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ORIGINAL PAPER

Transepithelial potential in the Magadi tilapia, a fish living in extreme alkalinity

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Abstract We investigated the transepithelial potential (TEP) and its responses to changes in the external medium in *Alcolapia grahami*, a small cichlid fish living in Lake Magadi, Kenya. Magadi water is extremely alkaline (pH = 9.92) and otherwise unusual: titratable alkalinity (290 mequiv L^{-1}, i.e. HCO_3^- and CO_3^{2-}) rather than Cl^- (112 mmol L^{-1}) represents the major anion matching Na^+ = 356 mmol L^{-1}, with very low concentrations of Ca^{2+} and Mg^{2+} (<1 mmol L^{-1}). Immediately after fish capture, TEP was +4 mV (inside positive), but stabilized at +7 mV at 10–30 h post-capture when experiments were performed in Magadi water. Transfer to 250% Magadi water increased the TEP to +9.5 mV, and transfer to fresh water and deionized water decreased the TEP to −13 and −28 mV, respectively, effects which were not due to changes in pH or osmolality. The very negative TEP in deionized water was attenuated in a linear fashion by log elevations in $[Ca^{2+}]$. Extreme cold (1 vs. 28°C) reduced the positive TEP in Magadi water by 60%, suggesting blockade of an electrogenic component, but did not alter the negative TEP in dilute solution. When fish were transferred to 350 mmol L^{-1} solutions of $NaHCO_3$, NaCl, $NaNO_3$, or choline Cl, only the 350 mmol L^{-1} $NaHCO_3$ solution sustained the TEP unchanged at +7 mV; in all others, the TEP fell. Furthermore, after transfer to 50, 10, and 2% dilutions of 350 mmol L^{-1} $NaHCO_3$, the TEPs remained identical to those in comparable dilutions of Magadi water, whereas this did not occur with comparable dilutions of 350 mmol L^{-1} NaCl—i.e. the fish behaves electrically as if living in an $NaHCO_3$ solution equimolar to Magadi water. We conclude that the TEP is largely a Na^+ diffusion potential attenuated by some permeability to anions. In Magadi water, the net electrochemical forces driving Na^+ inwards (+9.9 mV) and Cl^- outwards (+3.4 mV) are small relative to the strong gradient

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driving HCO_3^- inwards (−82.7 mV). Estimated permeability ratios are $P_{Cl}/P_{Na} = 0.51–0.68$ and $P_{HCO_3}/P_{Na} = 0.10–0.33$. The low permeability to HCO_3^- is unusual, and reflects a unique adaptation to life in extreme alkalinity. Cl^- is distributed close to Nernst equilibrium in Magadi water, so there is no need for lower P_{Cl}. The higher P_{Na} likely facilitates Na^+ efflux through the paracellular pathway. The positive electrogenic component is probably due to active HCO_3^- excretion.

Keywords Gill permeability · P_{Cl}/P_{Na} ratio · P_{HCO_3}/P_{Na} ratio · TEP · Na diffusion potential · Electrogenic potential · Calcium-dependent potential · *Alcolapia grahami*

Introduction

Lake Magadi, in the southern Rift Valley of Kenya, may be the most extreme aquatic environment on earth supporting fish life. As first documented by Coe (1966), the Magadi tilapia (*Alcolapia grahami*), a small cichlid, thrives here in extreme alkalinity, with a water pH close to 10, and a titratable base content (HCO_3^- and CO_3^{2-}) of about 300 mequiv L^{-1}. In addition to the high alkalinity, the ionic regime is unique, presenting an osmolality of about 525 mosm kg^{-1} with high $[Na^+]$ (twofold plasma levels) and much lower $[Cl^-]$ (60% of plasma levels), yet with very low Ca^{2+} and Mg^{2+} concentrations (<1 mmol L^{-1}) similar to those of fresh water (Leatherland et al. 1974; Maloiy et al. 1978; Skadhauge et al. 1980; Eddy et al. 1981; Eddy and Maloiy 1984; Wright et al. 1990; Wood et al. 1989, 1994, 2002). Precipitation of $CaCO_3$ and $MgCO_3$ prevents the concentration of these "hardness" cations from rising in Magadi water. Temperatures as high as 42°C, daytime hyperoxia, severe night-time hypoxia, substantial ultraviolet radiation, and intense avian predation present additional challenges. Magadi tilapia exhibits a host of interesting physiological and behavioral adaptations to these extreme conditions, which have been reviewed recently by Pörtner et al. (2010). Perhaps the most prominent is 100% ureotelism; the fish have solved the problem of excreting ammonia-N against a severe pH gradient by instead producing and excreting only urea-N (Randall et al. 1989).

The focus of the present study was on one aspect of ionoregulation in this unusual environment, the transepithelial potential (TEP) across the gills. This is one of only two factors determining the true electrochemical gradients which drive passive ion fluxes across the branchial epithelium and against which active transport occurs, the other being the concentration gradient of the relative ion. A robust early literature described the theoretical origin of the TEP in most teleosts residing in fresh water, sea water, or intermediate salinities. A "Na^+ diffusion potential" dominates in most circumstances—i.e. a higher permeability of the gill epithelium to Na^+ than to Cl^- ($P_{Na}/P_{Cl} > 1$) for passive fluxes creating a negative potential in low salinities and fresh water, and a positive potential in higher salinities (Kerstetter et al. 1970; Potts and Eddy 1973; House and Maetz 1974; Eddy 1975; McWilliams and Potts 1978; Potts 1984; Potts and Hedges 1991; Potts et al. 1991). In sea water, there is also an electrogenic component due to the active extrusion of Cl^- which makes the TEP even more positive. In most cases, the TEP is sensitive to the water Ca^{2+} concentration, such that as Ca^{2+} increases, P_{Na} is reduced more than P_{Cl}.

