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Ammonia and urea dynamics in the Lake Magadi tilapia, a ureotelic teleost fish adapted to an extremely alkaline environment

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Abstract. The tilapia *Oreochromis alcalicus grahami*, which thrives under harshly alkaline conditions in Lake Magadi, Kenya, was studied in its natural environment (pH = 10, total CO_2 = 180 mmol/L, osmolality = 525 mOsm/kg, 30–36.5 °C). At rest, this species excretes all nitrogenous waste as urea. This is the first known instance of complete ureotelism in an entirely aquatic teleost fish. Very small 'apparent' ammonia excretion (< 5% of overall N excretion) was attributable to faecal/bacterial production. Ammonia excretion could not be induced by feeding, reduced temperature, or exposure to pH 7. Exhaustive exercise induced only a small efflux of ammonia. Urea output was inhibited completely by pH 7 water and partly by exhaustive exercise, and greatly stimulated by exposure to 500 μmol/L NH_3 (at pH 10). A related species, nominally *Oreochromis nilotica*, which lives in freshwater at circumneutral pH in the same geographic region, excretes 85% ammonia-N and 15% urea-N at pH 7 in the standard teleost fashion. Urea-N efflux increased to 33% upon transfer of *O. nilotica* to pH 10 in freshwater. Urea output in this species was only marginally stimulated by exposure to 500 μmol/L NH_3 (at pH 7). Plasma and white muscle urea levels were 4- to 5-fold higher in *O. a. grahami* than in *O. nilotica*, and plasma levels increased between caudal and cardiac sampling sites, indicating hepatic ureagenesis. Blood pH and P_{NH3} levels, when corrected for sampling artifact, were unusually high in *O. a. grahami*. We hypothesize that complete ureotelism in *O. a. grahami* is an evolutionary response to the problems of excreting ammonia into highly buffered water at pH 10 and/or acid–base balance in this extreme environment.

Acid–base balance; Ammonia; High pH; Ornithine-urea cycle; Tilapia; Urea

The tilapia, *Oreochromis alcalicus grahami* (formerly *Tilapia grahami*), thrives under extreme water quality conditions in some of the remote lakes of the Great Rift Valley of Africa. Lake Magadi (Kenya) is an alkaline 'soda' lake fed by hotsprings and almost

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entirely covered by a solid crust of Na_2CO_3 and $NaHCO_3$. Here, this small cichlid lives in very great numbers in shallow pools of open water formed along the margins of the lake by the hot, alkaline springs; temperatures may reach more than 40 °C, osmolality more than 600 mOsm/kg, and pHs more than 10. The basic biology of the species in Lake Magadi has been described by Coe (1966), and its resistance to extremes of temperature, salinity, and pH documented by Reite, Maloiy and Aasehaug (1974). Previous studies have concentrated on the ionoregulatory and acid–base physiology of the Magadi tilapia (Leatherland *et al.*, 1974; Johansen *et al.*, 1975; Maloiy *et al.*, 1978; Maetz and DeRenzis, 1978; Skadhauge *et al.*, 1980; Eddy *et al.*, 1981; Eddy and Maloiy, 1984). The purpose of our study was to investigate the excretion of nitrogenous wastes under extreme alkaline conditions.

Since the classic work of Smith (1929), it has been known that teleost fish excrete most of their waste nitrogen across the gills as ammonia, together with small amounts of urea and negligible quantities of other substances. There are several reasons to predict that ammonia excretion will be difficult in Lake Magadi water. First, blood-to-water diffusion gradients for NH_3 may be extremely low when the environmental pH is above the pK (9.0–9.5) of the NH_3/NH_4^+ reaction (Randall and Wright, 1989). Second, Lake Magadi water is extremely well buffered. Hence the catalysed CO_2 hydration reaction which normally acidifies the gill water boundary layer in less buffered environments (Wright *et al.*, 1989) cannot facilitate the conversion of NH_3 to NH_4^+ in water passing over the gills. Third, Na^+/NH_4^+ exchange at the gills may be inhibited by high environmental pH (Wright and Wood, 1985). Wright and Wood (1985) found that ammonia excretion was reduced by 80% when rainbow trout were acutely exposed to a pH of 9.5 in freshwater. These considerations led us to question how Lake Magadi tilapia cope with nitrogen excretion under such extreme conditions.

In the first part of our study, we demonstrated that Magadi tilapia have a complete hepatic complement of the ornithine–urea cycle enzymes and excrete large amounts of urea, rather than ammonia (Randall *et al.*, 1989). This is the only known instance of ureotelism in a completely aquatic teleost fish. It presumably represents a de-repression of the ornithine–urea cycle genes so as to facilitate the efflux of nitrogenous waste in an environment where ammonia excretion is difficult or impossible. The purpose of the present study was to follow up this initial observation with a detailed examination of ammonia and urea dynamics in *O. a. grahami* in its natural environment.

Our first objective was to confirm that only urea and no ammonia excretion occurs under resting conditions, and to test whether significant ammonia excretion could be induced by conditions which might be expected to favour it. These included decreased environmental pH (Randall and Wright, 1989), exhaustive exercise (Dobson and Hochachka, 1987; Wright *et al.*, 1988), reduced temperature (McLean and Fraser, 1974), and measurements on actively feeding fish (Brett and Zala, 1975). A second objective was to document the internal levels of ammonia and urea in the animal, and the nature of their distribution between intra- and extracellular compartments. We also wished to determine whether differences in plasma composition indicative of hepatic urea synthesis could be detected across the liver. A third objective was to determine

internal acid–base status, for a previous report of extremely high blood pH in Magadi tilapia (Johansen *et al.*, 1975) would have profound consequences for NH_3 levels within the animal. A final objective was to test whether ammonia loading would stimulate ureagenesis in a fish with an active ornithine–urea cycle; this treatment is reported to have little or no effect on the low levels of urea excretion which other teleost fish normally produce by uricolysis (Olson and Fromm, 1971).

In view of the difficulties in performing physiological experiments on such small (~5 g), wild-caught fish under field conditions, and the resulting variability in the data, simultaneous control experiments were always conducted with animals collected at the same time, in the same batch. We also sampled blood via identical methods from similarly sized rainbow trout (*Salmo gairdneri*) and compared it with blood drawn from larger trout via chronic catheters, in order to gauge what effect the somewhat traumatic sampling technique might have had on measured plasma variables. Finally, in order to test whether the responses observed were unique to Magadi tilapia, or representative of the genus in general, we performed a largely parallel series of experiments on a closely related *Oreochromis* species (nominally *O. nilotica*) which lives only under circumneutral conditions in the same geographic region.

