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C. M. Wood

P. Wilson

Harold Bergman
University of Wyoming, bergman@uwyo.edu

A. N. Bergman

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Chris M. Wood[1,2,*]
Paul Wilson[3]
Harold L. Bergman[4]
Annie N. Bergman[4]
Pierre Laurent[5]
George Otiang'a-Owiti[6]
Patrick J. Walsh[2]

[1]Department of Biology, McMaster University, Hamilton, Ontario L8S 4K1, Canada; [2]Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, National Institute of Environmental Health Science, Marine and Freshwater Biomedical Sciences Center, University of Miami, Miami, Florida 33149; [3]Department of Chemistry, Wildlife Forensic DNA Laboratory, Trent University, Peterborough, Ontario K9J 7B8, Canada; [4]Department of Zoology and Physiology, University of Wyoming, Laramie, Wyoming 82071; [5]Centre d'Ecologie et de Physiologie Energétique, Centre National de la Recherche Scientifique, 23 Rue Becquerel, BP 20 CR, 67037 Strasbourg, France; [6]Department of Veterinary Anatomy, University of Nairobi, Nairobi, Kenya

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ABSTRACT

Alcolapia grahami is a unique ureotelic tilapia that lives in the highly alkaline, saline Lake Magadi, Kenya (pH, ~10.0; alkalinity, ~380 mmol L^{-1}; Na^+, ~350 mmol L^{-1}; Cl^-, ~110 mmol L^{-1}; osmolality, ~580 mosm kg^{-1}). The fish survived well upon gradual exposure to dilute lake water (down to 1%, essentially freshwater). Urea excretion continued, and there was no ammonia excretion despite favorable conditions, indicating that ureotelism is obligatory. Levels of most ornithine-urea cycle enzymes in the liver were unchanged relative to controls kept for the same period in 100% lake water. The fish exhibited good abilities for hypo- and hyperregulation, maintaining plasma Na^+, Cl^-, and osmolality at levels typical of marine and freshwater teleosts in 100% and 1% lake water, respectively. Plasma total CO_2 did not change with environmental dilution. Routine oxygen consumption (Mo_2) was extremely high in 100% lake water but decreased by 40%–68% after acclimation to dilute lake water. At every fixed swimming speed, Mo_2 was significantly reduced (by 50% at high speeds), and critical swimming speed was elevated in fish in 10% lake water relative to 100% lake water. Osmotic and Cl^- concentration gradients from water to plasma were actually increased, and osmotic and Na^+ gradients were reversed, in 10% and 1% dilutions relative to 100% lake water, whereas acid-base gradients were greatly reduced. We suggest that approximately 50% of the animal's high metabolic demand originates from the cost of acid-base regulation in the highly alkaline Lake Magadi. When this load is reduced by environmental dilution, the energy saved can be diverted to enhanced swimming performance.

* Corresponding author; e-mail: woodcm@mcmail.cis.mcmaster.ca.

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Introduction

The tilapia (*Alcolapia grahami*, formerly *Oreochromis alcolapia grahami*; Seegers and Tichy 1999) of Lake Magadi, Kenya, thrives in one of the most alkaline aquatic environments on earth (pH, ~10.0; total CO_2 [i.e., HCO_3^- plus $CO_3^=$], ~220 mmol L^{-1}; alkalinity, ~380 mmol L^{-1}; Coe 1966; Reite et al. 1974; Johansen et al. 1975; Wood et al. 1989). Accompanying this extreme alkalinity is a most unusual ionic regime, with high Na^+ (~350 mmol L^{-1}), low Cl^- (~110 mmol L^{-1}), and an osmolality (~580 mosm kg^{-1}) equivalent to that of approximately 50% seawater. These fish eat a cyanobacterial diet rich in nitrogen and live at extremely high temperatures, tolerating up to 42°–43°C (Coe 1966; Narahara et al. 1996). Remarkable physiological specializations have been identified, especially in nitrogen metabolism, which allow this successful existence in an environment that would quickly kill most other fish.

Most important, this teleost species is 100% ureotelic when living in Lake Magadi water, excreting urea-N at an extremely high rate via the gills, yet not excreting ammonia-N at all (Randall et al. 1989; Wood et al. 1989, 1994). This strategy overcomes the difficulty of maintaining a diffusion gradient for NH_3 into the highly buffered branchial water where the pH is fixed at about 10.0, well above the pK (9.3) of the $NH_3 \leftrightarrow NH_4^+$ dissociation. Several authors have suggested that this ureotelism may also serve to "consume" excess bicarbonate (which floods into the organism from this highly alkaline environment) by metabolic urea production (Meijer et al. 1990; Atkinson 1992), but experimental examination suggested that

Table 1: Measured water chemistry values for tap water, 100% lake water (from Fish Springs Lagoon), and for the various dilutions made from these media

	Tap Water	100%	50%	10%	2.5%	1%
Na^+ (mmol L^{-1})	3.5	355	164	49.8	10.4	8.1
Cl^- (mmol L^{-1})	.3	113	55	15.9	3.8	1.2
Total CO_2 (mmol L^{-1})	.9	216	96	19.3	6.0	4.7
pH	6.96	9.86	9.78	9.74	9.45	9.15
Osmolality (mosm kg^{-1})	11	581	329	76.1	30.0	18.9
Alkalinity (meq L^{-1})	.9	378	154	21.5	6.8	5.0

this was not the case (Wood et al. 1994). Regardless, urea-N production and excretion rates are massive (up to 20-fold greater than the total-N excretion rates of "standard" ammoniotelic teleosts). These high urea-N production rates are achieved by expression of the ornithine-urea cycle (OUC) not only in the liver (Randall et al. 1989; Walsh et al. 1993) but also throughout the entire muscle mass (Lindley et al. 1999) so that most of the body is ureagenic. The high urea-N excretion rates are achieved by the expression in branchial tissue of a recently identified UT-A type urea transporter, which facilitates rapid efflux of urea across the gills (Walsh et al. 2001).

