

THE EFFECTS OF REDUCING WATER pH AND TOTAL CO₂ ON A TELEOST FISH ADAPTED TO AN EXTREMELY ALKALINE ENVIRONMENT

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Summary

The teleost fish *Oreochromis alcalicus grahami* is well adapted to an extremely alkaline environment (pH 10, total CO₂ 184 mmol l⁻¹) in Lake Magadi, Kenya. *O. a. grahami* excretes all nitrogenous wastes as urea, and exposure to neutral water (pH 7, total CO₂ < 1 mmol l⁻¹) results in a complete inhibition of urea excretion. In the present study, we further characterized the physiological effects of transferring *O. a. grahami* from alkaline to neutral water. Exposure to neutral water resulted in a significant decrease in blood pH, a large reduction in plasma HCO₃⁻ levels, a severe impairment of swimming ability, an increase in Na⁺ influx, but no change in O₂ consumption. A closely related species of tilapia, *O. nilotica*, living in neutral waters of the Sagana River, was relatively unaffected by acute exposure to tapwater at pH 10, but showed a marked alkalosis in Lake Magadi water prior to death. These results demonstrate that *O. a. grahami* thrive in an environment of high pH and bicarbonate/carbonate concentration, conditions that are fatal to other species adapted to neutrality. *O. a. grahami*, however, is extremely unusual amongst aquatic animals in its adverse response to neutral water.

Introduction

In rainbow trout, exposure to highly alkaline conditions (pH > 9.5) causes severe physiological disturbances, including blood alkalosis, inhibition of Na⁺ influx, impairment of branchial ammonia excretion and a decrease in swimming speed (Wright and Wood, 1985; Ye, 1986). It is unusual, therefore, that a species of

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teleost fish, *Oreochromis alcalicus grahami*, is able to thrive in water of pH 10 (Lake Magadi, Kenya). In two previous studies, we have reported that *O. a. grahami* produces urea by the ornithine-urea cycle and excretes all nitrogenous wastes as urea (Randall *et al.* 1989; Wood *et al.* 1989), presumably enabling the fish to survive in alkaline water.

Lake Magadi is almost entirely covered by a solid crust of $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$. The fish live on the perimeter in shallow lagoons fed by underground hot springs (Coe, 1966). The total CO_3^{2-} plus HCO_3^- concentration is 184 mmol l^{-1} and the osmolality is about 50% that of sea water. We propose that, unlike most other aquatic animals, exposure to water of pH 7 will present a number of physiological problems to *O. a. grahami*. By reducing the pH of Lake Magadi water, the total CO_2 concentration will also be reduced as HCO_3^- is titrated to form CO_2 , which is then removed from solution by aeration. In neutral water, therefore, the tilapia will be exposed to a reversal of both the H^+ and HCO_3^- gradients. Reite *et al.* (1974) reported the pH tolerance of *O. a. grahami* to be between 5 and 11. Their study, however, dealt only with survival and did not address the physiological responses to altered water pH. In the present study, we have determined the acid-base status, blood ion composition, Na^+ influx rate, O_2 consumption and swimming ability in *O. a. grahami* at pH 10 and 7 to assess the effects of reduced water pH and total CO_2 levels. As a comparison, we have also studied the effects of alkaline exposure in *O. nilotica*, a closely related species of tilapia adapted to the neutral waters of the Sagana River, Kenya.

Materials and methods

Experimental animals

O. a. grahami (1–12 g) were collected from Lake Magadi and held outdoors at the Magadi Soda Co. in aerated lake water (water pH=9.98, 30–37°C, total $\text{CO}_2=184 \text{ mmol l}^{-1}$, $[\text{Na}^+]=342 \text{ mmol l}^{-1}$, $[\text{Cl}^-]=108 \text{ mmol l}^{-1}$, $[\text{K}^+]=2.22 \text{ mmol l}^{-1}$, osmolality= $525 \text{ mosmol kg}^{-1}$). To determine the effects of neutral water pH, lake water was acidified by the addition of concentrated HCl and vigorously aerated to reduce total CO_2 from the normal value of approximately 180 mmol l^{-1} to less than 1 mmol l^{-1} , as measured by a Corning 965 CO_2 analyzer. In these experiments it was not possible to separate the effects of reduced water pH from those of reduced total CO_2 levels. Thus, fish were exposed to water of pH 7 and reduced total CO_2 levels of less than 1 mmol l^{-1} and this water composition will be termed 'neutral water'.