Materials and Methods

Experimental animals. *Oreochromis alcalicus grahami* (Magadi tilapia; 1–12 g; experimental N = 76) were collected by seine in late January and early February, 1988 from Fish Springs Lagoon at the edge of Lake Magadi, Kenya (see Coe, 1966). The fish were held in groups of 10–20 in 20 L plastic buckets filled with Lagoon water. The composition of this water is given in table 1. Note that ammonia levels in freshly collected Lake Magadi water were less than 1 μmol/L, the detection limit of the assay. The water was vigorously aerated and changed twice each day. Nevertheless, the condition of the fish deteriorated rapidly after the first few days in captivity (darkening, sluggishness, and eventual death). For this reason experiments were always performed using animals collected the same day or the previous one. The fish were maintained and experiments performed outdoors on the balcony of the chemistry laboratory of the Magadi Soda Works. Ambient temperature ranged from 30 to 36.5 °C, slightly below the 37 °C at the collection site.

Oreochromis nilotica (Sagana tilapia; 6–15 g, experimental N = 47) were collected once only (February 8, 1988) by seine from the breeding ponds of the Sagana River Tilapia Hatchery. While this species is nominally *O. nilotica*, intense cross-breeding with *O. zillii* and possibly other tilapia species occurs in the hatchery ponds, so its exact taxonomic status is uncertain. The fish were transported to the University of Nairobi where they were held indoors in dechlorinated Nairobi tapwater under similar conditions as for Magadi tilapia. Ambient temperature was 22 °C, close to that at the collection site (23 °C). The composition of Nairobi tapwater was similar though not

TABLE 1

Physical and chemical characteristics of the natural habitat of *O. a. grahami* (Lake Magadi Fish Springs) and *O. nilotica* (Sagana River). The composition of Nairobi tapwater is included for comparison.

	Lake Magadi	Sagana River	Nairobi tapwater
pH	9.98	7.13	7.22
Total CO_2 (mM/L)	184	1.31	0.56
P_{CO_2} (Torr)	0.3	2.6	0.9
P_{O_2} (Torr)	130	–	–
Temp (°C)	37	23	22
Ammonia (μmol/L)	<1	–	3
Na^+ (mmol/L)	342	0.76	0.29
Cl^- (mmol/L)	108	0.31	0.09
K^+ (mmol/L)	2.22	0.03	0.03
Ca^{2+} (mmol/L)	0.65	0.14	0.09
Mg^{2+} (mmol/L)	0.04	0.19	0.06
SO_4^{2-} (mmol/L)	1.65	0.10	0.08
Osmolality (mOsm/kg)	525	<5	<5

identical to that of the Sagana River ponds, and very different from that of Lake Magadi (table 1). All experiments were performed in the three days following collection.

Salmo gairdneri (rainbow trout; 1–6 or 265–325 g) were employed to evaluate the effect of the blood sampling procedure on the measured plasma variables. Trout were obtained from Spring Valley Trout Farm, Petersburg, Ontario, Canada, and held in flowing Hamilton tapwater at 15 °C (see Wright and Wood, 1985, for water chemistry). Blood was sampled from small trout (N = 27) in a manner identical to that for the Kenyan tilapia (see below); large trout (N = 8) were fitted with chronic dorsal aortic catheters (Wright and Wood, 1985) and allowed to recover two days prior to blood sampling via the catheter. In both groups, feeding was suspended for 1 week prior to sampling to minimize any dietary influence on blood acid–base or nitrogen status.

Experiments.

(i) *Fluxes.* Fluxes of nitrogenous wastes were determined routinely by placing individual animals in 250 ml of the appropriate medium and analysing changes in water composition over 3 h periods. Addition/recovery tests demonstrated that significant losses of ammonia (~50%) but not urea occurred from the Lake Magadi water at pH 10 if the experiments were performed in open, aerated beakers. Thus in experiments where ammonia measurements were of critical importance, the fish were placed in 250 ml water within sealed bottles. Air was bubbled through the water having first been passed through an acid trap (1.0 N HCl) to remove any inflowing ammonia. Air exiting the bottle was then passed through a second trap (0.1 N HCl) to collect any volatilized ammonia. Losses of ammonia from this system over 3 h were only 6.4 ± 1.4% (SEM), N = 6) at a water ammonia level (100 μmol/L, pH 10) much higher than the actual levels

encountered in our experiments. Simultaneous controls of untreated fish collected in the same batch were run for all experimental trials. The possibility of ammonia and urea production or removal by bacteria etc. was evaluated by conducting identical fluxes on fish faeces (collected over a 3 h period) and dead fish.

(ii) *Influence of feeding.* Magadi tilapia eat mainly epiphytic blue-green algae growing on rocks in the lagoon (Coe, 1966). To test whether the lack of feeding in captivity prevented ammonia output in our experiments, flux measurements were started at lakeside within 5 min of capture for one batch of fish.

(iii) *Influence of environmental pH.* The effect of environmental pH on nitrogenous waste excretion (3 h flux measurements) was tested by transferring Magadi tilapia from Lake Magadi water at pH 10 to Lake Magadi water adjusted to pH 7 with HCl, and Sagana tilapia from Nairobi tapwater at pH 7 to Nairobi tapwater adjusted to pH 10 with KOH. (Sagana tilapia died within 1 h when transferred to Lake Magadi water at pH 10, so the effects of this transfer on excretion could not be evaluated.) Lake Magadi water was titrated to pH = 7 with HCl and vigorously aerated until the total CO_2 concentration, as measured by a Corning 965 analyzer, decreased from the normal value of ~180 mM/L to less than 1 mM/L. This avoided complications caused by elevated P_{CO_2}.

(iv) *Influence of environmental temperature.* Nitrogenous waste excretion was determined in the standard manner in one batch of Magadi fish, half of which were held overnight and tested at 24 °C, the other half of which were kept at 30 °C.

(v) *Influence of severe exercise.* Magadi fish were chased vigorously with a small stick in 1 L beakers for 10 min, by which time they were near exhaustion. They were then transferred to the flux bottles for measurements of nitrogenous waste output from 0–0.5, 0.5–1, and 1.0–2.0 h post-exercise.

