J. exp. Biol. ***180****, 323-387 (1993)*
Printed in Great Britain © The Company of Biologists Limited 1993

SHORT COMMUNICATION

EFFECTS OF AMMONIA ON SURVIVAL, SWIMMING AND ACTIVITIES OF ENZYMES OF NITROGEN METABOLISM IN THE LAKE MAGADI TILAPIA *OREOCHROMIS ALCALICUS GRAHAMI*

PATRICK J. WALSH[1], HAROLD L. BERGMAN[2], ANNIE NARAHARA[2], CHRIS M. WOOD[3], PATRICIA A. WRIGHT[4], DAVID J. RANDALL[5], J. N. MAINA[6] and PIERRE LAURENT[7]

[1]*Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL 33149, USA,* [2]*Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071, USA,* [3]*Department of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1,* [4]*Department of Veterinary Pathology, Guelph University, Guelph, Ontario, Canada N1G 2W1,* [5]*Department of Zoology, University of British Columbia, Vancouver, BC, Canada V6T 2A9,* [6]*Department of Veterinary Anatomy, University of Nairobi, Chiromo Campus, Nairobi, Kenya* and [7]*Laboratoire de Morphologie Fonctionnelle et Ultrastructurale des Adaptations, CNRS, Strasbourg, France*

Accepted 22 March 1993

The Lake Magadi tilapia, *Oreochromis alcalicus grahami*, is remarkable among teleosts in that it flourishes under extremely well-buffered alkaline water conditions (pH10, C_{CO_2} 180mmol l^{-1}) at temperatures of 30–40˚C (Wood *et al.* 1989). As expected from current models in teleosts, ammonia excretion into such water would be difficult at best (Wood, 1993). Part of the survival strategy of the Lake Magadi tilapia is that it has a complete ornithine–urea cycle (O–UC) in the liver and excretes virtually all of its waste nitrogen as urea (Randall *et al.* 1989). Ammonia toxicity in ammoniotelic teleosts has been studied extensively, and typical values for unionized ammonia (NH_3) 96h LC_{50} (the concentration at which half of test subjects die after 96h) are well below 100 μmol l^{-1} (Haywood, 1983; Thurston *et al.* 1983*a*,*b*; Campbell, 1991). Surprisingly, no ammonia LC_{50} values are available for ureogenic teleost fish, and one would predict that fish synthesizing and excreting urea for whatever purpose would have higher LC_{50} values than their ammoniotelic counterparts. Additionally, since ammonia exposure has been implicated in the functional response of urea excretion in the Lake Magadi tilapia (Wood *et al.* 1989) and another ureogenic teleost (the gulf toadfish *Opsanus beta*) (Walsh *et al.* 1990), we reasoned that ammonia exposure in the Lake Magadi tilapia might reveal insights into the biochemical regulation of the O–UC in this species; in particular that it

Key words: O–UC enzyme activities, ammonia LC_{50}, brain, liver, gill, alkaline lake, tilapia, *Oreochromis alcalicus grahami*, nitrogen metabolism.

might induce enzyme activity. We report here that the Lake Magadi tilapia has a rather high ammonia LC_{50} compared to values for other teleosts, but that short-term ammonia exposure has very limited effects on the activities of the enzymes of nitrogen metabolism and on swimming performance.

All tests were performed with juveniles or adults of the Lake Magadi tilapia, *Oreochromis alkalicus grahami*, in January and February of 1992 (except for swimming speed measurements, which were performed in January 1988). Fish were caught by net at the previously described Lake Magadi 'Fish Springs' site (Wood *et al.* 1989) and quickly transported to an outdoor laboratory on the balcony of the chemistry laboratory of the Magadi Soda Company. Juvenile fish, weighing less than 100mg, were exposed to ammonia by adding stock solutions of NH_4Cl to Lake Magadi water (mean pH9.94) to give final nominal total ammonia concentrations of 0, 500, 658, 866, 1140 and 1500 $\mu mol\,l^{-1}$, which were checked by assay (Verdouw *et al.* 1978). Typically, 10 fish were placed in darkened plastic containers with 800ml of test water. Metabolic rates and water volumes were such that aeration was not required for the juvenile fish, and test solutions were partially replaced every 8h for up to 48h. Ten adult fish per treatment (approx. 2–3g) were placed in 15l of aerated test water prepared with 0, 500 and 1000 $\mu mol\,l^{-1}$ total ammonia, and fish were exposed for up to 24h with a complete water change at 12h. In both cases, measurement of water ammonia at the start and at water exchange showed that less than 15% had been lost (presumably due to a combination of degassing and metabolic processing). As the experiments were performed outdoors, temperature could not be precisely controlled but varied from 30 to 36°C (mean 34°C), over the day, which was similar to the diurnal variation at the collection site. Experiments were terminated at 24h (adults) or 48h (juveniles) because, beyond these periods, 'natural' mortality in the control treatments became unacceptably high (>20%). This mortality has been reported previously (Wood *et al.* 1989) and appears to be due to the combined effect of the absence of feeding *vis à vis* the very high metabolic rate of these fish and to non-specific stress associated with holding wild fish in captivity. LC_{50} values were calculated using a moving averages method (Thompson, 1947) for juveniles and by a simple graphical method for adults.