But this theoretical background does not provide a framework for predicting the TEP in a fish adapted to the unique environment of Lake Magadi, one which it has likely inhabited for only a short period of evolutionary time (<10,000 years; see Pörtner et al. 2010). What will the TEP be in an environment where HCO_3^- rather than Cl^- is the dominant anion in the water, and its entry would have dire consequences for acid–base regulation (Johansen et al. 1975)? Will P_{HCO_3} be lower or higher than P_{Na}? Will P_{HCO_3} be lower or higher than P_{Cl}? Will there still be a positive electrogenic component to TEP when the need is to actively excrete HCO_3^- rather than Cl^-? In the absence of knowledge about P_{Na}/P_{HCO_3}, how will TEP respond to dilution? Will the TEP respond to variations in Ca^{2+} concentration which probably never changes in the fish's natural environment? In the present investigation, we have addressed these and other questions about the nature of the TEP in the Magadi tilapia. The results reveal new insights into the unusual ionoregulatory mechanisms of this unique organism, and yet more information on fundamental differences from other teleost fish in the physiology of this species. These mechanistic differences have arisen in a relatively short period of evolutionary time (Pörtner et al. 2010), and have facilitated adaptation to an environment which is so harsh as to be lethal to other fish, even closely related cichlids (Randall et al. 1989; Wood et al. 1989; Wright et al. 1990).

Methods

All experiments complied with the laws of Kenya, and were performed under a research permit issued by the National Council for Science and Technology of the Ministry of Higher Education, Science, and Technology of the Republic of Kenya.

Experimental animals

Experiments were performed on approximately 165 Magadi tilapia (*Alcolapia grahami*, formerly *Oreochromis alcalicus grahami*, formerly *Sarotherodon alcalicus*

grahami, formerly *Tilapia grahami*) with a mean weight of 6.34 g (range 3.45–11.30 g). Fish were collected in August 2010 from Fish Springs Lagoon on the edge of Lake Magadi (see Coe 1966; Bergman et al. 1996 for maps) with a seine net. Fish were collected between 6:30 and 8:30 am, and quickly transported (15 min) to an outdoor laboratory set up on the balcony of a house kindly provided by the Magadi Soda Company in the nearby Magadi township. The fish were held for a maximum of 24 h prior to cannulation, in groups of 10–20 in aerated 20-L plastic buckets filled with water from Fish Springs Lagoon (hereafter termed Magadi water). Ambient temperature during holding was 26–32°C, similar to that at the Lagoon.

Cannulation

Intraperitoneal catheters for TEP measurement were implanted as first described by Potts and Eddy (1973) and Pic (1978). Wood and Grosell (2008) validated this approach by showing it yielded identical TEP values to blood catheters. Methods were the same as those of Wood and Grosell (2008), except that cold anaesthesia was used as recommended by Pic (1978). Fish were immersed in ice-cold (1°C) Magadi water for 5 min, which rendered them unconscious. A saline-filled PE50 catheter (Clay-Adams; Becton–Dickinson, Sparks, MD, USA) was inserted 1–2 cm through the peritoneal wall into the coelom via a puncture site made with a 19 gauge needle, just lateral and anterior to the rectum. A 1-cm PE160 sleeve, heat-flared at both ends, was glued to the PE50 with cyanoacrylate resin and anchored to the body wall with several silk sutures, to prevent the catheter from changing depth. The fish were revived by returning them to 28°C Magadi water and then transferred to individual 0.75-L chambers immersed in a water-bath at 28°C, where they were held prior to TEP measurements. These highly active fish recovered normal activity quickly, but tended to deteriorate by 8 h after cannulation, so all measurements were started by 3 h and finished by 6 h, after which the fish were euthanized. Within individual fish, TEPs were stable in this time window and among fish, the numerous control measurements were very uniform, so we are confident that there was no physiological impairment over this period.

TEP measurements

To minimize stress, the fish was gently transferred without air-exposure to a 0.5-L plastic bowl in which TEP measurements were made. In each series, TEP measurements were first made in freshly collected Magadi water at 28°C, which was then replaced by four exchanges of the test solution. TEP measurements were taken 5 min after the solution change, but in practice were stable within 2 min.

TEP was measured using 3 M KCl-agar bridges connected by Ag/AgCl electrodes to a high impedance electrometer (Radiometer pHM meter, Copenhagen, Denmark). The measurement bridge was connected to the coelomic catheter (out of the water), and the reference bridge was placed in the surrounding water. The electrodes were checked for symmetry. TEP values (mV) were expressed relative to the water side as 0 mV after correction for junction potential.

Experimental series

All experimental series comprised at least six different fish, and each fish was used in only one series. The standard protocol was to first record the TEP in Magadi water, and then to record the TEP in the experimental solution. In those experiments where there was more than one experimental solution, the order of presentation was randomized for each fish.

Each of the following test solutions comprised a series:

(i) De-ionized water.
(ii) Fresh water at its normal pH of 8.31 (Table 1). This was Magadi township tapwater which had been aerated for 24 h to remove any chlorine.
(iii) Fresh water which had been similarly treated but in which the pH was raised to 9.92 (the same pH as Magadi water) by slight addition of NaOH (Table 1).
(iv) Magadi water in which the pH had been lowered to 8.31 by the addition of HCl together with vigorous aeration (Table 1). This took several days before a stable pH was achieved.
(v) 250% Magadi water. This was made by evaporating Magadi water in the sun in shallow dishes for several days, until the Cl^- concentration had increased by approximately 2.5-fold (Table 1).
(vi) 350 mmol L^{-1} $NaHCO_3$ in de-ionized water.
(vii) 350 mmol L^{-1} NaCl in de-ionized water.
(viii) 350 mmol L^{-1} $NaNO_3$ in de-ionized water.
(ix) 350 mmol L^{-1} choline Cl in de-ionized water.
(x) 600 mmol L^{-1} glucose in de-ionized water. This was chosen to approximate the osmolality of Magadi water, but in the absence of ions.
(xi) 50% Magadi water, 175 mmol L^{-1} $NaHCO_3$ (i.e. 50% of test solution vi), and 175 mmol L^{-1} NaCl (i.e. 50% of test solution vii), each in de-ionized water.
(xii) 10% Magadi water, 35 mmol L^{-1} $NaHCO_3$ (i.e. 10% of test solution vi), and 35 mmol L^{-1} NaCl (i.e. 10% of test solution vii), each in de-ionized water.
(xiii) 2% Magadi water, 7 mmol $L^{-1}$$NaHCO_3$ (i.e. 2% of test solution vi), and 7 mmol L^{-1} NaCl (i.e. 2% of test solution vii), each in de-ionized water.