(vi) *Influence of ammonia loading.* Urea output was measured using the open beaker system in Magadi tilapia subjected to elevated NH_3 levels in Lake Magadi water (pH 10), and in Sagana tilapia exposed to elevated NH_3 levels in Nairobi tapwater at pH 7. The objective was to create the same environmental NH_3 levels (approximately 500 μmol/L) in the two experiments after accounting for differences in temperature, ionic strength, and pH. Thus Magadi tilapia were exposed to 500 μmol/L NH_4Cl, and Sagana tilapia to 111 mmol/L NH_4Cl. Urea output was measured over a 3 h control period followed by three successive 3 h periods and a final 8 h period of ammonia loading. In Lake Magadi water at pH 10, measured ammonia levels fell by as much as 50% during each period due to volatilization and entry into the fish, so the loading media was renewed prior to each flux measurement. In Nairobi tapwater at pH 7, this was not a problem, but the same pattern of media replacement was followed for the sake of consistency.

(vii) *Blood and tissue sampling.* Blood samples (15–200 μl/fish) for acid–base, ammonia, and urea analysis were drawn anaerobically from freshly stunned Magadi and Sagana tilapia using 50 or 100 μl gas-tight syringes (Hamilton). The fish were exposed to the air during sampling. Pre-hepatic samples were taken by blind caudal puncture of the haemal arch; post-hepatic samples were taken from the exposed ventricle. Each fish

yielded only one sample, either pre-hepatic or post-hepatic, never both. The sampling generally took several minutes. Caudal samples only were taken in an identical manner from small rainbow trout and compared with those drawn from large trout by catheter. White muscle samples (~300 mg) were excised from freshly killed tilapia, frozen immediately in a dry ice/ethanol mixture at −80 °C, and transported to Canada on dry ice for analysis of ammonia and urea.

Analytical techniques. Ammonia levels in water were determined by the salicylate-hypochlorite method of Verdouw *et al.* (1978), in plasma by a commercial kit (Sigma no. 170-UV) based on the l-glutamic dehydrogenase/NAD method, and in muscle by the same enzymatic method after homogenization and deproteinization (1 : 20) in 8% $HClO_4$, as described by Wright *et al.* (1988). Urea levels in water and plasma were determined routinely by conversion to ammonia using jack-bean urease (Sigma or BDH). As different water qualities affected both the colour reaction of the Verdouw *et al.* (1978) assay and the enzymatic conversion efficiency for urea, standard curves were always constructed using the appropriate media. The low levels of urea in water produced by the Sagana tilapia were measured by the diacetyl monoxime method (Crocker, 1967); this method was also used to measure urea in the $HClO_4$ extracts of muscle tissue. Uric acid excretion to the water was checked by the uricase/phospho-tungstate method of Henry *et al.* (1957).

Blood acid–base status was measured on samples from individual animals, but for plasma ammonia and urea determinations it was generally necessary to pool blood from 2–6 fish to obtain adequate volume. Blood pH was measured first using a Radiometer E5021 micro-electrode assembly and pHM 72 or 82 meter thermostatted to the experimental temperature; the sample was then recovered from the electrode and assayed for total CO_2 using a Corning 965 analyzer. P_{CO_2} and HCO_3^- levels were calculated by the Henderson-Hasselbalch equation using values of pK′ and αCO_2 appropriate to the temperature and ionic strength of the samples, from Boutilier *et al.* (1984). Values of NH_3, NH_4^+, and P_{NH_3} were similarly calculated using appropriate constants from Cameron and Heisler (1983).

Values are reported as means ± 1 SEM (N) where N represents the number of fish (fluxes) or the number of sample pools (blood data). The significance of differences was evaluated using Student's two-tailed *t*-test, paired or unpaired design as appropriate.

Results

Resting fluxes. Magadi tilapia (*O. a. grahami*) excreted enormous amounts of urea in Lake Magadi water at pH 10. Typical values were around 5000–10000 μmol-N/kg/h (table 2), but there was considerable variation in absolute rates amongst different batches of fish. Note, for example, the much higher control rates in fig. 2 and table 3. This variability presumably reflected the problem of working with wild-caught, voraciously feeding fish at very high temperature. Measured ammonia appearance in the

TABLE 2

Changes in ammonia-N and urea-N concentrations in 250 ml water over 3 h created by *O. a. grahami* (3.68 ± 0.40 g) in Lake Magadi water, and *O. nilotica* (11.44 ± 0.54 g) in Nairobi tapwater, and calculated flux rates, compared with those created by dead fish or faeces (3 h production). Means ± 1 SEM (N).

	Ammonia-N		Urea-N	
	Concentration (μmol/L)	Flux (μmol/kg/h)	Concentration (μmol/L)	Flux (μmol/kg/h)
O. a. grahami				
Live fish	15.5	298	391.0	7771
(N = 6)	±4.5	±75	±39.0	±849
Dead fish	20.8	687	9.0*	269*
(N = 3)	±9.6	±367	±10.4	±331
Faeces	15.8	357	4.2*	95*
(N = 4–5)	±4.6	±104	±45.0	±1019
O. nilotica				
Live fish	113.1	788	20.0	152
(N = 12)	±10.6	±78	±2.4	±19
Faeces	3.5*	25*	2.0*	14*
(N = 6)	±3.8	±27	±0.8	±6

* Significantly different ($P < 0.05$) from corresponding value for live fish.

water was always less than 5% of urea on a nitrogen basis, and sometimes not significantly different from zero. Control experiments with dead fish and faeces demonstrated that these low levels of ammonia production could be entirely attributed to bacterial action (table 2). There was also no measurable uric acid excretion (detection limit ~200 μmol-N/kg/h). Thus *O. a. grahami* appeared to be entirely ureotelic; there was no evidence of any ammonia excretion at rest.

Sagana tilapia (*O. nilotica*) excreted about 85% ammonia-N and 15% urea-N in Nairobi tapwater at pH 7 (table 2). Uric acid excretion was again undetectable. Absolute nitrogen efflux rates were less than 30% of those in Magadi tilapia, a difference at least partially due to the 10 °C lower temperature. Tests with faeces of Sagana tilapia demonstrated negligible production of urea and ammonia (table 2). Thus *O. nilotica* appeared to be largely ammoniotelic in the standard teleost pattern, excreting only small amounts of urea at rest.

Influence of feeding. The lack of ammonia excretion in Magadi tilapia was not due to food deprivation. In a batch of 5 fish for which a flux measurement was started at lakeside within 5 min of capture, ammonia excretion was only 178.6 ± 14.7 μmol-N/kg/h, comparable to that of faeces alone (*cf.* table 2). Urea excretion in these same fish was 5520.4 ± 2829.8 μmol-N/kg/h.