O. nilotica (6–15 g) were collected from the Sagana River Tilapia Hatchery in Kenya. While this species is nominally *O. nilotica*, intense cross-breeding with *O. zillii* and possibly other tilapia species occurs in the hatchery ponds; therefore its exact taxonomic status is uncertain. Fish were held indoors at the University of Nairobi in aerated, dechlorinated Nairobi tapwater (water pH=7.22, 22°C, total $\text{CO}_2=0.56 \text{ mmol l}^{-1}$, $[\text{Na}^+]=0.29 \text{ mmol l}^{-1}$, $[\text{Cl}^-]=0.09 \text{ mmol l}^{-1}$, $[\text{K}^+]=0.03 \text{ mmol l}^{-1}$, osmolality $<5 \text{ mosmol kg}^{-1}$). To determine the effects of alkaline

exposure on *Sagana tilapia*, fish were transferred from Nairobi tapwater at pH 7 (neutral tapwater) to tapwater adjusted to pH 10 with KOH (alkaline tapwater). In this case, there was no change in total water CO₂ levels (approximately 0.5 mmol l⁻¹). A complete description of fish-holding facilities and water composition is given in Randall *et al.* (1989) and Wood *et al.* (1989).

Experiments

Blood sampling

Blood samples (15–200 µl fish⁻¹) for acid–base, ammonia, urea and ionic analyses were drawn anaerobically from stunned *Magadi* and *Sagana tilapia*. Blood samples were collected from both species of *tilapia* in their normal environmental water within 24 h of captivity. In transfer experiments, blood samples were taken after 12 h in neutral water (*O. a. grahami*) and after 10 h in alkaline tapwater (*O. nilotica*). Fish were exposed for several minutes to the air during sampling. Samples were taken by blind caudal puncture of the haemal arch. For details and discussion on the validity of the blood-sampling protocol and measurements, the reader is referred to Wood *et al.* (1989).

Critical swimming speed

Magadi tilapia were swum in a miniature swim tunnel to determine the effect of neutral water on swimming performance. The apparatus was constructed from the barrel of a disposable 20 ml syringe connected to a 3 A submersible pump (Little Giant), with the plunger-end of the syringe connected to the outlet of the pump. A wire mesh was placed between the pump and syringe, and the needle-end of the syringe was perforated in several places to create a more even cross-sectional flow. The pump and syringe were placed in a 20 l reservoir (O₂ tension = 16 ± 2 kPa, 30.9 ± 0.4 °C). Flow was varied by attaching the pump to a rheostat. Velocity of flow was determined by collecting the volume of water per unit time leaving the syringe. Fish of similar size (length 4.3 ± 0.2 cm, mass 1.4 ± 0.1 g, N = 5) were divided into two groups. One group was kept in Lake *Magadi* water, the other group was transferred to neutral water for 2–3 h before the start of the experiment. Fish were placed individually in the syringe and swum for 30 min at low velocity, followed by 15 min at each increased water velocity until the fish fatigued and was no longer able to maintain its position in the current. Critical swimming velocity was calculated as described by Brett (1964).

Metabolic rate and Na⁺ influx

Oxygen (O₂) consumption rate and Na⁺ influx were assessed in *O. a. grahami* transferred from Lake *Magadi* water to neutral water. To measure O₂ consumption, fish were placed in individual sealed chambers. After an initial 10 min period of adjustment, water samples (1 ml) were removed at 0 and 20 min for measurement of water P_{O₂} (P_{wO₂}). O₂ consumption was calculated from the change in P_{wO₂} over the 20 min experimental period using appropriate O₂ solubility coefficients (Boutilier *et al.* 1984).