Table 1 Ionic composition of the blood plasma of Magadi tilapia (mean ± 1 SEM) and of the various experimental waters used in experimental trials

	pH	Na^+ (mmol L^{-1})	Cl^- (mmol L^{-1})	Ca^{2+} (mmol L^{-1})	Mg^{2+} (mmol L^{-1})	Titratable base (mequiv L^{-1})
Plasma ($N = 25$)	8.08[a]	175.0 ± 1.9	168.0 ± 3.5	3.06 ± 0.19	0.98 ± 0.05	15.3[a]
Magadi water	9.92	356	112	0.65	0.04	290
Magadi water, pH 8.3	8.31	352	368	0.66	0.04	1.8
250% Magadi water	9.75	772	286	0.60	0.03	490
Fresh water, pH 8.3	8.31	1.22	0.17	0.15	0.14	1.1
Fresh water, pH 9.9	9.92	1.96	0.60	0.15	0.14	1.8

[a] Data for plasma acid–base status from Wood et al. (1994). The value for titratable base is the measured plasma HCO_3^- concentration

(xiv) 0.1 mmol L^{-1}, 1.0 mmol L^{-1}, and 10 mmol L^{-1} $Ca(NO_3)_2$ in de-ionized water.
(xv) 20 mmol L^{-1} $NaNO_3$ in de-ionized water (i.e. same concentration of NO_3 as in 10 mmol L^{-1} $Ca(NO_3)_2$.
(xvi) 100% Magadi water at 28°C and 100% Magadi water at 1°C.
(xvii) 2% Magadi water at 28°C and 2% Magadi water at 1°C.

Plasma samples

Plasma samples were taken by caudal puncture in a separate group of 25 non-cannulated fish which had been kept in Magadi water in individual 0.75-L chambers at 28°C for 12–24 h post-capture. The fish were anaesthetized in ice-cold (1°C) Magadi water, and a blood sample (30–100 µl) was drawn using a specially modified 100-µl Hamilton gas-tight syringe in which the needle had been shortened and beveled. The syringe was pre-rinsed with 1,000 i.u. ml^{-1} lithium heparin (Sigma-Aldrich, St. Louis, MO, USA). Samples were spun at 5,000*g* for 2 min, then the plasma was decanted and stored at −20°C for later analysis of ion concentrations. These data were used for calculating equilibrium potentials and electrochemical gradients across the gills.

Analyses

Water pH was measured using a Radiometer GK401C combination electrode (Copenhagen, Denmark) calibrated with Radiometer precision buffers. Plasma and water Na^+, Ca^{2+}, and Mg^{2+} concentrations were determined by flame atomic absorption spectroscopy (Varian SpectrAA-220FS, Mulgrave, Australia), and Cl^- concentrations by coulometric titration (Radiometer CMT 10, Copenhagen, Denmark). The titratable base content of selected water samples was carried out by the double titration method recommended by Hills (1973) and described in detail by Bergman et al. (2003).

Modeling

Modeling of the data was performed using the Nernst equation (Eq. 1) and the Goldman–Hodgkin–Katz equation (Eq. 2) to estimate the equilibrium potentials (E) across the gills (Goldman 1943; Sten-Knudsen 2002):

$$E = -\frac{RT}{zF}\ln\frac{[C]_{in}}{[C]_{out}} \quad (1)$$

$$E = -\frac{RT}{F}\ln\frac{P_C[C]_{in}+P_A[A]_{out}+\cdots}{P_C[C]_{out}+P_A[A]_{in}+\cdots} \quad (2)$$

where z is the valence, R, T, and F are the standard thermodynamic constants, $[C]_{in}$ is the activity of a particular cation (i.e. Na^+) in the blood plasma, $[C]_{out}$ is the activity of the same cation in the environment, P_C is the permeability of the gills to that cation, $[A]_{in}$ is the activity of a particular anion in the blood plasma, $[A]_{out}$ is the activity of the same anion in the environment, P_A is the permeability of the gills to that anion, etc. In Eq. 1, $[C]_{in}$ and $[C]_{out}$ would be reversed for anions (i.e. Cl^-, HCO_3^-). Activity coefficients were taken from Robinson and Stokes (1959).

Statistics

Data have been expressed as mean ± 1 SEM (N). The significance ($P < 0.05$) of most differences was assessed using Student's paired two-tailed t test, as each fish was first tested in Magadi water, and therefore, used as its own control. When there was more than one such comparison, the Bonferroni correction was applied. For comparisons amongst experimental data from different series, one-way ANOVA followed by Fisher's LSD test was applied.

Results

Water and plasma chemistry

The chemistry of Magadi water collected from Fish Springs Lagoon was similar to previous reports (Wood

et al. 1989, 1994; Bergman et al. 2003; Wilson et al. 2004), with a pH of 9.92, and a Na^+ concentration (356 mmol L^{-1}) equal to that of about 70% seawater, and approximately threefold greater than the Cl^- concentration (Table 1). Thus, the major anion was represented by titratable base, comprising HCO_3^- and CO_3^{2-}. Ca^{2+} and Mg^{2+} concentrations were very low. The tap water of Magadi township used as fresh water in these experiments had Na^+, Cl^-, and titrable base concentrations which were less than 0.4% of those in Magadi water. Ca^{2+} levels were lower but Mg^{2+} levels were higher than in Magadi water.

The 250% Magadi water was made by evaporating the water in the sun, while monitoring Cl^- concentration. Note that while Cl^- concentration was elevated by 2.5-fold, increases were rather less for Na^+ (2.2-fold) and titratable base (1.7-fold) whereas the low Ca^{2+} and Mg^{2+} concentrations did not change (Table 1). This lack of proportionality is likely explained by the visible precipitation which occurred. Magadi water appears to be saturated with respect to $CaCO_3$ and $MgCO_3$; the additional $CaCO_3$ and $MgCO_3$ which formed would precipitate, leaving the small concentrations of dissolved calcium and magnesium unchanged. However, the major precipitate was likely Na_2CO_3 as seen by the large deficits of sodium and titratable base (Table 1).

Plasma Na^+ concentrations in Magadi tilapia (175 mmol L^{-1}) were only about half of those in Magadi water, whereas Cl^- concentrations were comparable to plasma Na^+, and much higher than those in Magadi water (Table 1). Plasma Ca^{2+} and Mg^{2+} concentrations were also higher than in Magadi water. Plasma acid–base status was not measured in the present study, but representative values from a previous investigation (Wood et al. 1994) are included in Table 1. These indicate a plasma pH and base content (HCO_3^-) that are both much lower than the values in Magadi lake water.