Influence of environmental pH. An environment of lower pH should both enhance blood-to-water NH_3 diffusion gradients and favour Na^+/NH_4^+ exchange. Acute transfer of Magadi tilapia to reduced pH 7 in Lake Magadi water was performed to test whether ammonia excretion could be induced under these more favourable conditions. This did not occur; indeed the very low levels of ammonia excretion were significantly reduced (fig. 1A), probably due to an inhibitory effect of the altered pH on microbial activity. Surprisingly, neutral pH also reduced urea output by the fish to a level not significantly different from zero (fig. 1A). The fish tended to raise water pH and HCO_3^- levels during the 3 h fluxes at pH = 7, indicating an output of base, whereas there were no changes in water pH during fluxes in Lake Magadi water at pH 10.

The reciprocal transfer of Sagana tilapia to pH 10 in Nairobi tapwater did not abolish ammonia excretion but actually raised it non-significantly (fig. 1B). Moreover, this

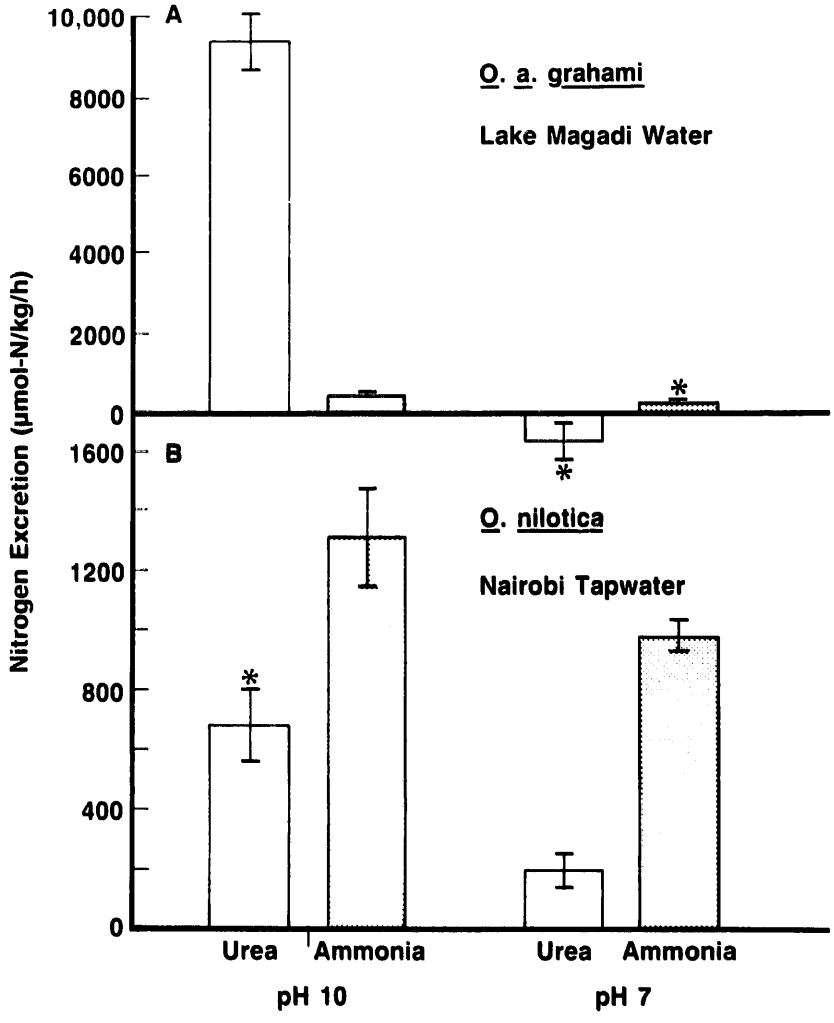

Fig. 1. The effect on urea-N and ammonia-N excretion rates of acute transfer of (A) *O. a. grahami* from pH 10 (control, N = 12) to pH 7 (N = 12) in Lake Magadi water at 32 °C, and of (B) *O. nilotica* from pH 7 (control, N = 6) to pH 10 (N = 6) in Nairobi tapwater at 22 °C. Means ± 1 SEM. Asterisks indicate significant differences ($P < 0.05$) from corresponding value at control pH. Note the difference in scale between (A) and (B).

TABLE 3

Effects of a reduction in ambient temperature on the excretion rates of urea-N and ammonia-N by *O. a. grahami* in Lake Magadi water. Means ± 1 SEM (N).

	Ammonia-N (μmol/kg/h)	Urea-N (μmol/kg/h)
30 °C	272	22,272
	±90	±3,215
	(5)	(5)
24 °C	247	10,593*
	±34	±1,192
	(6)	(6)
Q10	-	3.44

* Significantly different ($P < 0.05$) from corresponding value at 30 °C.

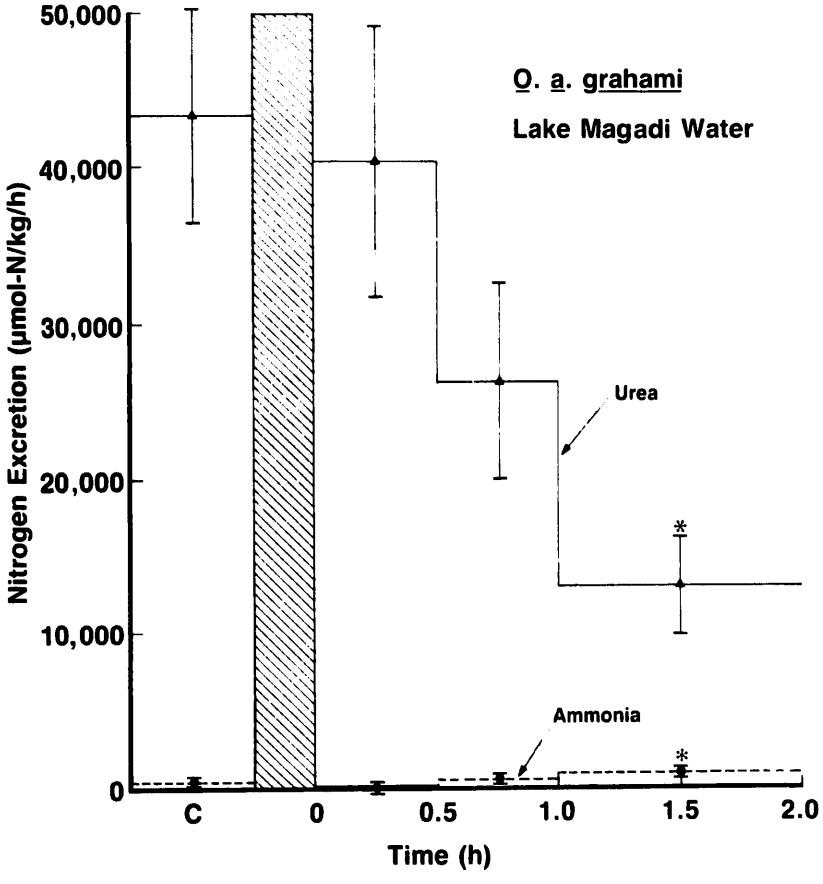

Fig. 2. The influence of 10 min of severe exercise (chasing, at bar) on ammonia-N and urea-N excretion rates in *O. a. grahami* in Lake Magadi water (pH = 10) at 36.5 °C (N = 6). There were no significant changes over time in the excretion rates of resting control fish (N = 6) run simultaneously. The mean control rates are shown (C). Means ± 1 SEM. Asterisks indicate significant differences ($P < 0.05$) from simultaneously measured flux in control group.