At present, we have only a partial understanding of how ionoregulation and acid-base regulation are achieved. Despite the unusual ionic regime of the lake water (high Na^+, high HCO_3^- plus $CO_3^=$, low Cl^-), gill chloride cell structure appears typical of marine teleosts (Laurent et al. 1995). In some reports (Leatherland et al. 1974; Eddy and Maloiy 1984; Wright et al. 1990), plasma ions and osmolality are also fairly typical of those in marine teleosts, while in others, plasma ion concentrations and ratios are unusual (Maloiy et al. 1978; Skadhauge et al. 1980). There is one report that drinking of the medium occurs at a much higher rate than in marine teleosts (Maloiy et al. 1978). Several studies agree that the species regulates exceptionally high internal pH and HCO_3^- levels (Johansen et al. 1975; Johnston et al. 1983; Wood et al. 1989, 1994). Nevertheless, there appear to be strong electrochemical gradients for the loss of H^+, Cl^-, and water and for the entry of Na^+, OH^-, HCO_3^-, and $CO_3^=$ at the gills (Eddy et al. 1981; Wood et al. 1994), and additional basic anions may enter through the intestinal tract as a result of drinking (Skadhauge et al. 1980).

Given the fact that ureotelism is much more expensive than ammoniotelism (2 ATP per urea-N excreted; Wood 1993), and the fact that the organism is regulating against strong ionic, osmotic, and acid-base gradients, it is perhaps not surprising that *A. grahami* living in Lake Magadi water exhibit the highest routine metabolic rates ever recorded in teleost fish of this size (Franklin et al. 1995; Narahara et al. 1996). However, we have recently discovered isolated populations living in lagoons with markedly lower alkalinity and salinity relative to "standard" lake water (P. A. Wilson, C. M. Wood, P. J. Walsh, A. N. Bergman, H. L. Bergman, P. Laurent, and B. N. White, unpublished manuscript). Furthermore, simple dilution of the full strength Lake Magadi water may also occur periodically in nature, for occasional rainstorms have been reported to greatly influence lake water levels (Coe 1966). There are several reports that Magadi tilapia can be acclimated in the laboratory to diluted lake water, though there is conflicting information on whether they can be acclimated to true freshwater (Coe 1966; Leatherland et al. 1974; Maloiy et al. 1978; Maina 1990). This raises the interesting question as to what happens to metabolism when the species is acclimated to more dilute environments. Under such conditions, ionic, osmotic, and acid-base gradients will be altered, and with reduced environmental pH, alkalinity, and buffering, it should become possible and indeed advantageous to excrete ammonia across the gills. Will ureotelism continue? Will metabolic costs be reduced? Will there be greater scope for other activities, such as swimming performance?

In this study, we addressed these questions by gradually exposing Magadi tilapia to reduced alkalinity and salinity by progressive environmental dilution, in the end achieving acclimation to 1% lake water, which is essentially freshwater. Metabolic rate, urea and ammonia production, and ionic, acid-base, and osmotic gradients were assessed at several points in the dilution regime, and aerobic swimming performance was compared between 100% lake water and a dilution (10%) at which metabolic costs appeared to be minimal. The results reveal a species with an even more unusual physiology than previously believed and cast particular light on the high cost of living in the normal environment.

Material and Methods

Experimental Animals

Adult *Alcolapia grahami* (typically 1–4 g) were collected in January–February 1997 by beach seine from the Fish Springs Lagoon at the edge of Lake Magadi, Kenya. This was the standard collecting site used in almost all previous physiological studies on this species (see Coe 1966 and Narahara et al. 1996 for a detailed description of the site), and it provided the standard reference water termed "100% lake water" in Table 1. The fish were brought immediately to an outdoor laboratory set up on a balcony kindly provided by Magadi Soda, where the fish

were subsequently held and all experiments were performed. Ambient temperature varied from about 30° to 36°C over the day, similar to the diurnal fluctuation at the collection sites (cf. Narahara et al. 1996).

Measurements of Water Chemistry

Water pH, temperature, total CO_2, and chloride were measured at the outdoor laboratory using equipment and methods identical to those described by Wood et al. (1994). Alkalinity, expressed as HCO_3^- equivalents (i.e., $[HCO_3^-] + 2[CO_3^=]$), was calculated from pH and total CO_2 measurements using values for αCO_2 and pK^I and pK^{II} at the appropriate temperature and chlorinity from Skirrow (1975). Samples were frozen and transported back to McMaster University for later measurement of sodium (by atomic absorption spectroscopy [AAS]; Varian 1275-AA) and osmolality (by vapor pressure osmometry; Wescor 5100A).

Progressive Acclimation to Reduced Salinity/Alkalinity

These acclimations were performed twice. In each case, a batch of fish caught on a single day was divided into two groups (control and experimental) of 25 fish, and each group was placed in a polyethylene bucket filled with 100 L of 100% lake water from Fish Springs Lagoon. Vigorous aeration was provided. Fish in each tank were fed 1 g of a commercial cichlid diet twice per day (8:00 A.M. and 8:00 P.M.), which corresponds to a ration of approximately 3.2% body weight per day (dry food/wet weight). As all food was eaten, control and experimental groups consumed the same amount. The water was changed daily by removing and replacing 90 L approximately 2 h after the morning feeding. One group, designated control, was kept in 100% lake water throughout (composition as in Table 1). The second group was gradually acclimated to dilute media by reductions of 10% every 24 h, achieved by the addition of the appropriate proportion of Magadi tap water (composition as in Table 1). Once 10% (i.e., 90% dilution) was reached, subsequent daily steps were 5%, 2.5%, and 1%. The last was essentially freshwater. Samples for analysis of actual water chemistry were collected at each acclimation dilution; measured values (Table 1) were consistent with the nominal mixing ratios. Note that total measured ion concentrations exceed osmolality in 100% lake water because the osmotic activity coefficient of a solution high in HCO_3^- and $CO_3^=$ is much reduced through ion-pairing (Table 1). As the solution becomes more dilute, the activity coefficient increases and the osmolality becomes higher than the sum of the ions because not all ions were measured.