To determine Na^+ influx (J_{Na}^n), 10 μCi of ^{22}Na (as NaCl , Amersham) was added to 250 ml of water in aerated beakers. After a 10 min mixing period, water samples (10 ml) were removed at 0 min and 3 h. The fish were killed with an excess of anaesthetic (MS-222). J_{Na}^n was determined by the appearance of ^{22}Na in the fish, taking into account water Na^+ specific activity.

Analytical techniques

Water and plasma ammonia and urea levels and blood acid–base parameters were measured as described by Wood *et al.* (1989). P_{CO_2} and HCO_3^- levels were calculated by the Henderson–Hasselbalch equation using values of pK' and CO_2 appropriate to the temperature and ionic strength of the samples (Boutilier *et al.* 1984). Values of P_{NH_3} were similarly calculated using appropriate constants from Cameron and Heisler (1983). Plasma protein levels were determined using a Goldberg refractometer (American Optical). Plasma samples and whole fish were frozen on dry ice and transported to the University of Ottawa where ion concentrations, osmolality and ^{22}Na activities were measured. Plasma $[\text{Na}^+]$ and $[\text{K}^+]$ were determined by atomic absorption spectroscopy (Varian AA-10), plasma $[\text{Cl}^-]$ by coulometric titration using a chloridometer (Buchler–Cotlove), and plasma $[\text{Ca}^{2+}]$ by spectrophotometry using a commercial assay kit (Sigma). Plasma osmolality was measured on 7 μl samples using a vapour pressure osmometer (Wescor 5100B). Water and whole-body ^{22}Na activities were determined using a gamma counter.

Statistics

Values are reported as means ± 1 s.e.m. (N), where N represents the number of fish (flux experiments and blood acid–base data) or the number of sample pools (plasma ammonia, urea and ion data). Student's paired or unpaired t -tests were employed to evaluate the significance of differences between mean values ($P < 0.05$).

Results

The transfer of Magadi tilapia from alkaline to neutral water resulted in a severe metabolic acidosis (-0.43 pH units), an eightfold decrease in total CO_2 , and a threefold decrease in calculated P_{CO_2} after 12 h (Table 1). In contrast, when Sagana tilapia were transferred to alkaline tapwater, there was no significant change in acid–base status after 10 h. Acute transfer (1 h) to Lake Magadi water, however, resulted in a severe metabolic alkalosis measured shortly after death. This was reflected in a $+0.16$ unit rise in pH and a more than threefold increase in total CO_2 (Table 1). Chronic measurements could not be made as the fish died within 1 h of exposure to Lake Magadi water.

Plasma urea and total ammonia levels did not significantly change in Magadi tilapia after 12 h of exposure to neutral water (Table 2). In Sagana tilapia, plasma urea and ammonia concentrations also did not change after 10 h of exposure to

Table 1. Effects on blood acid-base status of transferring *Oreochromis alcalicus grahami* from Lake Magadi water to neutralized Lake Magadi water, and of transferring *Oreochromis nilotica* from neutral tapwater to alkaline tapwater

| Treatment | pH | Total CO ₂ (mmol l ⁻¹) | P _{CO₂} (Pa) |
|--------------------------------|-------------------|--------------------------------------------------|-------------------------------------|
| <i>O. a. grahami</i> | | | |
| Lake Magadi water (control) | 7.58±0.06 (5) | 9.74±1.15 (9) | 952±121 (5) |
| Neutral water† (12 h) | 7.15±0.11* (6) | 1.18±0.19* (6) | 289±53* (6) |
| <i>O. nilotica</i> | | | |
| Neutral tapwater (control) | 7.66±0.03 (7) | 6.48±0.62 (7) | 463±65 (7) |
| Alkaline tapwater‡ (10 h) | 7.59±0.08 (5) | 4.38±0.42 (6) | 393±51 (5) |
| Lake Magadi water (1 h) | 7.82±0.15 (2) | 22.23±3.21* (5) | 868±73* (2) |