TEP under control conditions and effects of transfer to different waters

TEP was always positive when the fish were held in Magadi water. In tilapia cannulated immediately after capture, with measurements made within 4 h, values were quite low, about +4 mV (Fig. 1). Thereafter, values approximately tripled to +12 mV at 4–8 h, before stabilizing at about +7 mV at 10–14 h, 18–24 h, and 24–30 h post-capture. Therefore, all experiments were performed in the 10–30 h post-capture time window.

Transfer of the fish to 250% Magadi water caused a small but significant rise in the positive TEP (Table 2). Transfer to de-ionized water caused reversal of the TEP to a highly negative value, about –28 mV, whereas transfer to fresh water yielded a negative TEP of about half this value (Table 2). The pH of this fresh water was normally 8.3

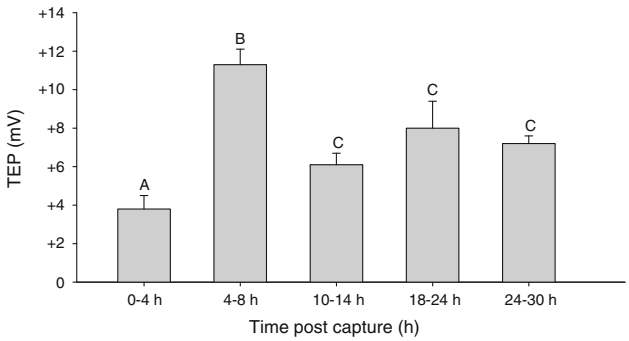

Fig. 1 The influence of time post-capture on the TEP of Magadi tilapia in Magadi water (mean ± 1 SEM; $N = 5$ at 0–4 h, $N = 5$ at 4–8 h, $N = 14$ at 10–14 h, $N = 8$ at 18–24 h, $N = 7$ at 24–30 h). Mean sharing the same letter are not significantly different from one another ($P > 0.05$)

Table 2 The effect of transfer from Magadi water to various other waters on the TEP (mV) in the Magadi tilapia (mean ± 1 SEM, $N = 6$)

	Magadi water control	Experimental water
De-ionized water	+8.4 ± 0.5	−28.3 ± 2.1*^{A}
Fresh water, pH 8.3	+ 7.8 ± 0.6	−13.0 ± 2.4*^{B}
Fresh water, pH 9.9	+ 7.3 ± 1.2	−15.3 ± 1.7*^{B}
Magadi water, pH 8.3	+ 6.5 ± 0.6	+4.6 ± 0.7*^{C}
250% Magadi water	+7.5 ± 0.9	+9.5 ± 0.4*^{D}

Each fish was tested first in Magadi water (i.e. control), then in the experimental water

Within the experimental water treatments, means sharing the same letter are not significantly different from one another ($P > 0.05$)

* Significant difference ($P < 0.05$) from the Magadi water control value

(Table 1). When tilapia were transferred to fresh water in which the pH had been raised to 9.9 to equal that of Magadi water (by addition of a small amount of NaOH), the TEP was the same as that in fish transferred to normal fresh water, suggesting that the change in pH was not the cause of the response. Furthermore, when tilapia were transferred to Magadi water at pH 8.3, the TEP remained positive, though it did exhibit a small significant fall (Table 2). Note that the addition of HCl to Magadi water in order to lower its pH to 8.3 resulted in the virtual elimination of base from the water, and its replacement with Cl^- (Table 1). Therefore, this treatment was similar to the exposure to 350 mmol L^{-1} NaCl as outlined subsequently.

Effect of transfer to different experimental solutions on the TEP

Magadi water had a Na^+ concentration of about 350 mmol L^{-1} (Table 1). When fish were transferred to a 350 mmol L^{-1} $NaHCO_3$ solution, the positive TEP was maintained

unchanged at about +7 mV (Table 3). However, transfer to 350 mmol L^{-1} NaCl resulted in a significant fall in TEP, though the value remained positive, similar to the response observed when fish were exposed to Magadi water titrated to pH 8.3 with HCl (c.f. Table 2). Transfer to 350 mmol L^{-1} $NaNO_3$ drove the TEP to a negative value, though this was not significantly different than that in 350 mmol L^{-1} NaCl. Transfer to 350 mmol L^{-1} choline Cl^- (chosen as a Na^+ replacement) also resulted in a slightly negative value. Exposure to 600 mmol L^{-1} glucose (chosen to duplicate the osmolality of Magadi water but without any ions present) resulted in a highly negative potential (Table 3), comparable to that seen in de-ionized water (c.f. Table 2). These results are consistent with a conclusion that the TEP is independent of the osmotic pressure of the medium, and that a component of the TEP is a Na^+ diffusion potential—i.e. gill permeability to Na^+ is greater than that to anions, with HCO_3^- permeability being significantly lower than that to Cl^- or NO_3^- (see "Discussion").

To further explore this interpretation, fish were exposed to volumetric dilutions (50, 10, and 2%, with de-ionized water) of Magadi water, of 350 mmol L^{-1} $NaHCO_3$, and of 350 mmol L^{-1} NaCl (Fig. 2). In all cases, TEP fell progressively as the Na^+ concentration was lowered, changing to slightly negative values at 50% (−1 to −6 mV), and highly negative values at 2% (−16 to −21 mV), consistent with a greater Na^+ than anion permeability (see "Discussion"). Notably, at every dilution, the TEP in $NaHCO_3$ solution was the same as in diluted Magadi water, whereas this was not the case with NaCl solution. Thus in both 350 mmol L^{-1} (100%) and 175 mmol L^{-1} NaCl (50%), TEP was significantly lower than in 100 and 50% Magadi water, respectively, whereas in 7 mmol L^{-1} NaCl, TEP was higher (i.e. less negative) than in 2% Magadi water. These data suggest the occurrence of a Na^+ diffusion potential over the entire concentration range, with again, HCO_3^- permeability being lower than Cl^- permeability.