In the first acclimation series, fish ($N = 8$–10) were periodically removed from the acclimation tanks for simultaneous measurements (see below) of urea-N ($M_{urea\text{-}N}$) and ammonia-N ($M_{amm\text{-}N}$) excretion and O_2 consumption rates (Mo_2). This occurred at 100% (start, freshly collected) and 24 h after 50%, 10%, 2.5%, and 1% dilutions had been achieved. Measurements were performed in the appropriate acclimation water, measurements on the control fish in 100% lake water were performed simultaneously, and all fish were returned to their respective acclimation tanks after the measurements were completed. In the second acclimation series, groups of fish ($N = 25$) were held for 10 d at 10% and 1% once these dilutions had been reached. A control group of fish was held in the laboratory at 100% for the same total period. As outlined below, these fish were either killed for plasma composition measurements (all groups) or for liver enzyme activity measurements (100% and 1% groups only) or used for swimming performance trials (100% and 10% groups only).

Measurements of Urea-N and Ammonia-N Excretion and O_2 Consumption Rates

For these measurements, fish were removed from their acclimation tanks just before the morning feeding, and so had not been fed for about 12 h. Urea-N ($M_{urea\text{-}N}$) and ammonia-N ($M_{amm\text{-}N}$) excretion and O_2 consumption rates (Mo_2) were measured simultaneously using the Tusker chamber system described by Wood et al. (1994)—530-mL amber bottles fitted with sampling ports and aeration lines. Each chamber contained one fish and the appropriate acclimation water. After an initial 1-h settling period, measurements were started. Total flux periods were 3–5 h, with water samples drawn for the determination of urea-N and ammonia-N at the start and end; in the middle of the period, aeration was suspended and the chamber sealed for 40 min to allow the measurement of O_2 consumption. A chamber containing appropriate water but no fish was included in each run to serve as a blank. It was not possible to control temperature in the outdoor laboratory (which typically increased from about 31° to 35°C over the 4–6 h experiment), but in order to standardize conditions as much as possible, the experiment was conducted at the same time every day, and identical measurements were carried out simultaneously on fish ($N = 8$–10) in 100% lake water from the control tank. The fish were weighed once measurements were completed.

Urea-N and ammonia-N concentrations in water were measured colorimetrically on site by the diacetyl monoxime method (Rahmatullah and Boyde 1980; Price and Harrison 1987) and the salicylate-hypochlorite method (Verdouw et al. 1978), respectively. As salinity/alkalinity affected color development, it proved necessary to make up urea-N and ammonia-N standards in the appropriate dilution water in each case. The assays were modified by the addition of an extra 1 mL of dilution water to bring the volume to 3 mL so the samples could be read on a Spectronic 20 spectrophotometer (Bausch and Lomb). Nitrogen excretion rates ($M_{urea\text{-}N}$, $M_{amm\text{-}N}$) were calculated from the increases (blank-corrected) in water urea-N and

ammonia-N concentrations over the entire flux period, factored by mass and time. Samples for water Po_2 were analyzed using a Radiometer pHM 71 gas analyser and Radiometer O_2 electrode at the experimental temperature. Po_2 values were converted to O_2 concentrations using αO_2 values appropriate to the temperature and salinity from Boutilier et al. (1984). Mo_2 was calculated from the decrease (blank-corrected) in O_2 during the period of chamber closure, factored by mass, time, and volume.

Measurements of Plasma Composition

Fish were rapidly anesthetized by transfer to a solution of metomidate-HCl^- (5 mg L^{-1}) in the appropriate salinity/alkalinity. Each fish was then blotted dry with tissues and weighed. Blood samples were drawn by caudal puncture using a 100-μL gastight Hamilton syringe with a customized needle; the syringe was wetted with heparin (1,000 IU mL^{-1} in a simple saline, 200 mM NaCl, similar to Magadi tilapia plasma; Wright et al. 1990). Plasma was separated by centrifugation and frozen in liquid nitrogen for shipment back to McMaster University, using a dry shipper (Minnesota Valley Engineering), and later determination of plasma Na^+ (by AAS as above), Cl^- (by the colorimetric assay of Zall et al. 1956), total CO_2 (by Corning 965 analyzer), and osmolality (by vapor pressure osmometry, as above). Because of limited sample volumes, it was not always possible to measure all parameters on all fish.

Measurements of Liver Enzyme Activities

Fish were killed by cephalic concussion and weighed. The liver was immediately excised, freeze-clamped in liquid nitrogen, and weighed; hepatosomatic index (HSI) was calculated as the liver weight as a percentage of body weight. Frozen liver tissue was transported back to the University of Miami at liquid nitrogen temperature using a dry shipper (Minnesota Valley Engineering). There, the samples were stored for less than 2 mo at –80°C before analysis of enzyme activities. The activities of the following hepatic enzymes involved in the OUC and/or contributing to other aspects of nitrogen metabolism were determined at 30.0° ± 0.2°C according to Mommsen and Walsh (1989): glutamine synthetase (GSase), ornithine-citrulline transcarbamoylase, arginase (ARG), glutamate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase. Glutaminase was determined according to Curthoys and Lowry (1973), and arginosuccinate synthetase/arginosuccinate lyase (AS/AL; as a coupled reaction) and carbamoyl phosphate synthetase III metabolism were determined according to Anderson and Walsh (1995).

Measurements of Swimming Performance and Oxygen Consumption

The Blazka-style swimming respirometers described by Wilson et al. (1994) were used, and similar methods were employed. Each respirometer (volume 3.2 L) was submerged in a 60-L bath of the appropriate water, vigorously aerated, for continual water replacement and temperature control (31°–34°C). Individual fish that had been long-term acclimated to either 100% or 10% lake water were removed from their acclimation tanks before the morning feeding, measured for weight and fork length, transferred to the respirometers, and allowed to settle for a period of 45–75 min at a speed of 3 body lengths per second (BL s^{-1}). (These very active fish would not orient consistently into the current at lower speeds.) The front of the respirometer was shielded to provide cover for the fish; an underside mirror facilitated observation. After the settling period, velocities of 3, 4, 5, 6, 7, 8, and 9 BL s^{-1} were progressively imposed, each for a period of 40 min, until exhaustion occurred (exact time noted). There was a 5-min ramp-up period between each velocity. Exhaustion was defined by the fish falling back to the rear of the swim tunnel and failing to resume swimming after three trials when the velocity was briefly stopped and restarted. Critical swimming speed was calculated according to Brett (1964).