Blood samples were collected after 12 h in neutral water (*O. a. grahami*) and after 10 h in alkaline tapwater (*O. nilotica*).
 Data for *O. nilotica* were taken shortly after death in Lake Magadi water (within 1 h of transfer) are also included.
 Means±1 s.e.m. (N).
 * Significantly different ($P<0.05$) from control value.
 † Lake Magadi water acidified (pH 7) by the addition of concentrated HCl and aerated to reduce total CO₂ (<1 mmol l⁻¹).
 ‡ Nairobi tapwater adjusted to pH 10 with KOH, total CO₂ ≈0.5 mmol l⁻¹.

alkaline tapwater. Mean plasma NH₃ tensions (P_{NH_3}) were calculated from mean total ammonia (Table 2) and pH (Table 1) values. P_{NH_3} levels decreased threefold in Magadi fish exposed to neutral water, primarily as a result of the plasma acidosis.

Plasma ionic composition, osmolality and protein concentration for Magadi tilapia in Lake Magadi water and neutral water are given in Table 3. Exposure to neutral water resulted in a significant decrease in Na⁺ and an increase in Cl⁻, as well as a very large decrease in HCO₃⁻ levels. There were no significant changes in K⁺, Ca²⁺ and NH₄⁺ levels and osmolality. Plasma K⁺ values, however, were higher than expected for extracellular fluid (Guyton, 1981) in all fish, which may be related to the stress associated with the blood sampling method (for discussion see Wood *et al.* 1989). The plasma protein level decreased by 30% in neutral water, suggesting that a haemodilution occurred. This is supported by the observation that fish exposed to neutral water for 48 h had an extremely bloated appearance. Plasma ionic composition for Sagana tilapia is also given in Table 3 for comparison. The values are typical of other freshwater teleosts, but [Na⁺], [Cl⁻], [Ca²⁺] and osmolality are lower than in Magadi tilapia in either alkaline or neutral water.

Table 2. Effects on plasma urea and ammonia concentration, and calculated plasma P_{NH_3} , of transferring *Oreochromis alcalicus grahami* from Lake Magadi water to neutralized Lake Magadi water, and of transferring *Oreochromis nilotica* from neutral tapwater to alkaline tapwater

| Treatment | Urea (mmol l^{-1}) | Ammonia ($\mu\text{mol l}^{-1}$) | P_{NH_3} (Pa) |
|--------------------------------|----------------------------------|---------------------------------------|---------------------------|
| <i>O. a. grahami</i> | | | |
| Lake Magadi water (control) | 5.26 ± 0.53^1 (6) | 770 ± 103^1 (5) | 0.096 |
| Neutral water† (12 h) | 4.10 ± 0.67 (5) | 680 ± 199 (6) | 0.032 |
| <i>O. nilotica</i> | | | |
| Neutral tapwater (control) | 1.16 ± 0.11^1 (6) | 1043 ± 189^1 (6) | 0.065 |
| Alkaline tapwater‡ (10 h) | 1.06 ± 0.08 (2) | 1474 ± 8 (2) | 0.078 |

Blood samples were collected after 12 h in neutral water (*O. a. grahami*) and 10 h in alkaline tapwater (*O. nilotica*).

¹From Randall *et al.* (1989).

† See Table 1; ‡ see Table 1.

Means \pm 1 S.E.M. (N).

In neutral water, $J_{\text{Na}}^{\text{in}}$ was 55 % higher compared to control rates [Lake Magadi water, $2.1 \pm 0.2 \mu\text{mol g}^{-1} \text{h}^{-1}$ versus neutral water, $3.3 \pm 0.1 \mu\text{mol g}^{-1} \text{h}^{-1}$ ($N=6$)]. There was no increase in metabolic rate, as transfer from alkaline to neutral water did not change O_2 consumption [Lake Magadi water, $9.06 \pm 1.42 \text{ mmol kg}^{-1} \text{h}^{-1}$ versus neutral water, $11.39 \pm 0.82 \text{ mmol kg}^{-1} \text{h}^{-1}$ ($N=6$)].