For measurements of enzyme activities, brains, livers and gills were dissected from surviving adult fish at 24h, placed in cryotubes and quick frozen in liquid nitrogen, shipped to Miami in a dry shipper (Minnesota Valley Engineering) and stored at −80°C for less than 2 months prior to assays. Tissues were sonicated (brain) or homogenized (gill and liver) in approximately 10 volumes of homogenization buffer (20mmol l^{-1} K_2HPO_4, 10mmol l^{-1} Hepes, 0.5mmol l^{-1} EDTA, 1mmol l^{-1} dithiothreitol, 50% glycerol, adjusted to pH7.5 at room temperature). Samples were spun at 13000 ***g*** in a microcentrifuge and supernatants or 1:10 dilutions were used directly in assays. The following enzymes were assayed at 30±0.2°C by previously published methods; malate dehydrogenase (MDH), citrate synthase (CS), alanine aminotransferase (AlaAT), aspartate aminotransferase (AspAT), glutamate dehydrogenase (GDH), glutamine synthetase (GNS), arginase (ARG) and ornithine–citrulline transcarbamoylase (OCT) (Mommsen and Walsh, 1989). Measurements of cricital swimming speeds (U_{crit}) were performed on adult fish (1–2g) as previously described (Wright *et al.* 1990).

The Lake Magadi tilapia showed a substantial ability to survive ammonia challenge, in that total ammonia LC_{50} values ($\mu mol\,l^{-1}$ with 95% confidence limits; N=40 for juveniles, N=30 for adults) were: juvenile 24h LC_{50}=773.7 (705.9–838.3); juvenile 48 h LC_{50}=770.3 (677.9–856.5); adult 24h LC_{50}=750. Although the period of the tests was relatively short, the closeness of the 24h and 48h LC_{50} values gives confidence that acute toxicity due to ammonia had ceased and that these values are representative of incipient LC_{50} values. Considering that the water pH (9.94) and temperature (34˚C) in this study are both substantially higher than in most studies, it is important to convert these total ammonia LC_{50} values to NH_3 LC_{50} values of 681.7 (622.0–738.6) for juveniles at 24h, 678.7 (597.3–754.7) for juveniles at 48h and 660.8 $\mu mol\,l^{-1}$ for adults at 24h. These values are at least five times higher than those for typical ammoniotelic teleosts (Haywood, 1983; Thurston *et al*. 1983*a*,*b*; Campbell, 1991), but only slightly higher than those measured in preliminary observations for another ureotelic teleost (an approximate 96h LC_{50} value for the gulf toadfish, *Opsanus beta*, is 500 $\mu mol\,l^{-1}$ NH_3 in sea water of pH8.0; P. J. Walsh, unpublished data). Clearly, ureogenesis must confer this enhanced ability to survive ammonia challenge.

The Lake Magadi tilapia were able to swim unimpeded at ammonia concentrations which would be lethal to many ammoniotelic teleosts. We found no difference in U_{crit} [mean ± S.E. (N)] for control tilapia [4.53±0.31BL s^{-1} (5)] *versus* tilapia exposed to 100 $\mu mol\,l^{-1}$ total ammonia (88 $\mu mol\,l^{-1}$ NH_3) [4.88±0.48BL s^{-1} (5)], where BL is body lengths. The maintenance of this physiological trait is of obvious survival value in Lake Magadi, where avian predators are extremely abundant.