Table 3 The effect of transfer from Magadi water to various experimental solutions on the TEP (mV) in the Magadi tilapia (mean ± 1 SEM, $N = 6$–7)

	Magadi water control	Experimental solution
350 mmol L^{-1} $NaHCO_3$	+6.4 ± 1.0	+7.2 ± 1.2^{A}
350 mmol L^{-1} NaCl	+7.8 ± 1.6	+1.9 ± 0.5*^{B}
350 mmol L^{-1} $NaNO_3$	+6.7 ± 0.7	−3.5 ± 2.3*^{B}
350 mmol L^{-1} choline Cl^-	+6.1 ± 1.5	−1.9 ± 1.1*^{B}
600 mmol L^{-1} glucose	+7.9 ± 0.9	−22.1 ± 2.1*^{C}

Each fish was tested first in Magadi water (i.e. control), then in the experimental solution

Within the experimental solution treatments, means sharing the same letter are not significantly different from one another ($P > 0.05$)

* Significant difference ($P < 0.05$) from the Magadi water control value

Effect of Ca^{2+} on the TEP

Ca^{2+} concentration could not be manipulated in Magadi water because of precipitation, but was tested on the TEP response to acute transfer to de-ionized water (Fig. 3). At 0.1 mmol L^{-1} Ca^{2+} (as $Ca(NO_3)_2$) the response was essentially the same as in de-ionized water, a highly negative TEP of about –26 mV. However, when Ca^{2+} was raised to 1 mmol L^{-1}, the TEP was raised to approximately −14 mV, and at 10 mmol L^{-1} Ca^{2+}, it reached approximately 0 mV, in accordance with the properties of a Na^+ diffusion potential (see "Introduction"). Nevertheless, TEP in 10 mmol L^{-1} $Ca(NO_3)_2$ remained significantly lower than the positive TEP in Magadi water (Fig. 3). To ensure that these effects were due to Ca^{2+}, and not to the NO_3^- part of the salt, the same concentration of NO_3^- (20 mmol L^{-1}) was tested as $NaNO_3$ (20 mmol L^{-1}). The TEP remained highly negative, at about the level (−11 mV) predictable from the variation in Na^+ alone (c.f. Fig. 2).

Effect of cold on the TEP

Extreme cold (1°C) had no effect on the highly negative TEP seen after transfer to 2% Magadi water, but in 100% Magadi water, the positive TEP was reduced by about 60% (Fig. 4). This suggests that at least half of the positive TEP in full strength Magadi water may be of active, electrogenic origin, whereas there is no electrogenic component in dilute solution.

Discussion

The influence of stress on the TEP and comparison with previous measurements

The initially low TEP in the first 4 h after capture followed by a peak at 4–8 h (Fig. 1) is interpreted as an inhibitory response to stress, followed by a rebound overshoot. This lowering of the TEP in stressed animals was first reported by Potts and Eddy (1973) and Pic (1978) in seawater flounder and killifish, respectively. Potts and Eddy (1973) suggested that stress might tend to abolish the selectively low permeability of the gills to anions, which would tend to reduce the absolute values of Na^+ diffusion potentials in concentrated solutions. An additional factor could be an alpha-adrenergic inhibition of active electrogenic anion extrusion by stress-released catecholamines, as well documented in the opercular epithelium of seawater killifish (Mendelsohn et al. 1981; May and Degnan 1985; Marshall

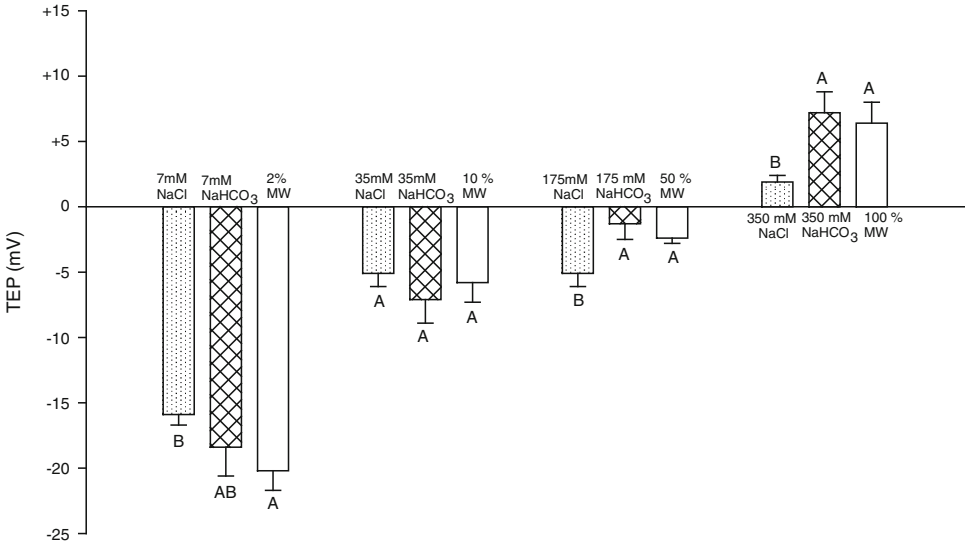

Fig. 2 The influence of comparable volumetric dilutions (100, 50, 10, and 2%, with de-ionized water) of Magadi water (MW), and of 350 mmol L^{-1} solutions of $NaHCO_3$ and NaCl on TEP of Magadi tilapia (mean ± 1 SEM; $N = 6$ throughout). Within a dilution, means sharing the same letter are not significantly different from one another ($P > 0.05$). Note that there were no significant differences between TEP in MW and in the $NaHCO_3$ solution at the same dilution

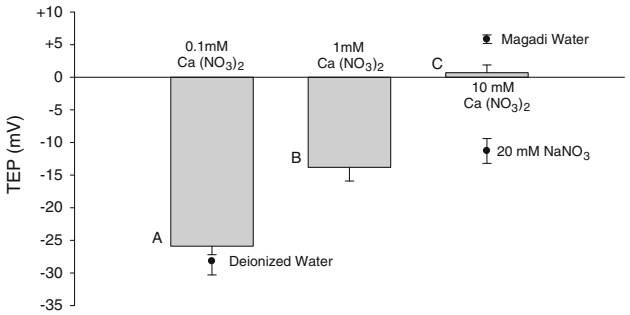

Fig. 3 The influence of progressive elevation of environmental Ca^{2+} concentration (as $Ca(NO_3)_2$) in a logarithmic series on TEP in Magadi tilapia in deionized water. The TEPs in Magadi water, de-ionized water alone, and 20 mmol L^{-1} $NaNO_3$ are also shown (mean ± 1 SEM; $N = 6$ throughout). Within the $Ca(NO_3)_2$ series, the three means were significantly different from one another ($P < 0.05$), as indicated by the different letters. The TEP in 0.1 mmol L^{-1} $Ca(NO_3)_2$ was not significantly different ($P > 0.05$) from that in de-ionized water. The TEP in 10 mmol L^{-1} $Ca(NO_3)_2$ was significantly below that in Magadi water, and significantly above that in 20 mmol L^{-1} $NaNO_3$ ($P < 0.05$)

