and digested in 5 ml. of concentrated nitric acid, and the activity of this solution was measured. The rate of efflux (K_e) of sodium from the fish was calculated using the formula

$$K_e = \frac{1}{t} \log_e \frac{A_0}{A_t},$$

where t is the time in hours of the period of efflux, A_t is the activity of the animal after efflux has continued for t hours and A_0 is the activity in the animal before efflux

has begun (calculated from the total activity in the efflux solutions plus that in the fish at the end of the experiment).

Measurement of drinking rates

The rate of drinking of *Tilapia* was measured by immersing fishes in a medium of the same salinity as that to which they were adapted, but containing a small amount of ^{35}S-labelled sodium sulphate. In the fresh water medium some of the chloride was replaced by sulphate. One hour after immersion the fishes were removed and placed in three changes of unlabelled medium for a total of 5 min., to wash any labelled sulphate off the skin and out of the mouth. Each animal was then dissected and the gut was removed and dissolved in a small amount of concentrated nitric acid. An aliquot of this was placed on a planchet, dried and the activity in it was measured, using an Isotope Developments Ltd. 663C scintillation counter. Samples of other tissues, skin, muscle, gill and kidney were digested and the amount of radioactive sulphate in them was also measured. In no instance did the total amount of activity in these other tissues exceed 10% of the total activity in the animal. The highest activity in the other tissues was in the kidney but this never exceeded 5% of the total in the gut. The gut appears, therefore, to be relatively impermeable to sulphate ions and the amount of activity present in the gut can be taken as a reasonable guide to the volume of medium swallowed by the animal. Other experiments showed that the increase in activity in the gut was linear for the first hour, so that loss from the anus during the first hour may be neglected.

Measurement of permeability to water

To measure the rate of water influx five to ten *Tilapia* were placed in a well-aerated container which was immersed in a temperature-controlled water bath at $25 \pm 1°$ C. At least 24 hr. were allowed for the fishes to become adapted to their surroundings. At the beginning of an experiment ^3H_2O was added to give a specific activity of 1 μc./ml. The fishes showed no signs of distress when treated in this manner. The animals were removed from the loading solution at the end of 30 min., rinsed quickly, dried, weighed and frozen.

To extract the water from the fishes a freeze-drying technique was used. The fishes were first cut into small pieces and then placed in a flask connected to a high-vacuum pump. Water vapour was condensed as ice in a flask surrounded by acetone and dry ice. All the water was removed from the fish in this manner. The extract was then thawed and 0·1 ml. samples were removed to liquid scintillation vials. The activity in the vials was measured in a Nuclear Chicago liquid scintillation counter. The activity in a sample of the loading solution was estimated by the same method. The rate of water influx was calculated using a formula analogous to that used in the sodium flux experiments. Influx measurements were made on fishes from three different salinities, fresh water, 100% sea water and 200% sea water; fifteen fishes were used at each salinity.

To estimate the rate of efflux single fish were loaded by leaving them in the loading solution for a time equivalent to ten times the biological half-life for water. The loaded fish was then removed, rinsed quickly and placed in 500 ml. of non-radioactive medium.

The activity of the medium was measured at ½, 1, 2, 4 and 24 hr. after starting the experiment.

The efflux constant can be derived (Rudy, 1966) from the following equation:

$$m = \frac{-\ln([\alpha-\beta]/\alpha_0)}{t([1/A]+[1/B])},$$

where m is the rate of water exchange (ml./hr.), A is the volume of exchangeable water in the fish, B is the volume of water in the external medium, t is time (hr.), α is the specific activity of the water in the fish (cts./ml.) at time t, α_0 is the specific activity of the water in the fish at zero time and β is the specific activity of the medium.

The efflux constant, K_e, equals m/A. To obtain a graphical representation of water efflux the fraction of the original activity remaining in the fish, $(\alpha-\beta)/\alpha_0$, is plotted against time on a semi-log scale.

RESULTS

Total sodium content

The total sodium content of the fishes increased slightly with increasing external salinity. In fresh water it was 45·9 μM/g. wet weight of fish (± 1·34 S.E., 12 determinations) while in 40% sea water it had increased to 53·7 μM/g. fish (± 1·43 S.E., 12 determinations). The figures for 40% and 100% sea water were almost identical, the latter being 53·4 μM/g. fish (± 1·06 S.E., 14 determinations). There was a further increase between 100% and 200% sea water to 59·9 μM/g. fish (± 1·12 S.E., 6 determinations) in the latter medium.