transfer significantly increased urea efflux on both an absolute and relative basis, so that it now accounted for 33% of nitrogen excretion in this species. While Sagana tilapia remained vigorous for at least 5 h in Nairobi tapwater at pH 10, they died within 1 h when transferred to Lake Magadi water at pH 10. The fish tended to lower water pH during the 3 h fluxes at pH 10 in Nairobi tapwater.

Influence of environmental temperature. A 6 °C reduction in temperature reduced urea excretion by more than 50% in Magadi tilapia, yielding a Q10 of 3.44 (table 3). The very low ammonia excretion remained unchanged at a level attributable to bacterial action.

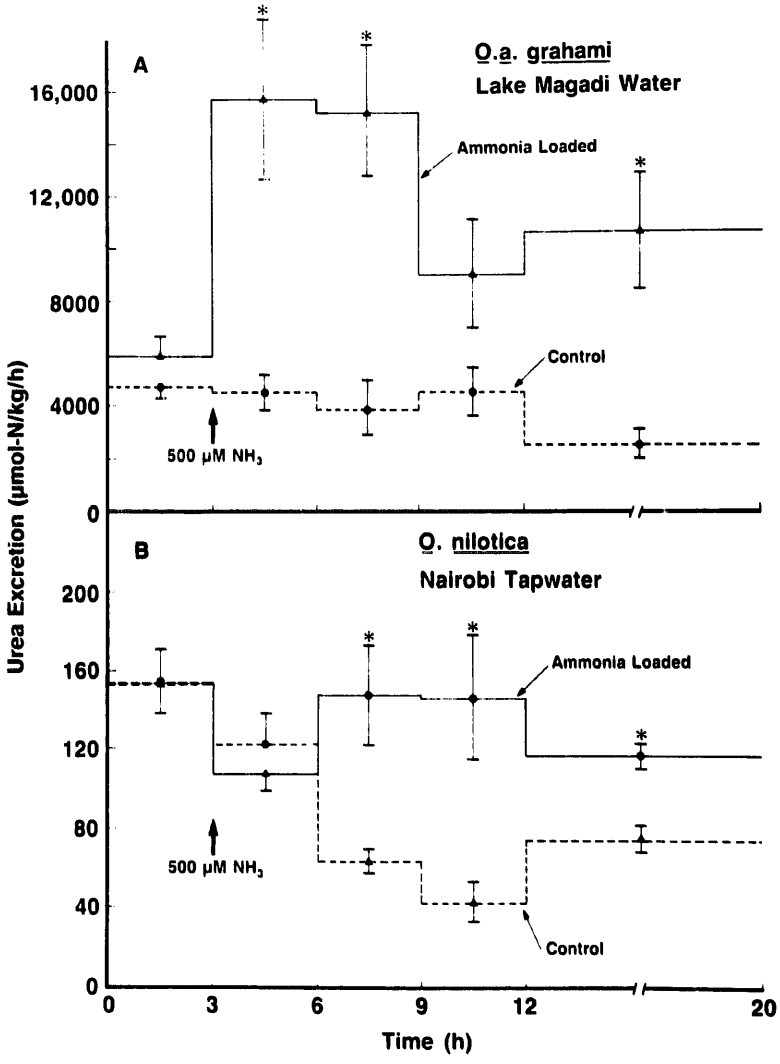

Fig. 3. The influence of ammonia loading on urea-N excretion rates in (A) *O. a. grahami* in Lake Magadi water (pH 10) at 30–34 °C and (B) *O. nilotica* in Nairobi tapwater (pH 7) at 22 °C. After an initial 3 h pre-exposure period, ammonia loaded fish were exposed to ~500 μmol/L NH_3 in the water for the remainder of the experiment; control fish remained in their normal medium. Means ± 1 SEM (N = 11–14 initially in each group, declining to 3–8 by 20 h). Asterisks indicate significant differences ($P < 0.05$) from simultaneously measured flux in control group. Note the difference in scale between (A) and (B).

Influence of severe exercise. Exercise was employed as a treatment which might favour ammonia excretion by increasing internal ammonia production rate. Ammonia output tripled in the two hour period after strenuous exercise, reaching 851.9 ± 272.1 (6) μmol-N/kg/h (fig. 2), the highest value seen in any experiment. While this may represent a true excretion of ammonia by the fish, we cannot exclude the possibility of increased defaecation and bacterial production after exhaustive activity. Ammonia still comprised only 6% of total nitrogen excretion. Over this same time course, urea efflux fell markedly reaching a level only 30% of that at rest by 1–2 h (fig. 2). Note, however, that resting urea excretion rates were unusually high in this particular batch of fish. There were no significant changes in ammonia or urea efflux in simultaneous controls over the same time course.

Influence of ammonia loading. The purpose of raising water NH_3 levels was to elevate internal NH_3 levels and thereby test the capacity of the fish for urea production. Exposure of Magadi tilapia to 500 μmol/L NH_3 in Lake Magadi water at pH 10 induced a 3-fold increase in urea excretion within the first 3 h (fig. 3A). The absolute level of this stimulation tended to decline with time, but so did the rates of urea output in the control group. Thus at 12–20 h of exposure, urea output remained 3-fold higher in the ammonia loaded fish than in the controls.

Exposure of Sagana tilapia to 500 μmol/L NH_3 in Nairobi tapwater at pH 7 caused no change in urea excretion over 20 h (fig. 3B). However, as urea efflux in the control group declined by about 50% over the same period, there appeared to have been a small relative stimulation of urea excretion in experimental fish as a result of ammonia loading. Efflux rates in the two groups were significantly different from 6 to 20 h. However, the absolute stimulation (at most ~110 μmol-N/kg/h) was only a small fraction of that in the Magadi tilapia (~11000 μmol-N/kg/h).