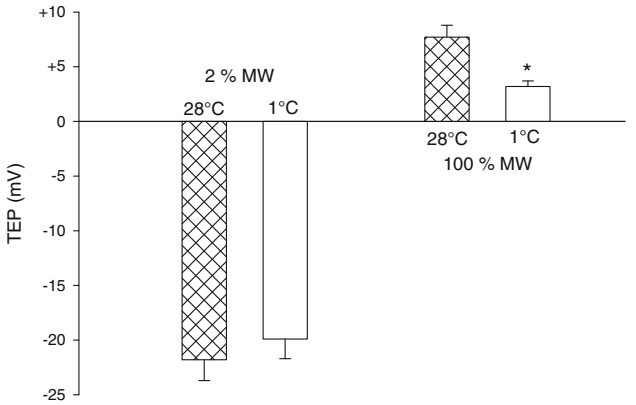

Fig. 4 The influence of acute transfer from 28 to 1°C on TEP in Magadi tilapia in 100% Magadi water (MW) or after acute simultaneous transfer to 2% MW (mean ± 1 SEM; $N = 6$ throughout). * indicates significant difference ($P < 0.05$) from TEP in the same water at 28°C

et al. 1998). Our experimental measurements were, therefore, made during the 10–30 h post-capture period where TEP stabilized at about +7 mV (Fig. 1). Significant post-capture elevations in plasma cortisol, glucose, and lactate are declining by this time (C.M. Wood, unpublished results).

We are aware of two previous measurements of TEP in the Magadi tilapia. Maloiy et al. (1978) reported a TEP averaging +1.4 mV in a few fish in Magadi water at 35°C, while Eddy et al. (1981) recorded a mean TEP of +1.8 mV in 16 animals in Magadi water at 20°C. Both reports are much lower than the +7 mV consistently recorded in Magadi water at 28°C in the present study. The difference may be due to stress, and in the case of Eddy et al. (1981), the much lower temperature that may have inhibited the active electrogenic component (see below). Notably, Eddy et al. (1981) reported a mean TEP of −11.9 mV when their fish were transferred to fresh water, very similar to the −13.0 mV measured in the present study (Table 2).

Responses of TEP to different waters and salt solutions

Transfer of Magadi tilapia to fresh water or de-ionized water induced an immediate changeover to a highly negative potential (Table 2). This is a very typical response for many marine teleosts when transferred to fresh water and usually reflects a higher permeability to Na^+ than to anions (usually Cl^-)—in other words, a Na^+ diffusion potential (see "Introduction"). In most euryhaline species (rainbow trout, Kerstetter et al. 1970; flounder, Potts and Eddy 1973;

brown trout, McWilliams and Potts 1978), the negative TEP persists after acclimation to fresh water, whereas in at least one, the common killifish, it does not (Wood and Grosell 2008). Magadi tilapia cannot survive the acute transfer to fresh water, as carried out in the present study, for more than a few hours, but they can be very gradually acclimated to 1% Magadi water (Maina 1990; Wood et al. 2002a, b). In future, it will be of interest to measure TEP in fish that have been acclimated for a long period to dilute water, a condition that causes marked changes in the morphology of the gills (Maina 1990; Wood et al. 2002a).

Transfer to 250% Magadi water caused a small but significant elevation in TEP (Table 2). This would again be predicted from the Na^+ diffusion potential hypothesis, though it is possible that an increased electrogenic component could also have contributed, as discussed subsequently. The tests with 600 mM glucose and altered pH suggest that osmolality and pH are relatively unimportant factors influencing the TEP, in accord with the observations of Potts and Eddy (1973) on euryhaline flounder, and Eddy (1975) on stenohaline goldfish, though more severe acid pH's were not tested (c.f. McWilliams and Potts 1978). Glucose was employed as a nonionic osmolyte because we were unable to obtain the more commonly used mannitol at our isolated field location. Nevertheless, as the TEP with a glucose solution isosmotic to Magadi water was a highly negative value (Table 3) comparable to that seen in de-ionized water (Table 2), the conclusion that the TEP is independent of the osmotic pressure of the medium seems secure.

Of the various salt solutions tested, 350 mmol L^{-1} $NaHCO_3$ was the only solution that fully supported the same positive TEP (i.e. + 7 mV) as measured in 100% Magadi water (Fig. 2; Table 3). Furthermore, volumetric dilutions of 350 mmol L^{-1} $NaHCO_3$ down to 2% produced identical TEPs to those achieved by the same volumetric dilutions of 100% Magadi water, whereas volumetric dilutions of 350 mmol L^{-1} NaCl did not (Fig. 2). This suggests that in Magadi water, the fish behaves electrically as though it is living in a $NaHCO_3$ solution at the same concentration, and that HCO_3^- is less permeable at the gills than either Cl^- or NO_3^-, as discussed subsequently.

The response to 350 mmol L^{-1} choline Cl^- was somewhat surprising. The bulky choline cation is usually considered to have low permeability. Therefore, we anticipated a highly negative TEP comparable to that in dilute Na^+ solutions (Fig. 2), as was reported for the flounder when exposed to an artificial sea water in which choline completely replaced Na^+ (Potts and Eddy 1973). In contrast, in the Magadi tilapia exposed to 350 mmol L^{-1} choline Cl^-, the TEP dropped only to a value slightly below that seen in 350 mmol L^{-1} NaCl (Table 3). Na^+ is the only cation that Magadi tilapia normally "see" in high concentrations in their environment (Table 1); note that K^+ is only 2.2–2.8 mmol L^{-1} (Leatherland et al. 1974; Maloiy et al. 1978; Skadhauge et al. 1980; Wood et al. 1989). Overall, this suggests that higher permeability to monovalent cations may be a general property for all cations, not just for Na^+, in this species. Similar high non-specific cation permeability has been reported in the seawater-acclimated killifish (Wood and Grosell 2008).