Sodium pool

The efflux of sodium from a sea-water-adapted fish loaded to equilibrium follows the equation

$$A_t = A_0\,(0{\cdot}68e^{-0{\cdot}74t} + 0{\cdot}32e^{-0{\cdot}032t}) \quad \text{(Figure 1.)},$$

where A_0 is the initial activity and A_t the activity at time t. The fast component contains 68% of the total activity (= 36·3 mM/kg.) and declines with a rate constant of 0·74/hr. (≡ 26·8 mM/kg. hr.). The slow component contains 32% (17·1 mM/kg.) and has a rate constant of only 0·032/hr. (≡ 0·55 mM/kg./hr.). If the slow component is internal and exchanges only with the fast component then the efflux rate will be 26·8 mM/kg./hr., whilst if the slow phase exchanges independently the total efflux will be 27·3 mM/kg./hr.

Flux rates of sodium

The rates of influx and efflux of sodium are shown in Table 1, both rates increase with increasing external salinity. The values of K_i and K_e in fresh water show some discrepancy. The differences between the other figures are slight and within the bounds of accuracy.

Table 1. *Rates of influx (K_i) and efflux (K_e) of sodium in various media from Tilapia* (± S.E.)

(Figures in parentheses are numbers of experiments.)

Medium	K_i	K_e
Fresh water	0·0346 ± 0·0018 (20)	0·049 ± 0·0063 (13)
40 % sea water	0·136 ± 0·0014 (22)	0·0985 ± 0·0232 (16)
100 % sea water	0·658 ± 0·071 (17)	0·5548 ± 0·0395 (18)
200 % sea water	0·902 ± 0·233 (21)	1·160 ± 0·222 (10)

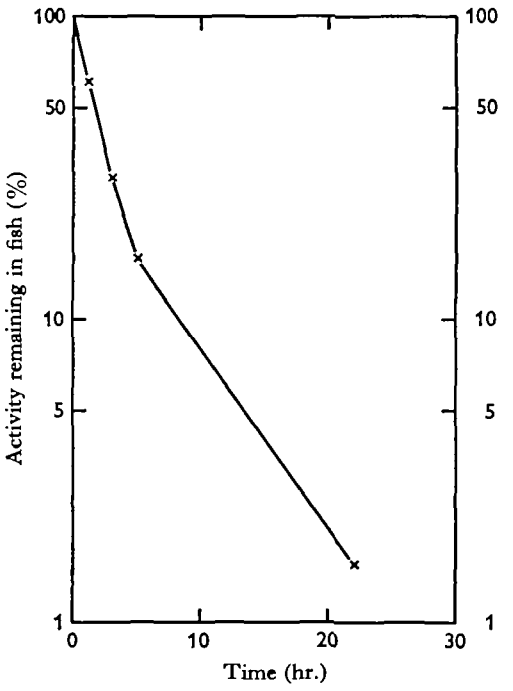

Fig. 1

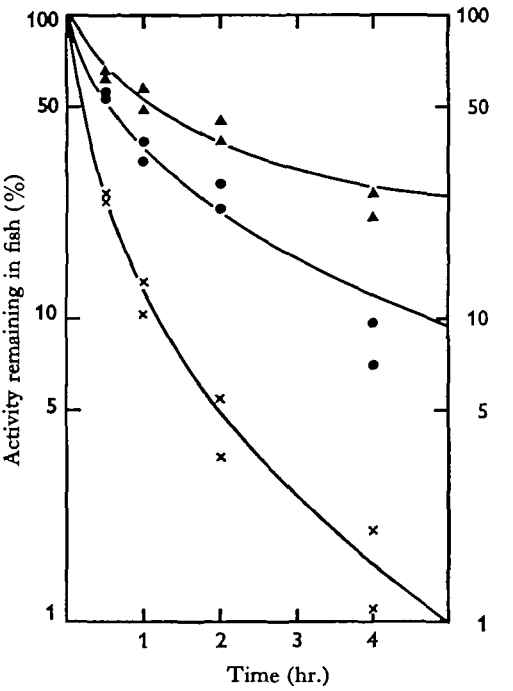

Fig. 2

Fig. 1. Rate of efflux of sodium from *Tilapia* adapted to 100 % sea water. The slope of the curve changes when the fast pool is emptied.