During each period of steady swimming, Mo_2 was monitored by sealing the respirometer and monitoring water Po_2 (as above) until it had declined to about 70% of air saturation, after which the system was flushed with saturated water. Mo_2 was calculated from the decrease (blank-corrected) in O_2 during the period of chamber closure, factored by mass, time, and volume.

Statistical Analyses

Data have been expressed as means ± 1 SEM (N), where N = number of fish. Comparisons to simultaneous control values were made by Student's unpaired t-test (two tailed); multiple comparisons were performed by one-way ANOVA followed by the Bonferroni post hoc test to identify specific differences. A fiducial limit of $P \leq 0.05$ was used throughout.

Results

Using the progressive dilution approach, it proved possible to acclimate Magadi tilapia to reductions in salinity/alkalinity right down to 1% lake water with minimal mortality (18% vs. 12% in controls, $P > 0.05$). Over the entire dilution range, these fish remained 100% ureotelic, but there were substantial changes in metabolic rate.

During this acclimation regime, both routine Mo_2 and $M_{urea\text{-}N}$ fell significantly in the 100% control group relative to the freshly collected fish at the start of the exposure (Fig. 1*A*, 1*B*). More important, Mo_2 and $M_{urea\text{-}N}$ declined to a much

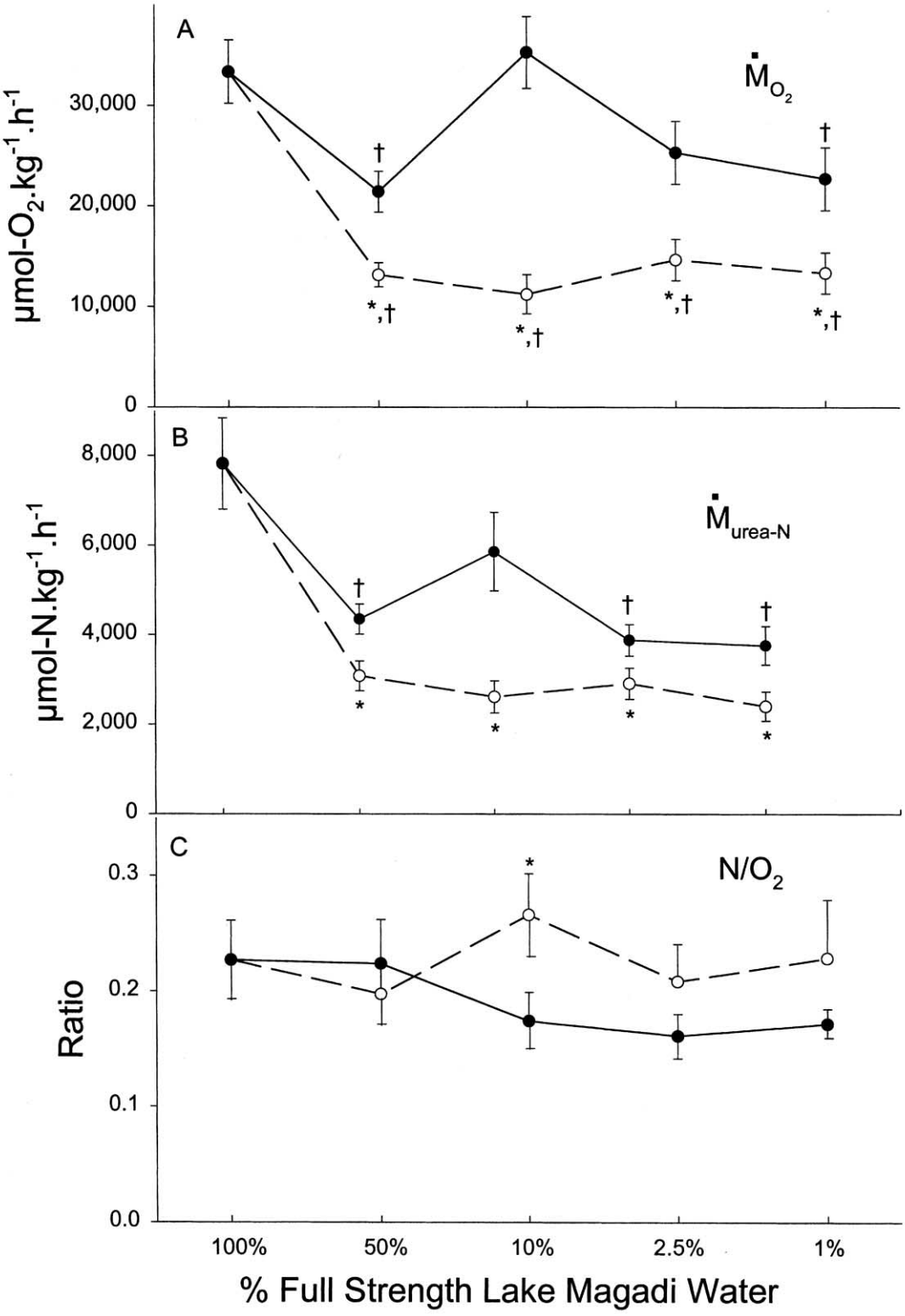

Figure 1. Routine rates of (*A*) oxygen consumption (Mo_2), (*B*) urea-N excretion ($M_{urea\text{-}N}$), and (*C*) the ratio of $M_{urea\text{-}N}$ to Mo_2 ($N:O_2$) in Magadi tilapia chronically acclimated to various concentrations of lake water (*open circles*) are compared with simultaneously measured control rates for fish held for the same length of time in 100% lake water (*closed circles*). In each panel, the left-hand closed symbol represents the initial rate in freshly collected fish in 100% lake water. Means ± 1 SEM ($N = 8–10$). Asterisks indicate significant differences ($P < 0.05$) from the simultaneous 100% control value; daggers indicate significant differences from the initial value of freshly collected fish in 100% lake water.

greater extent in the experimental fish exposed to progressive dilution and were always significantly lower than in simultaneous controls kept in 100% lake water for the same period (Fig. 1*A*, 1*B*). Mo_2 reached a minimum in 10% lake water, where it was depressed by 68%. At other dilutions (50%, 2.5%, and 1% lake water), the depression in Mo_2 was about 40%–45%. However, urea-N excretion persisted, and there was no detectable ammonia excretion relative to blanks at any dilution, even at 1%, which was essentially freshwater (Table 1). The relative fall in $M_{urea\text{-}N}$ was slightly less than that in Mo_2, but the $N : O_2$ ratio (normally around 0.20) was significantly elevated (to 0.27) only in 10% lake water (Fig. 1*C*).

