Urea excretion as a strategy for survival in a fish living in a very alkaline environment

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Ammonia is toxic to all vertebrates. It can be converted to the less toxic urea, but this is a metabolically expensive process[1] found only in terrestrial vertebrates that cannot readily excrete ammonia and marine fish that use urea as an osmotic filler. Freshwater fish mostly excrete ammonia[2,3] with only a small quantity of urea[4,5]. It seems the ornithine cycle for urea production has been suppressed in all freshwater teleosts[6] except for some airbreathers which, when exposed to air, increase urea synthesis via the cycle[7]. Here we show that the tilapia fish *Oreochromis alcalicus grahami*, the only fish living in Lake Magadi, an alkaline soda lake (pH = 9.6–10) in the Kenyan Rift Valley, excretes exclusively urea and has ornithine–urea cycle enzymes in its liver. A closely related species that lives in water at pH 7.1 lacks these enzymes and excretes mainly ammonia with small amounts of urea produced via uricolysis[4]. It dies within 60 min when placed in water from Lake Magadi. We suggest that urea production via the ornithine–urea cycle permits *O. a. grahami* to survive the very alkaline conditions in Lake Magadi.

Ammonia excretion by rainbow trout is impaired under alkaline conditions (pH > 9.5) (refs 8, 9). Trout are incapable of increasing urea production in the face of elevated ammonia levels, even via uricolysis[3] and die when exposed to extremely alkaline conditions. The teleost, *Oreochromis alcalicus grahami* survives in very alkaline (pH 9.6–10.0) volcanic spring-fed lagoons on the margins of Lake Magadi[10], because of its unique ability to excrete all nitrogenous waste as urea (Fig. 1*a*). These fish excrete no ammonia and no uric acid. Urea excretion rates were very high, probably reflecting the voracious feeding habits of these fish living at high temperatures (lagoon = 37 °C, laboratory = 30–34 °C). These rates declined by about 50% over 20 h, probably due to confinement stress and/or starvation. In contrast, Sagana river tilapia, *Oreochromis* sp., (probably *nilotica*, but of uncertain species because of intense cross-breeding), excretes most of its nitrogenous waste as ammonia (>80%) and only small amounts as urea (Fig. 1*b*), like other freshwater teleosts. These rates also declined by about 50% over 20 h, but total nitrogen excretion rates were only about 30% of that in *O. a. grahami*, reflecting the lower temperature (23 °C) of the Sagana River fish. In parallel to the differences in urea excretion, plasma urea levels were 4–5-fold higher in Magadi than Sagana tilapia, whereas plasma ammonia levels were similar (Table 1).

The liver of *O. a. grahami* contains significant levels of the ornithine–urea cycle enzymes (Table 1) in conjunction with considerable glutamine synthetase activity. By way of contrast and as in most teleost fish[12], *O. nilotica* from the Sagana river has only low or undetectable levels of the ornithine–urea cycle enzymes but much higher levels of a key uricolytic enzyme, allantoicase, compared to *O. a. grahami*.

The preferred substrate in the carbamoyl phosphate synthetase (CPS) assay was glutamine, subsequent addition of ammonia did not increase measurable enzyme activity. We conclude that *O. a. grahami* has both mitochondrial (CPS III linked to urea synthesis by the ornithine–urea cycle) and cytosolic (CPS II, involved in purine synthesis) forms of CPS, as in other fish (Mommsen and Walsh, in preparation). Magadi tilapia also have substantial activities of glutamine synthetase (GNS), an enzyme that delivers one of the substrates for CPS III catalysis, adding further support for urea synthesis via the ornithine–urea cycle in this fish. Also a high ratio of GNS/Allantoicase (6.67,

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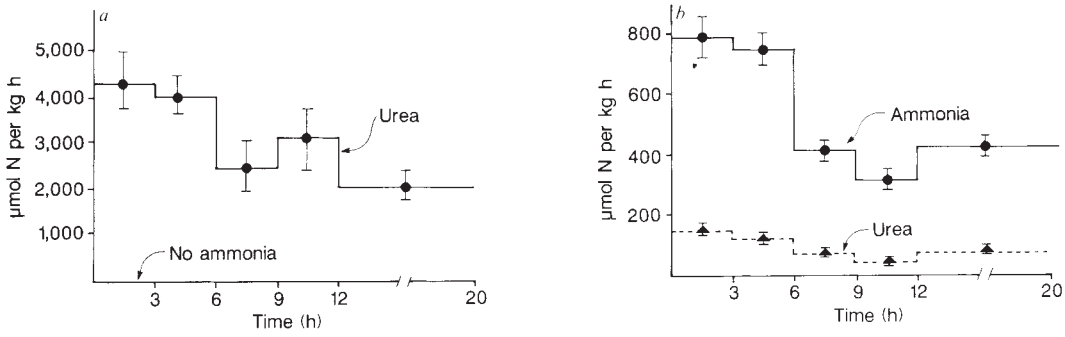