We measured critical swimming velocity in Magadi tilapia to determine the effect of neutral water on swimming ability. In Lake Magadi water, Magadi tilapia swam efficiently in the swimming apparatus [3.2 ± 0.2 body lengths s^{-1}], but when transferred to neutral water, critical swimming velocity decreased fourfold [0.7 ± 0.2 body lengths s^{-1} ($N=5$)].

Discussion

O. a. grahami are remarkable in their ability to survive in an extremely alkaline environment (pH=10, total $\text{CO}_2=184 \text{ mmol l}^{-1}$). The results of this study show that, unlike the majority of aquatic animals, *O. a. grahami* do not function well in neutral water (pH 7, total $\text{CO}_2 < 1 \text{ mmol l}^{-1}$). A good indicator of overall performance in fish is swimming ability. Critical swimming velocity was severely reduced when *O. a. grahami* were transferred from Lake Magadi to neutral water. In addition, there was a large metabolic acidosis and an ionic and fluid volume disturbance. Clearly, Magadi tilapia can survive but not function adequately in neutral water.

Table 3. Effects on plasma ionic composition, osmolality and protein concentration of transferring *Oreochromis alcalicus grahami* from Lake Magadi water to neutralized Lake Magadi water

| | <i>O. a. grahami</i> | | <i>O. nilotica</i> |
|---------------------------------------------------------|--------------------------------|--------------------------|-------------------------------|
| | Lake Magadi water (control) | Neutral water† (12 h) | Neutral tapwater (control) |
| Na ⁺ (mequiv l ⁻¹) | 208.6±7.4 (8) | 181.8±5.2* | 150.6±4.4 (9) |
| K ⁺ (mequiv l ⁻¹) | 7.8±0.6 (8) | 6.5±0.6 (6) | 7.5±0.7 (9) |
| Ca ²⁺ (mequiv l ⁻¹) | 7.4±0.5 (8) | 6.4±1.3 (6) | 3.2±0.5 (9) |
| Cl ⁻ (mequiv l ⁻¹) | 161.6±2.7 (8) | 200.0±10.1* | 117.3±8.3 (9) |
| HCO ₃ ⁻ (mequiv l ⁻¹) | 9.5±1.1 (9) | 1.1±0.2* | 6.3±0.6 (7) |
| NH ₄ ⁺ (mequiv l ⁻¹) | 0.8±0.1 (5) | 0.7±0.2 (6) | 1.0±0.2 (6) |
| Osmolality (mosmol kg ⁻¹) | 350.7±4.0 (9) | 347.8±4.1 (6) | 299.6±6.5 (8) |
| Plasma protein (g 100 ml ⁻¹) | 3.8±0.2 (6) | 2.6±0.1* (6) | 2.2±0.2 (6) |

Data from *Oreochromis nilotica* held in neutral tapwater are included for comparison.
 Blood samples were collected within 24 h of captivity in control fish and in *O. a. grahami* after 12 h of exposure to neutral water.
 * Significantly different from pH=10 value, $P<0.05$.
 † See Table 1.
 Means±1 S.E.M. (N).

The question as to why Magadi tilapia suffer a number of adverse affects in neutral water must be related to water composition. Acidification and subsequent aeration of Lake Magadi water resulted in the following changes: (1) a reduction in total CO₂ levels (184 to <1 mmol l⁻¹), (2) a reduction in calculated [HCO₃⁻] (64 to <1 mmol l⁻¹, see Materials and methods), (3) a 1000-fold increase in water H⁺ levels (pH 10 to pH 7), and (4) a calculated fourfold increase in Cl⁻ levels (108 to about 400 mmol l⁻¹). When Magadi tilapia were exposed to neutral water, the HCO₃⁻ gradient (now blood-to-water), H⁺ gradient (now water-to-blood) and Cl⁻ gradient (now water-to-blood) were reversed compared to the gradients in Lake Magadi water. Thus, one would predict the efflux of HCO₃⁻ from the plasma to the environment and the entry of Cl⁻ and H⁺. The data support this prediction: plasma [HCO₃⁻] and pH decreased significantly, and [Cl⁻] increased significantly in neutral water (Tables 1 and 3).