Internal ammonia and urea levels. Plasma urea levels were 4- to 5-fold higher in Magadi tilapia than in Sagana tilapia, whereas plasma ammonia levels were comparable in the two species (table 4). There was at least 10 times as much nitrogen carried as urea than as ammonia in the plasma of *O. a. grahami*, while in *O. nilotica* this difference was only 2-fold. In Magadi tilapia there was a significant increase in plasma urea levels between the pre-hepatic (caudal haemal arch) and post-hepatic (ventricle) sampling sites, suggesting a release of urea from the liver, but no change in plasma ammonia. Conversely, in Sagana tilapia, there was no difference in plasma urea levels between pre- and post-hepatic sites, but a significant reduction in plasma ammonia levels, suggesting an uptake of ammonia by the liver. Note that the pre-hepatic and post-hepatic samples were not paired, as each fish yielded only one sample, and samples from 2–6 fish were pooled for each assay.

Intracellular ammonia levels in the white muscle were similar in the two species, and about 10-fold higher than plasma levels (table 4). Intracellular urea concentrations were approximately equal to the respective plasma urea concentrations in each species.

TABLE 4

Concentrations of ammonia-N and urea-N in pre-hepatic (caudal) and post-hepatic (cardiac) blood plasma and white muscle in *O. a. grahami* in Lake Magadi water (32 °C, pH 10) and *O. nilotica* in Nairobi tapwater (22 °C, pH 7). Means ± 1 SEM (N).

	Ammonia-N (μmol/L)	Urea-N (μmol/L)
O. a. grahami		
Caudal plasma	770	10,520
	± 103	± 1,060
	(5)	(5)
Cardiac plasma	902	17,060*
	± 169	± 2,580
	(5)	(5)
White muscle**	9279*	14,676
	± 848	± 974
	(5)	(6)
O. nilotica		
Caudal plasma	1043	2,320
	± 189	± 220
	(6)	(6)
Cardiac plasma	396*	2,000
	± 80	± 80
	(4)	(4)
White muscle**	7673*	1,758
	± 331	± 238
	(6)	(6)

* Significantly different ($P < 0.05$) from corresponding value for caudal plasma in this species. ** Assuming an intracellular fluid volume of 85.5% (Wright *et al.*, 1988) of measured muscle water content (*O. a. grahami* = 756.3 ± 8.5 ml/kg; *O. nilotica* = 812.3 ± 8.3 ml/kg).

TABLE 5

Blood acid–base status, as determined from caudal blood samples, of *O. a. grahami* in Lake Magadi water (32 °C, pH 10) and *O. nilotica* in Nairobi tapwater (22 °C, pH 7). Means ± SEM (N).

	O. a. grahami (32 °C, Lake Magadi)	*O. nilotica* (22 °C, Nairobi tapwater)
pH	7.58	7.66
	± 0.06	± 0.03
	(5)	(5)
Total CO_2 (mmol/L)	9.74	6.48
	± 1.15	± 0.62
	(9)	(7)
P_{CO_2} (Torr)	7.14	3.47
	± 0.91	± 0.49
	(5)	(7)

Blood acid–base status. Blood pHs measured after caudal puncture from freshly collected Magadi tilapia at 32 °C were approximately 7.6, comparable to those measured in Sagana tilapia at 22 °C (table 5). However blood total CO_2 concentrations were about 50% higher, and calculated P_{CO_2} levels about 100% higher in the Magadi fish. Much lower blood pH and total CO_2 levels were measured in a group of Magadi tilapia which had been held in captivity in Lake Magadi water for several days, suggestive of metabolic acidosis.

Tests on possible artifacts due to blood sampling using rainbow trout. Blood drawn via arterial catheter from large rainbow trout contained only about 30% of the plasma ammonia levels seen in the blood of small trout sampled by caudal puncture (table 6). However, plasma urea levels were virtually identical by the two sampling procedures. This suggests that the relatively slow, traumatic sampling technique employed for the Kenyan fish elevated plasma ammonia levels considerably above normal *in vivo* values, but produced fairly realistic estimates of circulating urea concentrations (table 4).

Blood sampled by caudal puncture from small trout exhibited markedly depressed pH and elevated P_{CO_2} levels in comparison to that sampled by arterial catheter from large trout (table 6). Total CO_2 concentration was virtually unaltered. This indicates that retention of CO_2 and/or conversion of HCO_3^- to CO_2 occurred in the blood 'in a closed system', not surprising in view of the air exposure of the fish during sampling. In turn,

TABLE 6

A comparison of acid–base status, plasma ammonia-N, and plasma urea-N concentrations in the blood of *S. gairdneri* sampled by caudal puncture from freshly stunned, air-exposed small fish *vs* those in blood sampled by an indwelling arterial catheter from undisturbed large fish. Means ± 1 SEM (N).

	Caudal puncture (small fish)	Arterial catheter (large fish)
pH	7.22 ± 0.02 (15)	7.76* ± 0.03 (8)
Total CO_2 (mmol/L)	6.88 ± 0.38 (12)	6.78 ± 0.46 (8)
P_{CO_2} (Torr)	9.91 ± 0.91 (12)	2.72* ± 0.33 (8)
Ammonia-N (μmol/L)	682 ± 45 (5)	196* ± 27 (5)
Urea-N (μmol/L)	4240 ± 600 (5)	4400 ± 1220 (5)

* Significantly different ($P < 0.05$) from corresponding value by caudal puncture in small fish.

this suggests that the data collected on Kenyan fish (table 5) considerably overestimated P_{CO_2} and thereby underestimated pH, whereas the total CO_2 levels were fairly representative of *in vivo* values.

