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SHORT COMMUNICATIONS

Na^+ AND Cl^- EFFLUXES AND IONIC REGULATION IN *TILAPIA GRAHAMI*, A FISH LIVING IN CONDITIONS OF EXTREME ALKALINITY

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The osmoregularity ability of the cichlid *Tilapia grahami* enables this fish to live in conditions of extreme alkalinity (Reite, Maloiy & Aashaug, 1974; Maloiy *et al.* 1978). In this paper we describe the ionic balance of *T. grahami* from Lake Magadi (East African Rift Valley) which has a pH value of around 10, temperature in the range 30–40 °C and an osmotic pressure of about 500 mOsm, the principal inorganic ions being sodium carbonate and bicarbonate (Table 1). We also describe ionic balance of fish in water with a high NaCl concentration and in fresh water.

Fish were netted in pools at the edge of the lake and that day the air temperature was 26 °C, water temperature was 30 °C and water pH estimated using BDH pH papers was between 9–10. The fish were transported to Nairobi and kept in tanks of constantly aerated Lake Magadi water at 20 °C. After an initial high mortality the remaining fish survived for many months under laboratory conditions.

One group of fish was used to determine body water and ions (Table 1) while others were injected with known amounts of ^{22}Na and ^{36}Cl to determine effluxes of these ions (Table 2).

Whole fish, average weight 2·93 g, were homogenized in distilled water and after allowing two weeks for ion elution Na^+ and Cl^- were determined using an EEL 100 flame photometer and Cl^- using a Buchler Cotlove ampiometric titrator. Water content was determined by drying to constant weight in an oven at 100 °C.

Two further groups of fish were injected with known amounts of ^{22}Na and ^{36}Cl to determine effluxes of these ions (Table 2). Samples of the media were analysed for γ activity using a Packard 5120 spectrometer, and for β activity using a Tricarb Corumatic 200 liquid scintillation counter. One group (Table 2, experiment 1) were kept in 250 ml of 300 mM NaCl for 23 h and samples were taken at intervals. The medium was then changed to 500 mM NaCl + 10 mM $CaCl_2$ and further samples were taken after 2 h. The second group were kept in 250 ml of Magadi water for 19 h and samples were taken as before. The medium was then changed to tap water (1 mM NaCl, pH 7·5) and further samples taken at 3 h and 5 h (Table 2, experiment 2). Average weight of the fish in these experiments was 2·29 g.

Na^+ and Cl^- turnovers were calculated using equations based on those of Motais (1967) and effluxes were calculated from ion space values (Table 1). Most experiments

Table 1. *Ion and water content of* Tilapia grahami *whole body and tissues*

(Mean, standard error and number of determinations are indicated. Lake Magadi water also contains 116 mM CO_3^{2-} and 0·4 mM Ca^{2+}. Ion spaces calculated according to Holmes & Donaldson (1969). References indicated by numbers in parentheses.)

	Na^+	Cl^-	K^+	HCO_3^-	Water content (%)
Lake Magadi water (mM)	314 (1)	91 (1)	2·5 (1)	87 (1)	—
Blood plasma (mM)	217 (1)	110 (1)	8 (1)	6–9 (1)	—
	190 ± 4·2 (2)	156 ± 2·8 (3)	12·6 (2)	—	—
	175 ± 5·3 (3)	249 ± 20 (4)	—	—	—
	186 ± 12·5 (4)	—	—	—	—
Whole body m-mole/kg	141·5 ± 18·4 $n = 6$	66·6 ± 18·8 $n = 5$	133·2 ± 19 $n = 6$	—	87·8 ± 0·82 $n = 6$
Muscle m-mole/kg	117 (1)	—	465 (1)	—	—
Ion space (% wet wt.)	65·2	55·8	—	—	—

(1) Maloiy, Lykkeboe, Johansen & Bamford (1978); (2) Leatherland, Hyder & Ensor (1974); (3) Maetz & de Renzis (1978); (4) Skadhauge, Lechene & Maloiy (1980).

Table 2. *Na^+ and Cl^- turnover rates (K/h) effluxes (mM kg^{-1} h^{-1}) of* Tilapia grahami

(Mean, number of determinations and standard error of the mean are indicated.)

	Experiment 1		Experiment 2			
			Magadi water		Fresh water	
	300 mM NaCl 23 h	500 mM NaCl + 10 mM $CaCl_2$ 2 h	16·5 h	19 h	3 h	5 h
Sodium						
K/h	0·057	0·058	0·027	0·021	0·0067	0·0043
	± 0·022	± 0·02	7	5	5	5
	6	4	± 0·02	± 0·0085	± 0·0072	± 0·0034
mM kg^{-1} h^{-1}	8·04	8·12	3·80	2·96	0·94	0·61
Chloride						
K/h	0·033	0·045	0·025	0·024	0·008	0·0044
	6	4	5	5	5	5
	± 0·0041	± 0·023	± 0·017	± 0·0058	± 0·0057	± 0·0051
mM kg^{-1} h^{-1}	2·20	3·00	1·67	1·60	0·53	0·29

were carried out at 20 °C, but a few at 30 °C showed a marginal increase in ionic effluxes.

Handling and isotope injection stressed the fish, and this was reflected in unusually high and variable ionic effluxes during the first 15 h of the experiment. Subsequent values were much more uniform and only these are shown in Table 2. Media were changed with minimum disturbance to the fish.