Table 2 reports accompanying measurements of plasma chemistry. Plasma total CO_2 did not change significantly as the fish were acclimated to more dilute, less alkaline environments. Plasma total CO_2 represented only 7% of environmental values in 100% lake water, but 58% in 10% lake water, and 210% in 1% lake water (Fig. 2*A*). Overall, control tilapia acclimated to 100% lake water were clearly hyporegulating, maintaining plasma osmolality at about 65% and plasma Na^+ at about 50% of external lake water values (Fig. 2*B*, 2*C*). On the other hand, plasma Cl^- was substantially higher (145%) than lake water values (Fig. 2*C*). After acclimation to 10% lake water, plasma Na^+ and Cl^- fell slightly (by 10%–15%), but osmolality was perfectly regulated, so the animals were now clearly hyperregulating at internal ion levels several-fold higher than those in the dilute external environment (Fig. 2*B*, 2*C*). After acclimation to 1% lake water (essentially freshwater; Table 1), plasma ion levels exhibited no further change while osmolality fell only slightly, so strong hyperregulation continued at levels 20-fold (Na^+, osmolality) to 120-fold (Cl^-) higher than in the environment.

Since the reduction in routine metabolic rate was greatest in 10% lake water, swimming trials were performed in fish acclimated to this dilution and compared with fish acclimated to 100% lake water (Fig. 3). In 100% lake water, long-term laboratory-acclimated fish exhibited a critical swimming speed of 5.66 ± 0.41 BL s^{-1}; only four out of 12 tested fish achieved a speed of 7 BL s^{-1}, and none achieved a higher speed. However, Magadi tilapia acclimated to 10% lake water for the same period exhibited superior swimming performance. Indeed, seven of the nine fish tested achieved 7 BL s^{-1}. The mean critical swimming speed in 10% lake water was greater than 7.5 BL s^{-1}. However, the precise value could not be calculated for this treatment because two of these fish did not exhaust at 9 BL s^{-1}, the highest speed available in the respirometers.

Mo_2 tended to rise gradually with swimming speed in both treatments, except for a decrease in the initial step from 3 to 4 BL s^{-1}, which probably reflected a reduction in spontaneous activity (Fig. 3). At every swimming speed, Mo_2 was significantly lower in the 10% lake water group, a difference that reached about 50% at 6–7 BL s^{-1}. At such high swimming speeds, spontaneous activity was eliminated, so this difference in metabolic cost was due to the environmental conditions.

After long-term laboratory holding in 100% lake water, the activities (per gram liver weight) of all hepatic enzymes involved in the OUC and/or contributing to other aspects of nitrogen metabolism (Table 3) were significantly depressed by 55%–85% relative to freshly collected 100% fish at the start, with the single exception of the AS/AL. While this pattern apparently mirrored the decline in Mo_2 and $M_{urea\text{-}N}$ in these control fish (Fig. 1*A*, 1*B*), the hepatosomatic index (HSI) increased threefold (Table 3). Therefore, absolute amounts of enzymatic capacity may not have changed. In fish acclimated to 1% lake water and held for the same period in the laboratory as the long-term 100% control group, AS/AL and ARG activities were reduced and the HSI was smaller; there were no other significant changes (Table 3).

Discussion

Overview

Three important conclusions emerge from this study, one representing a clarification of previous uncertainty, and the other two representing entirely new findings. First, *Alcolapia grahami* possess euryhaline ionoregulatory abilities and can be acclimated to extreme environmental dilution, right down to essentially freshwater. Second, ureotelism appears to be obligatory in the Magadi tilapia because it continues to excrete entirely urea-N and no ammonia-N even when acclimated to freshwater. To our knowledge, no other teleost fish would continue to be 100% ureotelic under these conditions. Third, a substantial fraction of the very high routine metabolic rate of this species is due to the cost of homeostatic regulation against the

Table 2: Measured plasma chemistry of Lake Magadi tilapia after acclimation to various concentrations of lake water (means ± 1 SEM [*N*])

	Control (100%)	10%	1%
Plasma Na^+ (mmol L^{-1})	178.0 ± 6.9 (4)	155.6 ± 2.6 (7)*	153.0 ± 7.4 (4)*
Plasma Cl^- (mmol L^{-1})	163.9 ± 1.4 (7)	148.1 ± 2.4 (10)*	144.3 ± 7.0 (7)*
Plasma osmolality (mosm kg^{-1})	373.8 ± 8.4 (8)	372.2 ± 14.8 (7)	338.0 ± 14.1 (15)*
Plasma total CO_2 (mmol L^{-1})	14.85 ± 2.01 (6)	11.01 ± 2.59 (5)	9.85 ± 3.12 (7)

* $P < 0.05$ relative to 100% control.