Fig. 1 Changes in nitrogenous waste excretion with time. *a*, *O. a. grahami* (mean weight = 6.93 ± 0.70 g, 1 s.e.m., n = 25) in water from Lake Magadi (pH = 9.98). *b*, *Oreochromis* species from the Sagana river hatchery (mean weight = 10.76 ± 0.44 g, 1 s.e.m., n = 25) in Nairobi dechlorinated tapwater (pH = 7.22).

Methods. *a*, *O. a. grahami* excreted only urea at a high rate which declined with time. Experiments were carried out at ambient temperatures of between 30 and 34 °C on the balcony of the chemistry laboratory of the Magadi soda works. Water and fish were collected daily from the lagoons and hotsprings in which the fish lived. Fish were placed singly in bottles containing 250 ml of Magadi water to determine excretion rates. Air was bubbled through the water having first been passed through an acid trap (0.1 M HCl) to remove any ammonia. Air leaving the bottle was then passed through a second acid trap (0.1 M HCl) to collect any volatilized ammonia, this proved to be negligible. Water samples were taken and the water renewed every three hours for 12 h and then a final sample was taken at 20 h. To exclude the possibility of bacterial ammonia or urea production simultaneous control experiments were conducted. Three groups of three bottles each contained the following: one group contained only water, a second group contained dead fish, and a third group contained recently collected fish faeces. Ammonia was added to the water at the same rate by live and dead fish and fish faeces. The dead fish and faeces did not add any urea to the water in the bottle. Thus, we concluded that the live fish, which also produced faeces during the experiment, excreted no ammonia and that any ammonia appearing in the water was produced by the faeces. *b*, Sagana River tilapia, *O. nilotica*, were collected from the Sagana tilapia hatchery and maintained in neutral Nairobi dechlorinated tapwater. Sagana River tilapia excreted more than 80% of their nitrogenous waste as ammonia and less than 20% as urea at much lower rates of total nitrogenous excretion than *O. a. grahami*, these rates also decreased with time. Excretion rates in Sagana *Oreochromis* sp. were determined at the University of Nairobi by placing the fish singly in 250 ml of Nairobi tapwater at 22 °C in aerated beakers. Water was sampled and renewed over the same schedule as in *a*. Similar experiments with dead fish and faeces demonstrated that these produce negligible ammonia and urea. There was no uric-acid excretion by these fish. Ammonia was determined by the method of Verdouw, van Echted and Dekkers[14], urea-N by Chaney and Marbeck[15] or Crocker[16], and uric acid by the method of Henry, Sobel and Kim[17].

Table 1 Enzyme activities in tilapia livers

	O. a. grahami (Magadi tilapia)	*O. nilotica* (Sagana tilapia)
Urea cycle:		
Carbamoylphosphate synthetase:		
CPS II & III	1.24 ± 0.12 (5)	0.07 ± 0.02* (6)
CPS III	0.37 ± 0.01 (5)	0.02 ± 0.02* (6)
Ornithine carbamyl transferase (OTC)	7.32 ± 0.97 (6)	ND (6)
Arginase (ARG)	11.71 ± 0.38 (5)	13.25 ± 0.93 (6)
Intermediary metabolism:		
Glutamine synthetase (GNS)	6.14 ± 1.52 (6)	0.86 ± 0.73* (5)
Glutamate dehydrogenase (GDH)	13.87 ± 2.67 (6)	33.92 ± 5.88* (6)
Uricolytic pathway:		
Allantoicase	0.92 ± 0.15 (6)	2.65 ± 1.00 (6)
Plasma urea (mM l^{-1})	10.52 ± 1.06 (6)	2.32 ± 0.22* (6)
Plasma ammonia (mM l^{-1})	0.77 ± 0.10 (5)	1.04 ± 0.19* (6)