The effects of ammonia challenge on enzyme activities are given in Table 1. The activity of the standard metabolic enzymes CS and MDH are typical of values for other fish, with one exception, namely brain CS activity is some five- to seven-fold higher than that in a variety of other fish (see Table 12-2 in Hochachka and Somero, 1984). This difference is about what one would predict from the temperature difference between the studies (10 *vs* 30˚C), implying that the Lake Magadi tilapia has not adjusted its brain CS activity downwards in a compensatory response to increased rate from increased temperature. This ‘excess’ aerobic activity in the brain may be related to the extreme swimming activity and apparent visual dependence of this species in avoiding the intense avian predation. Otherwise, enzyme activity values are similar to those obtained previously for control fish (Randall *et al*. 1989).

Ammonia exposure had very little effect on the activities of enzymes examined: only a 13% reduction in AspAT activity in brain, and a 16% reduction in liver MDH were noted at 500 $\mu mol\,l^{-1}$ ammonia (Table 1). However, Wood *et al.* (1989) reported that urea excretion increased two- to threefold within the first 3h of exposure of the Lake Magadi tilapia to 500 $\mu mol\,l^{-1}$ NH_3 and remained significantly elevated at 20h (see Fig. 3 of Wood *et al.* 1989). There are three reasonable explanations for the lack of substantial changes in enzyme activities despite large increases in urea production rates: (1) we failed to look at the most important enzymes of ammonia detoxification; (2) 24h of exposure is not long enough to detect enzyme induction; (3) the ammonia detoxification pathways of amination and ureogenesis are operating continually and at near maximal capacity in this species. Of these three explanations, we favour the last for two reasons. First, we

Table 1. *Effects of ammonia exposure on enzyme activities in the Lake Magadi tilapia,* Oreochromis alcalicus grahami

	Enzyme activity (μmol min^{-1} g^{-1} wetmass)		
Tissue/Enzyme	0 μmol l^{-1} ammonia (N=6)	500 μmol l^{-1} ammonia (N=8)	1000 μmol l^{-1} ammonia (N=2)
Brain			
GDH	16.268±1.355	16.830±1.563	13.217±1.744
AspAT	73.249±1.489	66.515±2.407*	58.548±1.969
AlaAT	5.078±0.275	4.856±0.184	5.231±0.620
GNS	40.699±2.721	36.358±2.271	44.405±9.006
MDH	50.527±1.715	49.666±1.858	45.925±3.979
CS	10.151±0.425	10.079±0.332	9.345±0.457
Liver			
GDH	28.774±1.699	33.252±2.701	33.038±3.642
AspAT	68.641±6.381	77.621±8.127	67.728±16.236
AlaAT	35.903±4.120	31.940±3.893	22.258±9.835
GNS	2.157±0.127	2.492±0.204	2.477±0.275
OCT	10.819±1.422	13.376±2.053	10.033±1.560
ARG	40.893±5.278	39.343±4.795	41.361±3.014
MDH	514.449±16.815	432.167±16.236*	487.146±32.487
CS	10.354±2.115	9.020±0.428	6.849±0.455
Gill			
GDH	1.376±0.090	1.571±0.131	1.439±0.199
AspAT	12.216±1.144	12.578±1.316	11.674±0.028
AlaAT	1.566±0.142	1.613±0.135	1.863±0.847
MDH	16.041±1.107	16.972±1.204	15.232±1.126
CS	1.393±0.087	1.636±0.153	1.592±0.458

Values are means ± S.E.
*Significantly different from 0 ammonia value.
Enzyme abbreviations are explained in the text.

examined a variety of ammonia detoxification enzymes, including perhaps the one most vital to survival, GNS. In a prior study we have noted that both glucagon and dexamethasone treatment activate this enzyme in the liver of the ureogenic toadfish *Opsanus beta* (Mommsen *et al.* 1992). Second, the metabolic rate of this species is very high ($\dot{M}_{O_2}$=25.9±1.4mmol kg^{-1} h^{-1} at 34°C; P. J. Walsh *et al.*, unpublished results) and responses of enzyme activities to dietary changes were noted in as little as 24h (P. J. Walsh, unpublished results), indicating that these enzyme activities are probably plastic enough to change in this period. Furthermore, we have noted a distinct activation of liver GNS 24h after transferring toadfish from flowing to static water conditions (B. C. Tucker and P. J. Walsh, unpublished data). We would predict from our observations that control of ureogenesis in the tilapia is probably regulated substantially at the substrate level, namely in the supply to the liver of ammonia and amino acids from the diet, and bicarbonate from the environment, rather than by large changes in enzyme concentrations *per se*. This strategy may be contrasted with the response of the normally ammoniotelic, but potentially ureagenic, teleost *Heteropneustes fossilis*, an air-breathing fish with

hepatic O–UC capacity (Saha and Ratha, 1986). For *H. fossilis*, activation of increased urea production by high levels of environmental ammonia was rather slow (7 days) and was paralleled by the induction of several O–UC enzymes over this period.