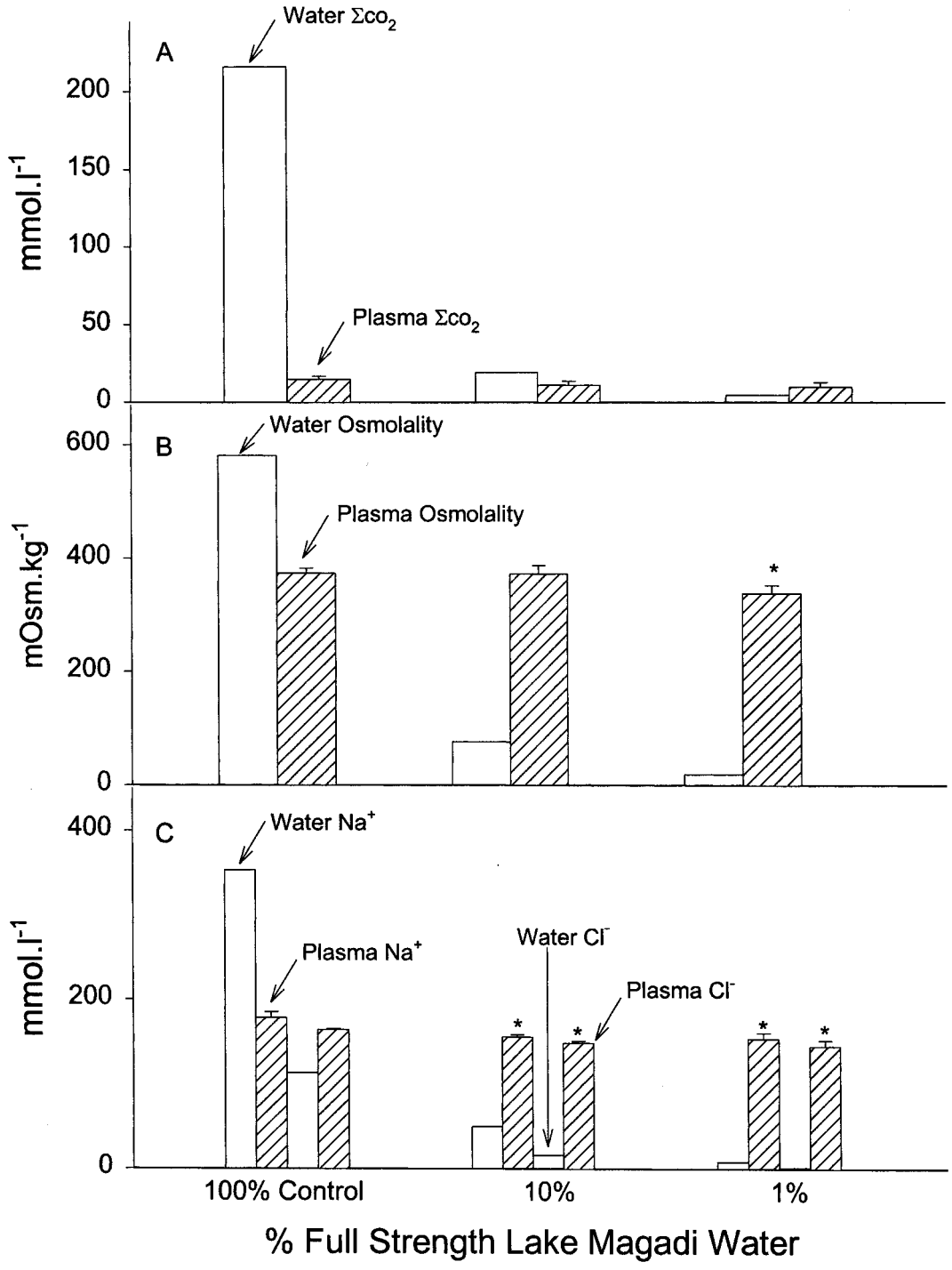

Figure 2. Concentrations of various moieties in lake water (*open bars*) and plasma (*hatched bars*) in Magadi tilapia chronically acclimated in the laboratory to various dilutions of lake water. The data for lake water and plasma are plotted side by side so as to illustrate the gradients. *A*, Total CO_2; *B*, osmolality; *C*, sodium and chloride. Means ± 1 SEM (*N* numbers as in Table 2). Asterisks indicate significant differences ($P < 0.05$) in plasma concentration from the 100% control value.

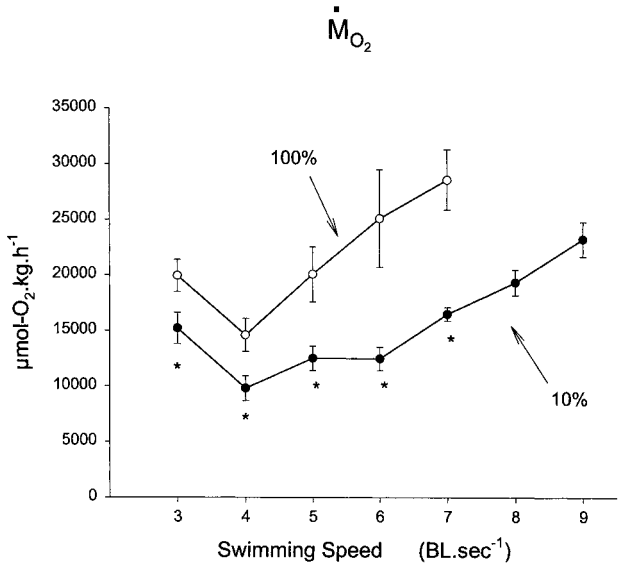

Figure 3. Relationships between aerobic swimming performance (body lengths per second; BL s^{-1}) and the rate of oxygen consumption (Mo_2) in Magadi tilapia chronically acclimated to either 100% (*open circles*) or 10% lake water (*closed circles*) in the laboratory. The fish were swum in their acclimation water. Means ± 1 SEM. In 100% lake water, $N = 12$, 12, 9, 7, and 4 at 3, 4, 5, 6, and 7 BL s^{-1}, respectively. In 10% lake water, $N = 9$ up to 7 BL s^{-1}, 3 at 8 BL s^{-1}, and 2 at 9 BL s^{-1}. Asterisks indicate significant differences ($P < 0.05$) from the 100% value at the same swimming speed.

extreme environmental chemistry. Acclimation to reduced salinity/alkalinity results in a 50%–60% drop in metabolic rate and an increased scope for swimming performance.