Modeling the TEP and estimates of relative Na^+, Cl^-, and HCO_3^- permeabilities

The calculated Nernst potential for Na^+ (E_{Na}; equation 1) ranged from +17.7 mV in 100%, +1.5 mV in 50%, −36.2 mV in 10%, to −76.4 mV in 2% Magadi water. Thus, the response qualitatively tracked the observed TEP (c.f. Fig. 2) but exhibited much greater extremes. The Nernst potential for Cl^- (E_{Cl}) in 100% Magadi water (+11.2 mV) was fairly close to E_{Na} in 100% Magadi water, but became progressively more positive with dilution, in contrast to the observed trend in TEP. The Nernst potentials for HCO_3^- (E_{HCO_3}) exhibited exactly opposite trends to E_{Na}, being negative at high concentrations (−74.9 mV), and positive (+18.0 mV) at the lowest concentration of Magadi water. Overall, this suggests that a Na^+ diffusion potential occurs in the gills of the Magadi tilapia, but is attenuated by some permeability to anions.

In order to assess the relative permeabilities of the gills to Cl^- versus Na^+, the Goldman–Hodgkin–Katz equation was applied to the TEP data for the 350 mmol L^{-1} NaCl dilution experiments (Fig. 2) and the plasma ion data of Table 1, and solved for P_{Cl}/P_{Na}, assuming that permeability to only these ions contributed to the TEP. The calculated P_{Cl}/P_{Na} ratio varied from 0.51 in dilute solutions to 0.68 in concentrated solutions. The same calculation was then applied to the data for the 350 mmol L^{-1} $NaHCO_3$ dilution experiments (Fig. 2), making a comparable assumption, and solved for P_{HCO_3}/P_{Na}, which ranged from 0.10 in dilute solutions to 0.33 in concentrated solutions. Thus P_{Cl} is clearly less than P_{Na} by 30–50%, but two to fivefold greater than P_{HCO_3}. Various values within these ranges for all three permeabilities (P_{Na}, P_{Cl}, and P_{HCO_3}) were then inserted into the Goldman–Hodgkin–Katz equation to see how well they predicted the actual TEP in dilutions of Magadi water in which all three ions are present. Examples for two extremes ($P_{Na} = 1.00$, $P_{Cl} = 0.51$, $P_{HCO_3} = 0.10$ vs. $P_{Na} = 1.00$, $P_{Cl} = 0.68$, $P_{HCO_3} = 0.33$) are shown and do a reasonable job of describing the observed data (Fig. 5a). The higher values of P_{Cl} and P_{HCO_3} appear to provide a better estimate in concentrated solutions, while the lower values give a better estimate in dilute solutions.

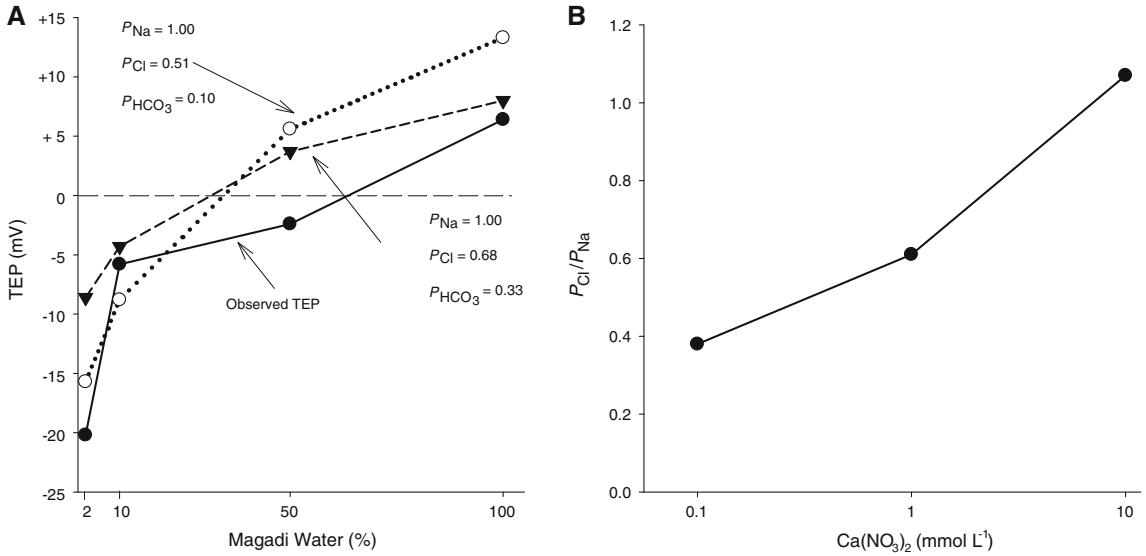

Fig. 5 Modeling, using the Goldman–Hodgkin–Katz equation (Eq. 2), of the TEP responses of the Magadi tilapia to **a** dilution of Magadi water and **b** logarithmic elevations of environmental [Ca^{2+}] (as $Ca(NO_3)_2$) in deionized water. In **a**, the observed TEP response to dilution (from Fig. 2) is compared to the predicted TEP response by the Goldman–Hodgkin–Katz equation using two sets of permeability ratios for sodium (P_{Na}), bicarbonate (P_{HCO_3}), and chloride (P_{Cl}). In **b**, the P_{Cl}/P_{Na} ratios required to explain the observed TEP response to elevations of environmental [Ca^{2+}] (from Fig. 3) have been calculated using the Goldman–Hodgkin–Katz equation. See text for details

The influence of Ca^{2+} on the TEP

Na^+ diffusion potentials are usually sensitive to Ca^{2+} concentration due to its relatively greater effect in reducing the permeability of the gills to cations versus anions (see "Introduction"), and this was clearly true in the Magadi tilapia, at least in dilute solutions (Fig. 3). This is remarkable considering that the dissolved Ca^{2+} concentration is probably invariant in the normal environment of this species. Again using the Goldman–Hodgkin–Katz equation, it can be calculated that P_{Cl}/P_{Na} increased from about 0.4 to 1.0 as Ca^{2+} concentration was raised from 0.1 to 10 mmol L^{-1} and TEP rose from −26 to 0 mV (Fig. 5b). Indeed these are larger changes in P_{Cl}/P_{Na} and TEP than seen in seawater killifish (Wood and Grosell 2008) or freshwater goldfish (Eddy 1975) subjected to similar protocols.

Electrogenic component

An acute drop in temperature from 28 to 1°C would undoubtedly block or reduce any active transport component, so the 60% fall in the positive TEP seen with this treatment in 100% Magadi water suggests that there is normally an electrogenic component. We cannot completely eliminate the alternate explanation that cold reduced the relative permeabilities of the gills to cations more than to anions, but this seems unlikely given the fact that acute 1°C exposure had no effect on the highly negative potential in 2% Magadi water (Fig. 4). As noted earlier, it is possible that the lower TEP in the first 4 h after capture (Fig. 1) was due to catecholamine-induced inhibition of the electrogenic component. Below, we argue that this electrogenic component is due to active HCO_3^- excretion at the gills.