We wish to thank the US National Geographic Society, NATO, NSERC Canada, NSF (IBN-9118819) and the University of Wyoming for providing support for this research. Tremendous logistic support was provided by Magadi Soda Co, PLC and by Mr George Muthee of the University of Nairobi. We thank The Tiffster and Mr Leonnard Intheory for their optimistic points of view.

References

CAMPBELL, J. W. (1991). Excretory nitrogen metabolism. In *Environmental and Metabolic Animal Physiology* (ed C. L. Prossser), pp. 277–324. New York: Wiley-Liss Inc.

HAYWOOD, G. P. (1983). Ammonia toxicity in teleost fishes: a review. *Can. Tech. Rep. Fish. aquat. Sci.* **1177**, 35pp.

HOCHACHKA, P. W. AND SOMERO, G. N. (1984). *Biochemical Adaptation*. Princeton, NJ: Princeton University Press. 537pp.

MOMMSEN, T. P. AND WALSH, P. J. (1989). Evolution of urea synthesis in vertebrates: the piscine connection. *Science* **243**, 72–75.

MOMMSEN, T. P., DANULAT, E. AND WALSH, P. J. (1992). Metabolic actions of glucagon and dexamethasone in liver of the ureogenic teleost, *Opsanus beta*. *Gen. comp. Endocr.* **85**, 316–326.

RANDALL, D. J., WOOD, C. M., PERRY, S. F., BERGMAN, H., MALOIY, G. M. O., MOMMSEN, T. P. AND WRIGHT, P. A. (1989). Urea excretion as a strategy for survival in a fish living in a very alkaline environment. *Nature* **337**, 165–166.

SAHA, N. AND RATHA, B. K. (1986). Effects of ammonia stress on ureogenesis in a freshwater air-breathing teleost, *Heteropneustes fossilis*. In *Contemporary Themes in Biochemistry*, vol. 6 (ed. O. L. Kon, M. C. M. Chung, P. L. H. Hwang, S. F. Leong, K. H. Loke, P. Thiyagarah and P. T. H. Wong), pp. 342–343. Cambridge: Cambridge University Press.

THOMPSON, W. R. (1947). Use of moving averages and interpolation to estimate median-effective dose. *Bacteriol. Rev.* **11**, 115–145.

THURSTON, R. V., RUSSO, R. C. AND PHILLIPS, G. R. (1983*a*). Acute toxicity of ammonia to fathead minnows. *Trans. Am. Fish. Soc.* **112**, 705–711.

THURSTON, R. V., RUSSO, R. C. AND VINOGRADOV, G. A.(1983*b*). Acute toxicity of ammonia to rainbow trout. *Trans. Am. Fish. Soc.* **112**, 696–704.

VERDOUW, H., VON ECHTED, C. J. A. AND DEKKERS, E. M. J. (1978). Ammonia determination based on indophenol formation with sodium salicylate. *Water Res.* **12**, 399–402.

WALSH, P. J., DANULAT, E. AND MOMMSEN, T. P.(1990). Variation in urea excretion in the gulf toadfish, *Opsanus beta*. *Mar. Biol.* **106**, 323–328.

WOOD, C. M. (1993). Ammonia and urea metabolism and excretion. In *The Physiology of Fishes* (ed. D. H. Evans). Boca Raton, FL: CRC Press. (in press).

WOOD, C. M., PERRY, S. F., WRIGHT, P. A., BERGMAN, H. L. AND RANDALL, D. J.(1989). Ammonia and urea dynamics in the Lake Magadi tilapia, a ureotelic teleost fish adapted to an extremely alkaline environment. *Respir. Physiol.* **77**, 1–20.

WRIGHT, P. A., PERRY, S. F., RANDALL, D. J., WOOD, C. M. AND BERGMAN, H. (1990). The effects of reducing water pH and total CO_2 on a teleost fish adapted to an extremely alkaline environment. *J. exp. Biol.* **151**, 361–369.