Euryhalinity

These results (Table 2) confirm earlier studies (Leatherland et al. 1974; Eddy and Maloiy 1984; Wright et al. 1990) showing that Magadi tilapia living in 100% lake water, an environment with an osmolality equivalent to about half-strength seawater, hyporegulate overall and maintain plasma Na^+, Cl^-, and osmolality at levels similar to those in marine teleosts in full-strength seawater (Holmes and Donaldson 1969). However, they do not confirm reports of very high (Maloiy et al. 1978) or very low (Skadhauge et al. 1980) plasma $Na^+ : Cl^-$ ratios. In opposition to Maina (1990), who reported that the species was essentially stenohaline, these findings also demonstrate that *A. grahami* can be gradually acclimated to extreme environmental dilution right down to essentially freshwater and hyperregulate well under these circumstances, maintaining plasma Na^+, Cl^-, and osmolality at levels (Table 2) typical of freshwater teleosts (Holmes and Donaldson 1969). This freshwater tolerance agrees with some earlier anecdotal reports of survival after transfer to freshwater (Coe 1966; Maloiy et al. 1978) and with Leatherland et al. (1974), who used a similar gradual dilution scheme for exposure to freshwater. The mechanisms for iono- and osmoregulation of the Magadi tilapia in various environments are explored elsewhere (Wood et al. 2002).

Urea Metabolism

The Magadi tilapia is clearly an obligate ureotele. Urea excretion continued in proportion to Mo_2, and there was no ammonia excretion, even when the external environment was diluted to 1% lake water (Fig. 1*B*). Since ammonia is present in the blood plasma at normal teleost levels (Randall et al.1989; Wood et al. 1989, 1994), the absence of ammonia excretion under these conditions, which would otherwise be favorable for ammonia diffusion, is likely due to ammonia scavenging by high GSase levels in the gills (Wood et al. 1989) or by direct incorporation into urea by branchial tissue. While the "traditional" location of the OUC is the liver (Randall et al. 1989), Lindley et al. (1999) have recently reported that direct ureagenesis from ammonia via the OUC also occurs in the white muscle, which represents 60% of the body mass of the Magadi tilapia. It is possible that branchial tissue is similarly ureagenic.

Thus, the Magadi tilapia differs from facultatively ureotelic teleosts such as the gulf toadfish (reviewed by Walsh 1997) and certain catfishes of the Indian subcontinent (reviewed by Saha and Ratha 1998), which switch on ureagenesis in adverse environments but rely primarily on ammoniotelism when environmental conditions are favorable for ammonia-N excretion. In *A. grahami*, ureotelism is "hard wired" into its metabolic pathways, and it does not appear to be capable of switching between the two strategies. However, the rate of urea-N excretion (and presumably production) does appear to vary in approximate proportion to the metabolic rate (Fig. 1). The latter finding is in agreement with an earlier experimental study (Wood et al. 1994), which concluded that the rate of urea-N production is set mainly by the ammonia load to the system, not by acid-base considerations, in opposition to Meijer et al. (1990) and Atkinson (1992). Indeed, had HCO_3^- loading from the environment been the driving force for ureagenesis, urea-N excretion should have virtually ceased after exposure to very dilute lake water (Table 1).

While the source of ammonia may occasionally be elevated environmental ammonia levels (e.g., Wood et al. 1989; P. A. Wilson, C. M. Wood, P. J. Walsh, A. N. Bergman, H. L. Bergman, P. Laurent, and B. N. White, unpublished manuscript), it more normally results from the aerobic metabolism of N-rich fuels such as protein, amino acids, and the unique N-rich polypeptides produced by cyanobacteria (Fay 1983). The normal $N : O_2$ ratio of the Magadi tilapia (around 0.20; Fig. 1*C*) is unusually high relative to most other teleosts (Wood et al. 1994). Based on metabolic theory (van Waarde 1983), this suggests that as much as 70% of routine aerobic metabolism is fueled by N-rich compounds. Thus, when metabolic rate fell

Table 3: Hepatic activities (μmol product min^{-1} g wet $weight^{-1}$) of the ornithine-urea cycle and related enzymes in Lake Magadi tilapia freshly collected from 100% lake water or long-term acclimated in the laboratory to either 100% or 1% lake water (means ± 1 SEM [$N = 6$])

Enzyme	100% (Freshly Collected)	100% (Long-Term Laboratory Acclimated)	1% (Long-Term Laboratory Acclimated)
GSase	7.26 ± 1.22	2.94 ± .63	3.80 ± .50
CPSase III	.044 ± .005	.018 ± .006	.016 ± .003
OTC	3.44 ± .17	.98 ± .14	.99 ± .08
AS/AL	.018 ± .005	.016 ± .003	.005 ± .002[A]
ARG	36.51 ± 1.87	17.04 ± 2.12	7.33 ± .47[A]
GLNase	.43 ± .19	.06 ± .03	.05 ± .05
GDH	19.49 ± 1.22	5.96 ± .62	7.71 ± .55
ALAat	19.35 ± 1.50	7.71 ± 1.37	7.31 ± .42
ASPat	79.43 ± 3.15	28.15 ± 3.01	29.50 ± 2.40
HSI	1.09 ± .07	3.48 ± .27	2.05 ± .15[A]

Note. GSase = glutamine synthetase; CPSase III = carbamoyl phosphate synthetase III; OTC = ornithine-citrulline transcarbamoylase; AS/AL = arginosuccinate synthetase/arginosuccinate lyase; ARG = arginase; GLNase = glutaminase; GDH = glutamate dehydrogenase; ALAat = alanine aminotransferase; ASPat = aspartate amino transferase; HSI = hepatosomatic index. Superscript letters indicate a significant difference between the two long-term acclimation treatments ($P < 0.05$). HSI and activities of all enzymes (except AS/AL) from 100% (long-term acclimated) fish are significantly different from those of 100% (freshly collected) fish.

(Fig. 1*A*) either as a result of long-term holding in the laboratory (as in the 100% lake water control group) or, much more markedly, as a result of acclimation to a more dilute environment (as in the experimental group), urea-N excretion also declined in approximate proportion (Fig. 1*B*) so the $N : O_2$ ratio remained more or less constant (Fig. 1*C*). The persistence of urea excretion with unchanged or increased $N : O_2$ ratio in dilute lake water (Fig. 1*B*, 1*C*) was in accord with the maintenance of plasma total CO_2 levels and, therefore, plasma HCO_3^- (Table 2). Wood et al. (1994) demonstrated that relative urea production (i.e., the $N : O_2$ ratio) is maintained when plasma HCO_3^- is in the normal physiological range and falls only at abnormally low plasma HCO_3^- concentrations in this species.