Fig. 2. Rate of efflux of tritiated water from the same two *Tilapia* adapted to different salinities. ▲, Animals in 200 % sea water; ●, animals in 100 % sea water; ×, animals in fresh water.

Drinking rates

The rates of drinking in the different experimental salines are given in Table 2, along with the percentage of the sodium influx which could be accounted for if all the sodium in the swallowed medium were absorbed. The rate of drinking increases rapidly with increasing external salinity to the extent that the rate in 200 % sea water is five times the rate in fresh water.

Table 2. *Rates of drinking in* Tilapia *and percentage of sodium influx from drinking* (± S.E.)

(Figures in parentheses are numbers of determinations.)

Medium	Drinking as % body wt./hr.	% Na influx from swallowed medium
Fresh water	0·26 ± 0·04 (12)	0·13
40% sea water	0·44 ± 0·095 (17)	10·0
100% sea water	1·11 ± 0·17 (15)	13·3
200% sea water	1·59 ± 0·206 (12)	24·8

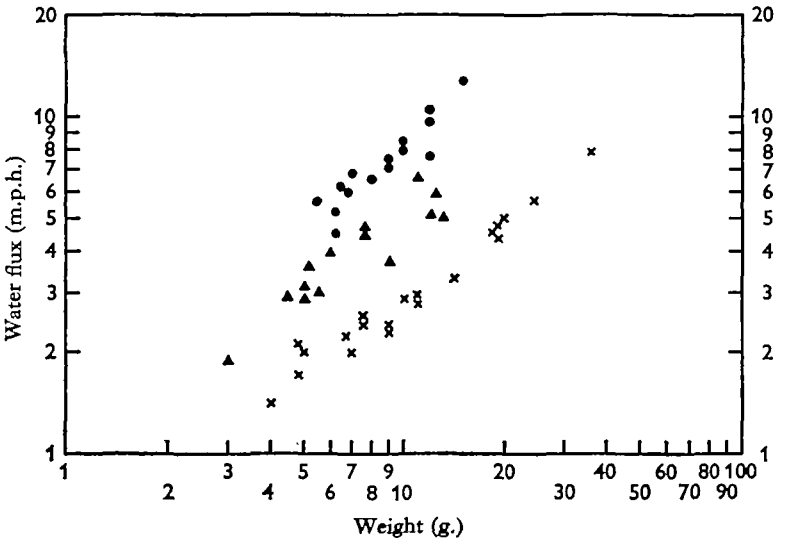

Fig. 3. Relationship between body size and rate of water exchange in *Tilapia*. ●, Fresh-water-adapted animals; ▲, 100%-sea-water-adapted animals; ×, 200%-sea-water-adapted animals.

Table 3. *Rates of influx* (K_i) *and efflux* (K_e) *of water in various salinities from* Tilapia (± S.E.)

(Figures in parentheses are numbers of determinations.)

Medium	K_i	K_e
Fresh water	1·15 ± 0·14 (15)	1·89 (3)
100% sea water	0·84 ± 0·06 (15)	1·02 (3)
200% sea water	0·42 ± 0·07 (15)	0·59 (3)

Permeability to water

The rate constants for water fluxes in the three salinities are shown in Table 3. There is a marked correlation between salinity and water fluxes. The most permeable fishes are those in fresh water which have influx rate constants of 1·15/hr. Fishes in 100% sea water have a smaller rate constant of 0·89/hr. Animals in 200% sea water reduce their water turnover even further and exchange water at one third of the rate of fishes in fresh water. Figure 2 shows the loss of tritiated water from fishes over a

period of time in three different salinities. The animals in the most saline conditions exchange their water more slowly. All efflux curves show a change in slope at about 1 hr. We believe that this results from a two-compartment system within the fish.