Enzyme activities in tilapia livers in μmol of substrate converted to product per gramme of liver tissue fresh weight in 1 min (1 h for CPS) at 22 °C under saturation conditions, ± s.e.m. (N). Liver were removed from freshly killed fish, frozen on dry ice and assayed within 20 d. Enzymes were assayed in 1:10 liver homogenates in HEPES buffer (50 mM, pH 7.5). CPS was assayed by a radiotracer technique[18] with (CPS II & III) and without (CPS II) *N*-acetylglutamate (NAG) in the presence of glutamine. CPS II + III prefer glutamine to ammonia as a nitrogen donor, whereas CPS I is specific for ammonia. In trial experiments the addition of ammonia to the assay medium had no effect on total CPS activity and ammonia was not used in subsequent assays. OTC (pH 8.5, ref. 19), ARG (pH 8.0, ref. 19), GNS (pH 6.7; ref. 20), GDH (pH 7.4, ref. 21), allantoicase (pH 7.4, ref. 22) were assayed spectrophotometrically. ND refers to nondetectable activities. Argininosuccinate synthetase and argininosuccinate lyase activities were not measured because of limited tissue availability. These enzymes, however, are known to be present in fish generally[6]. Comparisons of enzyme activities reported here with those in the literature are of limited value because of different assay conditions and also these livers were frozen and in transit for two weeks. The livers from *O. a. grahami* and *O. nilotica*, however, were treated identically and it is this comparison on which we base our conclusions. Plasma ammonia[23] and urea[14] were determined in freshly collected blood drawn by caudal puncture. * = significantly different ($P < 0.05$; Students t-test) for corresponding value in *O. a. grahami*.

Table 1) indicates that GNS is only involved to a minor degree in purine synthesis. CPS III activity was close to the level of detection and ornithine carbamyl transferase (OTC) could not be detected in the liver of Sagana River tilapia, indicating that urea production via the ornithine-urea cycle is insignificant in this fish. This is supported by the fact that the GNS/Allantoicase ratio is low, indicating that uricolysis is responsible for urea production in this fish. Allantoicase activity in Sagana *Oreochromis* was three times that in *O. a. grahami*, and yet urea production was less than 20% of total nitrogenous excretion. This indicates that the relative activities of enzymes may not be good indicators of the relative capacities of the two livers to produce urea by different pathways. It seems probable, however, that urea is formed only by uricolysis in *O. nilotica* and that the ornithine-urea cycle is the predominant pathway of urea production in *O. a. grahami*.

We found that *O. a. grahami* have a high tolerance to external ammonia, probably due to their high capacity to detoxify absorbed ammonia by urea production. These fish show a rapid (within a few hours) threefold increase in urea production when exposed to elevated environmental ammonia levels, whereas Sagana tilapia exhibited a negligible response to ammonia loading.

The hotspring water in which the fish lived had a total CO_2 content (bicarbonate and carbonate) of 160–200 mM compared with tilapia blood plasma level (bicarbonate) of 9.74 ± 1.15 mM (s.e.m., $n = 9$). Blood plasma pH was 7.58 ± 0.06 ($n = 9$) when water pH was 9.98. These large bicarbonate and pH differences may cause an alkaline load for the fish. Increased bicarbonate stimulates urea production in toadfish hepatocytes[13], and ureogenesis may play an important role in removing excess bicarbonate, reducing alkalinization of the fish.

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Female choice selects for a viability-based male trait in pheasants

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Recent theory on sexual selection[1–4] suggests that females in species without paternal care choose mates by their secondary sexual characters because these indicate genotypic quality which will be transmitted to the offspring. These ideas are not yet empirically supported as data quantifying the relationship between female mate choice and female reproductive success are lacking. Only in one case, in *Colias* butterflies, has it been demonstrated unequivocally that females choose 'good genotypes' as mates[5] and there is only one study, on *Drosophila*, demonstrating that mate choice increases one component of offspring fitness[6]. Spur length of male pheasants (*Phasianus colchicus*) correlates with various fitness-related properties[7]. We here present the first experimental field data showing that female pheasants select mates on the basis of male spur length and that female mate choice correlates with female reproductive success.

The pheasant is a precocial, sexually dimorphic species without paternal care. Male spur length is positively correlated with age, weight, size[7] (Table 1) and with access to food as a juvenile[8]. Males with long spurs attract more females per day to their territories, and they enjoy greater reproductive success[7]. In addition, the average spur length of adult males who survive to the next season exceeds that of males who die by 2.1 mm ($P < 0.05$), indicating that spur length correlates with male viability[7].

An isolated population of pheasants was studied in the Revinge area in southern Sweden from December 1983 to August 1987 (ref. 7). Each winter all pheasants in the study area (500 hectares) were trapped, marked, aged, biometrically measured,