Routine $M_{urea\text{-}N}$ and Mo_2 values in freshly collected fish were comparable to the very high values reported previously (Randall et al. 1989; Wood et al. 1994; Franklin et al. 1995; Narahara et al. 1996) but declined significantly during long-term laboratory holding without change in the $N : O_2$ ratio (Fig. 1). This probably occurred because the 3.2% daily ration of commercial cichlid food provided during holding did not supply the same energy and N-content as the natural cyanobacterial diet in these voraciously feeding fish. The reduction in activity levels of liver enzymes of N-metabolism during long-term holding (Table 3) was probably of the same origin, though it is unclear why there was a possibly compensating increase in HSI. During chronic acclimation to dilute lake water, $M_{urea\text{-}N}$ and Mo_2 fell to a much greater extent (Fig. 1). The reduction in hepatic AS/AL and ARG activities, as well as in relative HSI, in these fish (Table 3) may again reflect a suppression of production capacity, though neither of these enzymes is traditionally considered to be rate limiting to the OUC (Mommsen and Walsh 1989).

Cost of Living

Even after accounting for the very high temperature of their normal environment, *A. grahami* living in Lake Magadi water exhibit the highest routine metabolic rates ever recorded in teleost fish of this size (Franklin et al. 1995; Narahara et al. 1996). For example, other tilapia that tolerate comparable temperatures exhibit 25%–60% lower values of Mo_2 (see Narahara et al. [1996] for a detailed literature review). In this study, the marked decline in both $M_{urea\text{-}N}$ and $M_{urea\text{-}N}$ accompanying acclimation to dilute lake water indicated a large reduction in metabolic costs, reaching over 60% in 10% lake water and 40%–45% in 50%, 2.5%, and 1% lake water (Fig. 1). This difference between 100% and 10% lake water was also seen clearly during the swimming trials (Fig. 3) as a "loading stress"—that is, a stress that increased the cost of routine maintenance without reducing aerobic capacity, according to the definition of Brett (1958). When this load was reduced by environmental dilution, the fish were able to divert the saved

energy into enhanced swimming performance. Interestingly, exactly the same sort of loading stress was seen in swimming trials with rainbow trout under chronic low pH exposure (Wilson et al. 1994), so this may be a common feature of life in extreme acid-base environments.

Since urea-N production is metabolically costly (minimum of 2 ATP per urea-N), it is of interest to calculate the contribution of $M_{urea\text{-}N}$ to Mo_2. Using a standard $P : O_2$ ratio of 6.0 (Hultman et al. 1967) and data from Figure 1, we estimate that the cost of urea-N synthesis accounts for a small but significant fraction (about 5.5%) of the high routine metabolic rate in the Magadi tilapia, and the decline in $M_{urea\text{-}N}$ accounts for about 4.5% of the fall in Mo_2 seen after acclimation to 10% lake water (Fig. 1). Clearly, the fall in $M_{urea\text{-}N}$ would appear to be largely a correlate rather than a major cause of the fall in Mo_2. This, therefore, raises the question as to what aspect of the lake water environment accounts for the high metabolic rate and its fall upon environmental dilution. Based on physical tables (Riley and Skirrow 1975), the density and viscosity of Lake Magadi water would fall by only about 1.4% and 7%, respectively, upon dilution from 100% to 1% lake water, so it seems unlikely that the cost of ventilation (usually estimated at about 10% of routine Mo_2 in fish; Gilmour 1998) or locomotion is an important factor. Rather, we suspect some aspect of the water chemistry.

While plasma chemistry was not measured in fish acclimated to 50% lake water, it is likely that Na^+ and osmolality levels were similar to those in the external medium at this dilution (Table 1), and, thus, osmoregulatory costs were reduced. However, it is surprising that the reduction in Mo_2 persisted at more extreme dilutions where osmoregulatory costs were likely increasing again and that the apparent cost-saving was actually greatest in 10% lake water (Fig. 1). The analysis of Figure 2 shows that plasma-to-water osmotic and Cl^- gradients actually increased in 10% and 1% lake water relative to those in 100% lake water, while that for Na^+ was slightly reduced. Since the gradients for osmolality and Na^+ were reversed (though not for Cl^-), it is possible that some cost savings occurred because ionoregulation became more efficient under these conditions. However, it is difficult to believe that a reversal and enlargement of the ionic and osmotic gradients can account for a 45%–68% cost savings. Furthermore, studies on other tilapia species indicate that the costs of ionoregulation generally increase as salinity decreases over this range (Farmer and Beamish 1969; Febry and Lutz 1987).

It seems much more likely that the great reduction of the plasma-to-water alkalinity gradient coupled with a reduced base load from drinking—that is, a reduced cost of acid-base regulation—is the dominant explanation. In 10% lake water, external total CO_2 dropped from 216 to 19 mmol L^{-1} (and alkalinity from 378 to 22 meq L^{-1}; Table 1), whereas plasma total CO_2 remained unchanged at 11–15 mmol L^{-1} (Table 2), so passive influx of basic equivalents (or loss of acidic equivalents) across the gills undoubtedly decreased. Recently, we have confirmed the report of Maloiy et al. (1978) that the Magadi tilapia in 100% lake water do drink the medium, and, surprisingly, this continues at a more or less unchanged rate after acclimation to dilute lake water (Wood et al. 2002). Based on a drinking rate of 8 mL kg^{-1} h^{-1} and an intestinal absorption efficiency of 75% (Bergman 2000), intestinal uptake of basic equivalents would also have greatly decreased from about 2,270 to about 130 meq kg^{-1} h^{-1}. Thus, the requirement for net basic equivalent excretion across the gills likely dropped by about 90% between 100% and 10% lake water. Whether or not the branchial $HCO_3^-/CO_3^=$ excretion scheme proposed by Laurent et al. (1995) eventually proves to be the excretion mechanism, this undoubtedly represents a significant metabolic economy. While estimates of the cost of ionoregulation in fish are fairly plentiful (in the order of 2%–20% of resting metabolism, reviewed by Febry and Lutz 1987), to our knowledge, this is one of the first estimates of the cost of acid-base regulation. Its relatively high value, in the order of 50% of routine Mo_2, reflects the extreme alkalinity of the natural environment in Lake Magadi.

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