The relationship between body size and rate of water exchange is shown in Fig. 3. The rate of water exchange is related to weight in the following manner:

$$m = a\,(w)^{0.83},$$

where m is the rate of water exchange (ml./hr.), a is the rate of water exchange in a 1 g. fish and w is the weight of the fish in grammes. Values for a vary with salinity. In fresh water a = 0·82 ml./hr., in 100% sea water a = 0·60 ml./hr. and in 200% sea water a = 0·30 ml./hr.

In order to determine the net water fluxes which would result from the total water fluxes in these experiments the following procedure was used: in freshwater the total flux for a 1 g. fish is

$$0{\cdot}82 \times (1{\cdot}00)^{0.83} = 0{\cdot}82 \text{ ml./hr.}$$

The mole fraction of water in the external medium is 55·56/55·56, i.e. unity. If the body fluid has an osmotic concentration of 0·34 osmoles then the mole fraction of water within the fish is 55·56/55·90 or 0·9939. The difference on a mole fraction basis between water on the outside is 1·0000 − 0·9939 = 0·0061. The net flux is therefore 0·61% of the total flux (0·82 × 0·0061) or 0·50% body weight/hr. Net fluxes calculated in this manner are shown in Table 4.

Table 4. *Total and net fluxes of water in* Tilapia *in various salinities*

Medium	Total water flux (% body wt./hr.)	Net water flux (% body wt./hr)
Fresh water	82	0·50
100% sea water	60	0·64
200% sea water	30	0·80

DISCUSSION

Although the range of external salinity studied varied by a factor of 2500, the total body sodium is only 30% higher in 200% sea water than in fresh water. The sodium concentrations in the body fluids are probably kept even more constant since in the sea water there may be a considerable concentration of sodium salts in the gut. Long-term efflux experiments show that sodium in sea-water fishes is contained in at least two compartments with different rates of exchange. The identity of these compartments is uncertain, but as the rate constant of the exchange of sodium in tissues is of the order of 1/hr., the slower pool may be distributed in many parts of the body. The net rate of exchange at the body surface will be defined by the product of the rate of exchange of the fast pool and its size. In sea water and more concentrated solutions the sodium in the gut will also form a component of the slower pool. Motais & Maetz (1965) showed that when a flounder was placed in radioactive sea water the rising specific activity of sodium in the gut lagged behind the rising specific activity of sodium in the blood.

It is clear that in sea-water-adapted *Tilapia*, as in the flounder (Motais & Maetz, 1965) or *Fundulus* (Potts & Evans, 1967), the greater part of the influx of sodium takes place through the body surface rather than through the gut. As the vascularization of fish skin is low, most of the influx probably takes place through the gills. The area of the gill surface is many times greater than that of the rest of the body (Parry, 1966). The rate of drinking of *Tilapia* in sea water is equivalent to 1·54% of body weight per hour. This is similar to the rate of drinking in *Fundulus* but rather larger than the rates recorded for the eel and sculpin (*c.* 0·5%/hr.) by Smith (1930) or for the flounder (0·6% body wt./hr.) recorded by Motais & Maetz (1965). However, the experiments of both Smith and Motais were performed on fishes of weights 50–100 g., while the *Tilapia* studied here weighed less than 3 g. The relatively large drinking rate is sufficient to account for only 24% of the total sodium influx; the remaining 76% must take place through the body surface. In fresh water the rate of exchange across the gill is only 1 μM/g./hr. compared with an influx of 50 μM/g./hr. in sea water. If the permeability of the gill to sodium ions was the same in the two media and there was no potential difference across the gills, the passive efflux from the blood into the medium should be about one third of the passive influx into the blood in sea water as the sodium concentration in the blood of the freshwater fishes is approximately one third the sodium concentration of sea water. The fact that the flux in fresh water is very much less shows that either the permeability of the gills to sodium ions is smaller in sea water than in fresh water, or alternatively that a large part of the flux in sea water is due to exchange diffusion. A careful study by Motais, Garcia Romeu & Maetz (1966) has shown that both explanations apply in different euryhaline teleosts. However, the changes of efflux rate following immediate transfer between sea and fresh water (Potts, Foster, Rudy & Parry Howells, unpublished) indicate that the change in efflux rate is due primarily to a change in permeability.