Discussion

Complete ureotelism in Magadi tilapia. *Oreochromis alcalicus grahami* has significant levels of the ornithine–urea cycle enzymes in its liver (Randall *et al.*, 1989). The substantial increase in plasma urea concentration between pre- and post-hepatic sampling sites (table 4), together with the very high rates of urea excretion to the environment measured *in vivo*, indicate that this pathway is extremely active in the intact animal. Indeed, *O. a. grahami* appears to be unique amongst aquatic teleost fish in excreting all nitrogenous waste as urea. The present experiments confirm that no ammonia is excreted under resting conditions. Even when the environmental conditions are manipulated so as to favour ammonia efflux and/or to inhibit urea efflux, ammonia excretion remains negligible. The absolute rates of urea output (typically 5000–10 000 μmol-N/kg/h) are undoubtedly the highest values for nitrogen excretion ever recorded for resting fish. However, it must be remembered that Magadi tilapia eat a diet which is composed largely of blue-green algae (Coe, 1966), and which therefore may be extremely rich in nitrogen. The ability of Cyanophytes to 'fix' atmospheric nitrogen as ammonia, and to store large amounts of nitrogen in a unique polypeptide (multi-L-arginyl poly-L-aspartic acid) is well known (Fay, 1983). Furthermore, Magadi tilapia operate at temperatures close to that of the mammalian body and likely have very high metabolic rates. Their urea excretion rates are actually very similar to those reported for small rodents (*e.g.* Schmidt-Nielsen, 1955; Halperin *et al.*, 1986).

The only other aquatic teleost capable of significant hepatic ureogenesis via the ornithine–urea cycle is the toadfish (*Opsanus sp.*); however, this genus is reported to excrete mainly ammonia and virtually no urea *in vivo* (Read, 1971; T.P. Mommsen and P.J. Walsh, personal communication), so the significance of this capacity in the intact toadfish remains unclear. In the majority of aquatic teleosts, ornithine–urea cycle activities are low or undetectable (*e.g.* Huggins *et al.*, 1969; T.P. Mommsen and P.J. Walsh, personal communication). Nevertheless, most aquatic teleosts produce small but significant amounts of urea (*e.g.* Smith, 1929; Olson and Fromm, 1971; Sayer and Davenport, 1987), apparently via uricolysis or the degradation of dietary arginine (Forster and Goldstein, 1969; Vellas and Serfarty, 1974). One or both of these pathways is probably responsible for the low rate of urea production measured in *O. nilotica*. Indeed, the activity of hepatic allantoicase, a key enzyme of uricolysis, is higher in Sagana tilapia than in Magadi tilapia (Randall *et al.*, 1989). Several amphibious teleosts are capable of considerable urea excretion, especially during air exposure, and at least one such species (*Heteropneustes fossilis*) exhibits an active ornithine–urea cycle in its liver and kidney (Saha and Ratha, 1987). These authors have proposed that a derepression of the ornithine–urea cycle genes has occurred as an adaptation to dehydrating environments.

We suggest that the same de-repression has occurred in *O. a. grahami*, though for two very different adaptive reasons. The first is that ammonia excretion may be difficult or impossible in Lake Magadi water at pH 10, as outlined in the Introduction and below. The second is that urea production may be critical for acid–base balance in a fish surviving in a highly alkaline environment rich in CO_3^{2-} and HCO_3^-. Maetz and De Renzis (1978) and Eddy *et al.* (1981) have demonstrated that there are large electrochemical gradients for the entry of HCO_3^-, CO_3^{2-}, and OH^- ions across the gills into Magadi tilapia. In addition, the drinking rates measured by Maloiy *et al.* (1978) and Skadhauge *et al.* (1980) in this species in Lake Magadi water would bring approximately 5000 μequiv/kg/h of basic equivalents into the intestine. Some or all of this base load may be absorbed into the blood, or alternately neutralized by acidic equivalent secretion, which would have the same net effect on whole animal acid–base balance. Recently, Atkinson and Camien (1982) and Atkinson and Bourke (1987) have argued that urea synthesis serves as a mechanism for the removal of metabolic base, with two molecules of HCO_3^- consumed for each molecule of urea produced. This view of the role of urea production in acid–base balance in mammals remains controversial (see Walser, 1986, and Halperin *et al.*, 1986 for opposing interpretations). However, if correct, it would indicate that the high rates of urea production in Magadi tilapia could be important for the maintenance of acid–base homeostasis. NH_4^+ or other inorganic N contained in the blue-green algae diet (Fay, 1983) or produced by bacterial hydrolysis of urea in the intestine could provide the N source for this process. In this regard it is interesting that faeces from Magadi tilapia produced small but detectable amounts of ammonia (table 2).

In mammals urea production via the ornithine–urea cycle is inhibited by acidosis and stimulated by alkalosis (Bean and Atkinson, 1984; Kashiwagura *et al.*, 1984). This sensitivity of ureagenesis to pH is one possible explanation for the immediate abolition of urea excretion caused by exposure of Magadi tilapia to pH 7 (fig. 1A). Alternately the urea excretion mechanism itself may be sensitive to water pH. Post-exercise acidosis (Wright *et al.*, 1988) could account for the marked inhibition of urea output seen after strenuous activity (fig. 2). The shunting of blood flow away from the splanchnic circulation after exercise (Randall and Daxboeck, 1982) also may have impeded hepatic ureagenesis. Clearly these suggestions are highly speculative, and further research is needed before any firm conclusions can be drawn.

The dramatic increase in urea efflux induced by ammonia loading (fig. 3A) indicated substantial reserve capacity in the ureogenic enzyme system of *O. a. grahami*. This ability to convert absorbed ammonia to urea is characteristic of animals with an active ornithine–urea cycle (Campbell, 1973), and correlates with our observations of an unusually high ammonia tolerance of Magadi tilapia (unpublished results). In contrast, when *O. nilotica* were subjected to similar ammonia loading (fig. 3B), they were capable of only a marginal increase in urea output characteristic of standard teleosts which synthesize urea by uricolysis (Olson and Fromm, 1971).

The absence of ammonia excretion in Magadi tilapia. Branchial ammonia excretion at pH 10 is undoubtedly difficult due to the absence of diffusion trapping of NH_3 (Randall

and Wright, 1989) and interference with Na^+/NH_4^+ exchange (Wright and Wood, 1985). The results with Sagana tilapia suggest it is not impossible since *O. nilotica* were able to sustain ammonia output when acutely exposed to pH 10 (fig. 1B) in Nairobi tapwater. However, there was a massive difference in buffer capacity between Nairobi tapwater at pH 10, and Lake Magadi water at pH 10, due to the high HCO_3^- and CO_3^{2-} levels of the latter (table 1). Sagana tilapia were probably able to acidify the boundary layer next to the gill surface via CO_2 or acid efflux, as in the freshwater rainbow trout (Wright *et al.*, 1989). This option, however, is not available to Magadi tilapia in their highly buffered environment. This may have been an important factor in the selection for ureotelism in Magadi tilapia.