Implications for understanding the mechanisms of gill ion transport in the Magadi tilapia

Clearly, P_{HCO_3} is much lower than both P_{Na} and P_{Cl} in the gills of Magadi tilapia, and P_{Cl} is much lower than P_{Na}. These differences appear to be clearly adaptive. The true electrochemical driving force on an ion is the difference between the Nernst equilibrium potential and the TEP (+7.8 mV), signs considered (Kirschner 1970). Given $E_{Na} = +17.7$ mV, $E_{Cl} = +11.2$ mV, and $E_{HCO_3} = -74.9$ mV, the net forces tending to drive Na^+ inwards (+9.9 mV) and Cl^- outwards (+3.4 mV) are small relative to the strong electrochemical gradient tending to drive HCO_3^- inwards (−82.7 mV). Although internal pH and HCO_3^- concentrations are unusually high in Magadi tilapia relative to other teleosts at comparable temperature (Johansen et al. 1975; Wood et al. 1994), they would be far higher if there were not a very low P_{HCO_3} in the gills. We are aware of only one previous estimate of P_{HCO_3} in a teleost fish, and it was much higher than P_{Cl}. Using an artificial sea water in which HCO_3^- replaced Cl^-, Potts and Eddy (1973) reported $P_{Na} = 1.00$, $P_{HCO_3} = 0.5$,

$P_{Cl} = 0.03$. This highlights another difference between Magadi tilapia and other teleosts: at $P_{Cl}/P_{Na} = 0.40$–0.68, relative permeability to Cl^- is higher than previously measured in other teleosts where $P_{Cl}/P_{Na} = 0.03$–0.30 (Potts and Eddy 1973; House and Maetz 1974; Wood and Grosell 2008). This probably reflects the fact that Cl^- is normally distributed close to the Nernst equilibrium potential (E_{Cl}) in the unusual medium of Lake Magadi, so there is no need for lower P_{Cl}. Finally, the higher P_{Na} is likely needed to facilitate passive Na^+ efflux.

The morphology of the gill epithelium of the Magadi tilapia with respect to the fine structure (mitochondrial structure, micro-tubule density) of mitochondrial-rich and accessory cells, and the paracellular junctions between them, are essentially identical to those in standard marine teleosts (Laurent et al. 1995) and very different from those of standard freshwater teleosts (Maina 1991). It is therefore informative to compare ionoregulation in the Magadi tilapia in 100% lake water with that of marine teleosts in 100% sea water. Marine teleosts absorb large amounts of Na^+ and Cl^- through the intestine by drinking, in order to obtain and replace water which is lost osmotically across the gills. The need is therefore to excrete Na^+ and Cl^- at the gills. In these seawater fish, the electrogenic component of the TEP is part of the well-described NaCl excretion mechanism at the gills (the "Silva" model, originally proposed by Silva et al. 1977). Here electrogenic, secondary active Cl^- excretion through the transcellular pathway energizes passive Na^+ efflux through the paracellular "shunt" pathway (Wood and Marshall 1994; Marshall 2003).

Magadi tilapia similarly drink the medium at a high rate (~ 8 µl g^{-1} h^{-1}) to obtain water (Wood et al. 2002a; Bergman et al. 2003). However, in these fish the intestine absorbs large amounts of Na^+ and HCO_3^- and only smaller amounts of Cl^- (Bergman et al., 2003) reflecting the unusual composition of the lake water (Table 1). The need is therefore to excrete mainly Na^+ and HCO_3^- at the gills, whereas there is already a small net driving force (+3.4 mV) favoring outward Cl^- flux, as calculated earlier. For Na^+, the electrochemical gradient against which outward transport occurs is modest (+9.9 mV), but the opposing force is severe for HCO_3^- (−82.7 mV), just as it is for Cl^- in standard teleosts in sea water (approximately −50 mV). It is, therefore, very likely that the electrogenic component in Magadi tilapia is due to active HCO_3^- excretion. How this occurs is unknown, but Laurent et al. (1995) published a prescient hypothetical model (see Fig. 8 in that paper) in which electrogenic, secondary active HCO_3^- excretion through the transcellular pathway would energize a passive Na^+ efflux through the paracellular "shunt" pathway. This was essentially the classic model of Silva et al. (1977), with HCO_3^- replacing Cl^- at every step. The relative permeabilities and driving forces calculated in the current study are consistent with this model, but detailed molecular and pharmacological studies are now needed to provide additional support for these ideas. Other possibilities exist, such as HCO_3^- excretion through the sodium bicarbonate co-transporter (NBC).

Ecological and evolutionary significance

The harsh environment of Lake Magadi supports only a single teleost species and a very simple cyanobacteria-fish-bird ecosystem (Coe 1966). Despite intense avian traffic, no other fish have been able to invade, and even closely related cichlids die within a few hours when placed in Magadi water (Randall et al. 1989; Wood et al. 1989; Wright et al. 1990). The current study indicating unprecedented low HCO_3^- permeability and electrogenic HCO_3^- extrusion across the gills adds to the list of unique adaptations which allow only this species to survive in Lake Magadi. Others include ureotely, exceptionally high extracellular and intracellular pH, a high affinity blood O_2 curve insensitive to pH in the physiological range, extremely high gill O_2 diffusing capacity and metabolic rate, a stomach bypass which allows drinking of alkaline lakewater, and a capacity for facultative air-breathing via a physostomous air bladder (reviewed by Pörtner et al. 2010). Geological evidence (also reviewed by Pörtner et al. 2010) reveals that the founder populations can have been isolated for no more than 10,000 years, and this time frame is confirmed by molecular analyses of the base substitution rate for the mitochondrial DNA control region (Seegers et al. 1999; Wilson et al. 2000, 2004). Clearly, morphofunctional and physiological adaptations can occur over a very short evolutionary time frame, and genetic evidence suggests that further speciation is ongoing today in different isolated lagoon populations around Lake Magadi (Wilson et al. 2000, 2004). These *Alcolapia grahami* populations provide an invaluable resource for future studies of evolutionary and environmental physiology.

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