Although at first sight the permeability to water seems high, when gross fluxes are converted to net fluxes the permeabilities are consistent with the data from other fishes. If the permeability to ordinary water is the same as the permeability to tritiated water, the rate of exosmosis in sea water would be *c.* 0·5%/hr. The observed drinking rate in sea water (1%/hr.) is consistent with such permeability. Not all the sea water drunk will be available to balance the water lost by exosmosis. A considerable proportion must remain in the gut with the magnesium and sulphate ions present in the swallowed sea water and some more must be used to balance the small urine loss Similarly, in 200% sea water, the calculated loss by exosmosis is 1%/hr. but the observed drinking rate is 1·54%/hr. In fresh water the calculated gain by endosmosis would be 0·5%/hr., consistent with recorded rates of urine production in fresh-water fishes (Parry, 1966). However, this agreement can only be approximate since the fishes used in these experiments are so much smaller than those in which the rates of urine production have been directly determined and the differences in temperature introduce a further uncertainty. While some allowance might be made for differences in weight and temperature, extrapolation over 20-fold or 30-fold weight changes would be very unwise.

The approximate concordance of observed permeability and drinking rates does raise one interesting point. There is some evidence (Dainty & House, 1966; Rudy, 1967) that the diffusion permeability as measured by tritiated water in many animals

is approximately one quarter of the osmotic permeability as measured by the bulk flow under the influence of an osmotic gradient. This difference has been ascribed both to the presence of bulk flow through pores in the membrane (Solomon, 1959) and to the presence of an unstirred layer or layers bounding the membrane (Dainty & House, 1966). The concordance of the drinking rates and diffusion permeability measurements suggests that any discrepancy is much smaller in *Tilapia*. This could be due to the absence of pores through the limiting membrane or to the efficiency of the gill circulation and gill ventilation.

Water, like sodium, does not exchange as a single component (Fig. 2). Analyses of some organs of the brown trout, *S. trutta* show that during efflux the specific activity of the somatic muscles, liver and gut remains significantly higher than that of the blood, brain and kidney. No doubt the greater part of the slow compartment is contained in the somatic muscles. The limiting factor of exchange in the muscle may be the small blood flow through the muscle rather than a low permeability of the muscle cells to sodium.

One very interesting feature of the permeability to water is the change in the rate constant with salinity. This is very marked and occurs in both the influx and efflux experiments (Table 4, Fig. 2). It is difficult to account for this as an experimental artifact. Both the circulation through the gill and the ventilation of the gill are not likely to vary greatly in the different media, although hydration of the gill tissue might be lower in sea water. The apparent change in permeability is consistent with observations made recently on the effects of hypophysectomy and prolactin or urine production in other teleosts. It has been shown both in *Anguilla* (Jones, Henderson & Butler, 1965) and in *Fundulus kansae* (Stanley & Flemming, 1966) that hypophysectomy reduces the rate of urine flow in fresh water. Both species can survive after hypophysectomy in fresh water although this is not true of many other fishes. In both, prolactin restores urine flow to its original level. There is evidence that a prolactin-like hormone is produced by the pituitary in fresh-water fishes but not in sea-water fishes (Grant & Pickford, 1959). A hypophysectomized fish is, in this respect at least, analogous to a fish adapted to sea water. It could be argued that the presence of prolactin is associated with a higher permeability to water.

The evidence suggests that *Tilapia* in fresh water has a relatively low permeability to salts but a high permeability to water, while in sea water the permeability to salts is higher but to water is lower. While it is easy to see the advantages of a low permeability to water in hyperosmotic solutions and of a low permeability to salts in fresh water it is difficult to see the advantages of the high permeability to salts in sea water and of the high permeability to water in fresh water. On the other hand there is no doubt that *Tilapia mossambica* is a most successful and prolific fish in a wide variety of external media.

SUMMARY

1. The total body sodium increases from 45·9 μM/g. fish in fresh water to 59·9 μM/g. fish in 200% sea water.

2. The rate of exchange of sodium increases from 2 μM/g./hr. in fresh water to 60 μM/g./hr. in 100% sea water.

3. The rate of drinking increases from 0·26%/hr. fresh water to 1·6%/hr. in 400% sea water. Even in 200% sea water drinking accounts for only a quarter of the total sodium influx.

4. The permeability to water, as measured by tritiated water, is highest in fresh water and lowest in 200% sea water. The permeabilities to water measured in this way are consistent with the drinking rates determined in sea water and 200% sea water.

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