With the possible exception of strenuous exercise (fig. 2), none of the treatments reported to increase ammonia excretion in other teleosts had any effect in inducing ammonia excretion in *O. a. grahami*. The absence of ammonia excretion in Lake Magadi water at pH 7 was particularly surprising, for the HCO_3^-/CO_3^{2-} buffering of the environment was removed, and diffusion trapping of NH_3 should have then been possible. Furthermore, Na^+/NH_4^+ exchange should have been facilitated because Na^+ influx was increased by exposure to pH 7 (unpublished results). Four possible explanations may be considered.

The first is that there is no ammonia in the blood in the intact animal. While plasma ammonia levels were probably overestimated by the traumatic sampling technique (table 6), it is most unlikely that none was present. Indeed, plasma P_{NH_3} was probably very high, as outlined below. The second is that the gills are impermeable to ammonia. This seems to be ruled out by the results of the ammonia loading experiment (fig. 3A), for environmental ammonia was apparently absorbed and converted to urea by the fish. The third is that there is an ammonia uptake mechanism on the gills which short-circuits any ammonia efflux. This might be particularly advantageous for an organism which normally depends on NH_4^+ recycling and ureogenesis for acid–base homeostasis, as discussed earlier. The final possibility is that the gills may convert any ammonia which enters via the blood to another compound, such as glutamine or urea, so that ammonia efflux to the water cannot occur. In this regard, we have detected higher glutamine synthetase and ornithine transcarbamylase activities in gill tissue of Magadi tilapia compared to Sagana tilapia (unpublished results). These latter two possibilities certainly deserve further investigation.

Ammonia and pH levels in vivo in Magadi tilapia. The tests on rainbow trout (table 6) suggested that the slow blood sampling method employed on the very small Kenyan tilapia underestimated plasma pH and overestimated plasma ammonia. The former was due to CO_2 retention and/or conversion of HCO_3^- to CO_2 during air exposure, and the latter was probably caused by ammonia production via deamination of adenylates during the simultaneous anoxia (Dobson and Hochachka, 1987).

We have estimated the true *in vivo* plasma levels of ammonia in Kenyan tilapia using the following reasoning. The measured white muscle ammonia levels (table 4) are assumed to be correct, for the tissue was frozen at $-80\,^{\circ}C$ within seconds of sacrifice.

This is further supported by the fact that white muscle urea levels were equal to plasma urea levels (table 4), as expected for the passive distribution of this neutral molecule. In other teleost fish, ammonia is distributed between the blood plasma (extracellular fluid) and intracellular fluid of white muscle according to the membrane potential, rather than the pHe–pHi gradient; this reflects a significant cell membrane permeability to NH_4^+ (Wright *et al.*, 1988; Wright and Wood, 1988). Assuming the same situation in the Kenyan tilapia, and a normal white muscle membrane potential of – 83 mV (Wright *et al.*, 1988) then the true *in vivo* values for arterial plasma ammonia, as predicted from the measured muscle levels, would have been 394 μmol/L and 293 μmol/L for *O. a. grahami* and *O. nilotica*, respectively. These are 30–50% of the values measured by caudal puncture (table 4) and very similar to those which could be derived using a correction factor (30%) based on the rainbow trout data (table 6), thereby re-inforcing the validity of this extrapolation.

Previous blood acid–base data on Magadi tilapia are fragmentary. Johansen *et al.* (1975) measured a mean pH of ~8.05 ± 0.10 at 35 °C in about 30 samples drawn by cardiac puncture from freshly collected fish at lakeside. Total CO_2 and P_{CO_2} levels were not measured.As in the present study, Johansen *et al.* (1975) reported that captivity induced acidosis. Maetz and De Renzis (1978) measured caudal puncture pHs of 7.33 and 7.69, and total CO_2s of 6.7 and 7.3 mmol/L, in two Magadi tilapia kept for 7 days in artificial Lake Magadi water at 25 °C. The present data support the conclusion of Johansen *et al.* (1975) that blood pH is abnormally high in *O. a. grahami* in Lake Magadi. On the basis of the trout tests, the measured pHs (table 5) were recalculated to *in vivo* estimates (table 7) using the measured total CO_2 levels and an arbitrarily assumed Pa_{CO_2} = 2 Torr. If anything, this Pa_{CO_2} is on the high side as Lake Magadi water has a low P_{CO_2} (table 1) and should serve as a 'CO_2 vacuum' at pH 10. Based on these assumptions, pHa should be at least 8.14 in *O. a. grahami* at 32 °C (table 7). This is about 0.5–0.6 units above the standard fish values normally measured at this

TABLE 7

Estimated arterial plasma pH, NH_3, and NH_4^+ levels *in vivo* for *O. a. grahami* in Lake Magadi water (32 °C, pH 10) and *O. nilotica* in Nairobi tapwater (22 °C, pH 7). Measured values for *S. gairdneri* in Hamilton tapwater (15 °C, pH 8, large fish sampled by catheter) are included for comparison.

	O. a. grahami (32 °C, Lake Magadi)	*O. nilotica* (22 °C, Nairobi tapwater)	*S. gairdneri* (15 °C, Hamilton tapwater)
pHa	8.14*	7.89*	7.76
NH_4^+ (μmol/L)	358**	285**	193
NH_3 (μmol/L)	36**	8**	3
P_{NH_3} (μTorr)	1171**	199**	58

* Recalculated from data of Table 5, assuming *in vivo* Pa_{CO_2} = 2 Torr at unchanged total CO_2 concentration.
** Estimated from white muscle ammonia levels of Table 4 (see text for details), and appropriate pK and αNH_3 values from Cameron and Heisler (1983).

temperature or predictable from alpha-stat and relative alkalinity relationships (Cameron, 1984). It of course reflects an unusually high plasma HCO_3^- concentration ($\sim$9.5 mmol/L), not surprising in view of the large gradient for entry of HCO_3^-, CO_3^{2-}, and OH^- from the environment. Using the same assumptions, the estimated pHa in *O. nilotica* at 22 °C is 7.89, which conforms to measured values in other species and standard theory.

The above estimates of *in vivo* pHa and plasma ammonia levels have been used to partition the latter into NH_3 and NH_4^+ components (table 7). This analysis illustrates that Magadi tilapia have remarkably high plasma NH_3 and P_{NH_3} levels, due to a combination of the high pHa, and the low values of pK and αNH_3 at high ambient temperature. Intracellular NH_3 and P_{NH_3} levels are probably similarly elevated (Wright *et al.*, 1988; Wright and Wood, 1988). The tissues must be extremely resistant to NH_3 poisoning, a conclusion confirmed by toxicity tests (unpublished results). In light of these very high internal P_{NH_3} levels, the absence of ammonia excretion is even more remarkable in this most unusual ureotelic fish.

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