When Sagana tilapia were exposed to Lake Magadi water, they quickly died, exhibiting a marked metabolic alkalosis (Table 1). When Sagana tilapia were exposed to alkaline tapwater, however, the effects on blood acid-base balance

were negligible (Table 1), indicating that toxicity was related in some way to the high water carbonate alkalinity rather than to pH *per se*.

Exposure to neutral water resulted in a fluid volume disturbance in Magadi tilapia. There are two pieces of evidence that point to an increase in blood volume and possibly extracellular fluid volume. First, plasma protein levels decreased and, second, fish in neutral water had a bloated appearance. This expansion was due to an uptake of both ions and water as there was no change in osmolality in Magadi tilapia exposed to neutral water.

Changes in plasma acid–base status and ionic composition may have affected metabolic function. *O. a. grahami* excrete all nitrogenous wastes as urea (Randall *et al.* 1989) and, when exposed to neutral water, urea excretion was completely inhibited (Wood *et al.* 1989). The inhibition of urea excretion may have been due to an inhibition of urea production *via* the ornithine–urea cycle (Randall *et al.* 1989; Wood *et al.* 1989). The large decrease in plasma $[\text{HCO}_3^-]$ (Table 1) may have resulted in an inhibition of liver urea metabolism. In toadfish hepatocytes, a decrease in extracellular $[\text{HCO}_3^-]$ reduces the rate of urea synthesis (Walsh *et al.* 1989). Moreover, these data indicate an inhibition of protein metabolism because plasma ammonia levels (Table 2) and excretion rates (Wood *et al.* 1989) did not rise, despite an inhibition of urea production. Nitrogenous wastes in Sagana tilapia were excreted predominantly as ammonia, but during alkaline exposure, urea excretion increased threefold (Randall *et al.* 1989; Wood *et al.* 1989), with no change in plasma $[\text{HCO}_3^-]$ (Table 1) but an increase in plasma [ammonia] (Table 2). This response is found in some ammoniatic teleost fish which are able to increase urea production slightly *via* uricolysis in response to elevated body ammonia levels (Olson and Fromm, 1971).

Exposure to neutral water significantly increased Na^+ influx in *O. a. grahami*. To our knowledge, Na^+ influx rates in *O. a. grahami* have not been previously reported. In freshwater rainbow trout, Na^+ influx is pH sensitive: the optimum pH is between 7 and 8, and Na^+ influx decreases in acid or alkaline water (Wright and Wood, 1985; Randall and Wright, 1986). The pH sensitivities of the Na^+ transporters in trout and Magadi tilapia appear to be similar, but Na^+ flux rates in trout are an order of magnitude lower.

In summary, the results of this study show that exposure of *O. a. grahami* to neutral water results in a metabolic acidosis, an ionic and fluid volume disturbance, an increase in Na^+ influx, and a large reduction in critical swimming velocity. Reite *et al.* (1974) report the pH tolerance of *O. a. grahami* to be between 5 and 11: however, they did not measure any physiological parameters and were only interested in survival for 24 h. The adverse response of *O. a. grahami* to neutral conditions is unique since the majority of aquatic animals have an environmental pH optimum between 6 and 8. Finally, our results also indicate that Lake Magadi water is far more 'toxic' to *O. nilotica* adapted to neutral conditions than neutral water is to *O. a. grahami* adapted to Lake Magadi water. The Lake Magadi toxicity appears to be related to the high bicarbonate alkalinity rather than to pH *per se*. Thus, urea production *via* the ornithine cycle in *O. a. grahami* can be

viewed as a way to maintain nitrogenous excretion by consuming NH_4^+ and blood pH by consuming HCO_3^- .

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