After 24 h recovery from cannula insertion and anaesthesia (50 mg/l benzocaine, *P*-aminobenzoate), fish were placed in Magadi water until the potential (measured by the method of Potts & Eddy, 1973) was constant, usually about 5 min, and then the procedure was repeated in dilute Magadi water (Table 3). Twenty-two fish, average weight 3·35 g, were used.

Table 3. *Potential measurement for* T. grahami *in Lake Magadi water and in dilution of Magadi water*

(Polarity refers to the inside of the fish with respect to the external medium. Equilibrium potentials were calculated using the Nernst equation and plasma ion values in Table 1 and a blood pH value of 8·0 was used (Johansen, Maloiy & Lykkeboe, 1975). Mean potential (mV), standard error and number of determinations are indicated.)

Medium	Measured potential	Equilibrium potential			
		Na^+	Cl^-	HCO_3^-	OH^-
Magadi water	1·8 ± 0·25 (16)	9·3	14	−60	−116
75% Magadi water	−0·53 ± 1·17	2·1	23	−53	−68
50% Magadi water	−1·95 ± 1·34 (22)	−8·1	27	−41	−99
25% Magadi water	−2·16 ± 1·34 (22)	−25·6	50	−25	−81
Fresh water	−11·9 ± 1·13 (20)	−135	129	52	58

The ionic composition of whole body reflects the composition of Lake Magadi water, i.e. a high Na^+ content with a lower Cl^- content (Table 1). However, blood plasma Na^+ and Cl^- concentrations are of similar magnitude and the blood bicarbonate concentration is not usually high (Table 1). Ionic balance in *T. grahami* is unusual because ionic concentration differences between blood and the medium dictate that the fish will gain Na^+ while there is a tendency to lose Cl^-. The equilibrium potential for Cl^- is around 14 mV while that for Na^+ is 9·3 mV, both removed from the measured potential of 1·8 mV (Table 3), suggesting that both ions are actively regulated.

Sodium and chloride turnover rates in Lake Magadi water are about 2%/h (Table 2) and these are very low when compared to values for marine fish. Lake Magadi water corresponds to approximately 50% sea water in having an osmotic pressure of 539 mOsm (Maloiy *et al.* 1978) and Na^+ turnover rates for fish in half-strength sea water have been measured at 25%/h for sea-water adapted flounders (Motais, 1967), 14%/h for cod (Fletcher, 1978) and 10%/h for *T. mossambicus* (Potts *et al.* 1967). Even in 500 mM NaCl the Na^+ turnover rate for *T. grahami* is only about 6%/h (Table 2, experiment 1). Thus this fish is adapted to the Lake Magadi medium in having low branchial permeability to both Na^+ and Cl^-, which is far more characteristic of a freshwater fish than a marine one. The Cl^- efflux of 1·6 mM kg^{-1} h^{-1} is only about half the Na^+ value (Table 2), reflecting much higher Na^+ body content of this ion (Table 1).

The data allow a limited analysis of the ionic effluxes; if they are potential-dependent and if external and internal (blood plasma) ionic concentrations are known then it is possible to predict the potential using the Goldman equation, assuming it describes at least some of the gill's electrical properties (Potts & Eddy, 1973; Maetz & Bornancin, 1975). The potential predicted in Lake Magadi water is about 8 mV compared with the measured value of 1·8 mV (Table 3), indicating the effluxes are likely to be substantially independent of potential.

The unusually high blood pH values, 7·6–8·4 (Maloiy *et al.* 1978; Johansen, Maloiy & Lykkeboe, 1975), might arise from efficient removal of metabolic CO_2 from the gills into a virtually CO_2-free medium. It might also arise from imbibed HCO_3^- and CO_3^{2-} which may amount to about 1 mM kg^{-1} h^{-1} (Potts *et al.* 1967), but Skadhauge, Lechene & Maloiy (1980) suggest that these salts do not interfere with Na^+, Cl^- and water absorption, but are probably neutralized in the gut, although CO_2 released would need to be buffered. A further possibility is that the gills are permeable to HCO_3^- and/or OH^-, which could diffuse inwards along their concentration gradients. However, when $NaHCO_3$ was added to the medium (1/10 sea water) a more positive potential resulted, indicating greater branchial permeability to Na^+ than to HCO_3^- or OH^- (Maetz & de Renzis, 1978).

Na^+ and Cl^- turnovers in fresh water (Table 2) are similar to those reported for freshwater fish (Maetz, 1971). However, Na^+ efflux is about twice the Cl^- value, and electrical neutrality would need to be achieved by Na^+/H^+ (NH_4^+) and Cl^-/HCO_3^- exchanges proposed for freshwater fish (Maetz, 1971).

As well as showing adaptation to its unusual environment, *Tilapia grahami* showed effective ionic regulation after several hours at around pH 7 in either tap water or a solution similar to sea water (Table 2), achieving euryhalinity in a different way from other fish so far studied; for example flounders, which are characterized by high salt effluxes in sea water and low effluxes in fresh water (Motais, 1967; Potts & Eddy, 1973). *T. grahami* achieves euryhalinity by possessing gills which remain relatively impermeable to Na^+ and Cl^- in a wide variety of media containing different salt concentrations.

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