ACID-BASE RESPONSES AND AMMONIA TRANSFERS DURING EXPOSURE TO ELEVATED AMBIENT AMMONIA IN A MARINE TELEOST (*MYOXOCEPHALUS OCTODECIMSPINOSUS*)

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In two previous studies, we examined the effects of an internal ammonia load (infusion of NH_4Cl or NH_4HCO_3) on the acid-base balance and transbranchial ammonia transfers in a seawater teleost, the long horned sculpin (Claiborne & Evans, Bull. MDIBL 24:24-25, 1984; Bull. MDIBL 25:31, 1985). Both studies indicated that at least a portion of the total ammonia ($T_{AMM} = NH_3 + NH_4^+$) excreted to the water by this species, crosses the gills in the form of NH_3. Recently, a role for the ionic exchange of internal NH_4^+ for either Na^+ or H^+ has been postulated in two freshwater teleosts. High external ammonia concentrations were necessary to elicit these ion exchange mechanisms (Cameron & Heisler, J. Exp. Biol. 105:107-125, 1983; Cameron, J.N., J. Exp. Zool. 239:183-195, 1986). To test this proposition in a marine teleost, we exposed sculpin to seawater in which the T_{AMM} had been increased (~1 mM NH_4Cl). Acid-base parameters (plasma pH, T_{CO_2}, and T_{AMM}), and net ammonia (ΔNH_4^+), bicarbonate (ΔHCO_3^-) and acid (ΔH^+) transfers between the animal and the water, were monitored during a 2-3 hour control period, a 6 hour high external ammonia (HEA) period, and a subsequent 15 hour recovery period (for details see Claiborne and Evans, ibid., 1984).

HEA induced several effects on acid-base balance and ion transfers in these animals (169 ± 22 grams, n=6, mean ± S.E.). While plasma pH varied around the control measurement of 7.78 ± 0.02, plasma T_{CO_2} was slightly elevated ($p<0.01$) from 5.29 to 6.42 mM after 2 hours. This parameter returned to control levels by hour 6, and remained stable throughout the recovery period. After 30 minutes in HEA, plasma T_{AMM} had increased by nearly 3X from a control of 230 ± 30 to 690 ± 103 uM. T_{AMM} reached a maximum of 839 ± 157 uM at hour 6 (but remained below that of the external bath: 1175 ± 43 uM), and then decreased to 552 uM one hour after normal seawater was reinstated. A control ammonia efflux (.271 ± 0.042 $mmole \cdot kg^{-1} \cdot h^{-1}$) was reversed to an influx as a ΔNH_4^+ of -1.216 ± 0.390 was observed during the first hour of HEA (Fig. 1). This uptake resulted in a calculated net ΔNH_4^+ (the measured cumulative transfer - the control rate) gain of ~2.0 mmole/kg after 4 hours. From hours 4-6, ΔNH_4^+ returned to an efflux once again (0.442 ± 0.088 $mmole \cdot kg^{-1} \cdot h^{-1}$, $p<0.01$). During the first 3 hours of the recovery period, a large net ΔNH_4^+ loss was observed which equalled the ammonia gained in the preceding period. ΔHCO_3^- transfer was reversed from a control uptake of 0.10 ± 0.05 $mmole \cdot kg^{-1} \cdot h^{-1}$ to a slight excretion of 0.22 ± 0.08 $mmole \cdot kg^{-1} \cdot h^{-1}$ at hour 2 of HEA. A large but variable efflux totaling

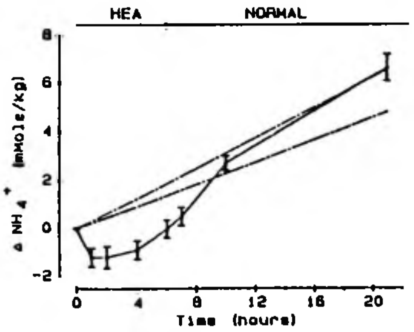

Fig 1. Changes in cumulative NH_4^+ transfer during and after exposure to high external ammonia (HEA; mean ± S.E., n=6). Hashed lines represent the measured control rate of efflux (± S.E.) extended as a reference over the subsequent experimental periods.

3.39 ± 1.20 mmole/kg was observed at the end of the recovery period. The measured ΔNH_4^+ and ΔHCO_3^- transfers resulted in a negative net ΔH^+ (a net base uptake) of 2.74 ± 0.58 mmole/kg from hours 1-4 of HEA. H^+ efflux then resumed from hours 4-6 and was maintained through the remainder of the experiment. Importantly, alterations in the rate of ammonia transfer (Fig. 1) accounted for about 90% of the calculated ΔH^+ during the first few hours of HEA. This result, in combination with the negligible effects of the HEA on plasma pH, may suggest that a large portion of the T_{AMM} which entered the animal was in the form of NH_4^+. Had NH_3 been the predominant form taken up by the animal, a larger change in ΔHCO_3^- and a blood alkalosis might be predicted. These results are not unexpected since the ionic permeability of the gills in marine species is thought to be relatively high (Evans, D.H., in "Comparative Physiology of Osmoregulation in Animals", ed. G.M.O. Maloiy, Vol. 1, pp. 305-390, 1979). Indeed, evidence for a role of ionic NH_4^+ diffusion across the gills of this species has been described previously (Goldstein et al., J. Exp. Zool. 219:395-398, 1982).

To study the gradients driving the movement of NH_3 across the gills (Fig. 2), we utilized the measured plasma and water pH and T_{AMM} values as well as appropriate solubility and pK' constants derived for trout plasma and seawater (Cameron and Heisler, 1983, op. cit.). Under control conditions, a positive (from fish to water) NH_3 diffusion gradient of about 60 uTorr was measured. Within 1 hour after exposure to the HEA, however, plasma P_{NH3} was not significantly different from that of the surrounding seawater. Thus, there was no net gradient for NH_3 diffusion between the animal and the water during this time period. When the external bath was flushed with normal seawater, plasma P_{NH3} decreased (but remained above the control average), and a positive diffusion gradient was established once more. Seawater NH_3 decreased during the first 2 hours of HEA as ammonia entered the fish, and then significantly increased again during hours 2-6. That a ΔNH_4^+ efflux (see above and Fig. 1) could be maintained during a period when NH_3 gradients between the animal and the water were near zero and NH_4^+ gradients were negative, suggests that the sculpin is capable of actively extruding ammonia (perhaps via Na^+/NH_4^+ exchange; see review by Evans & Cameron, J. Exp. Zool. 239:17-23, 1986) under these extreme conditions. On the other hand, the sculpin cannot maintain internal ammonia levels below that of the external environment as has been observed in the two freshwater teleosts (Cameron & Heisler, 1983, op. cit.; Cameron, 1986, op. cit.). The present data, in combination with our previous findings (Claiborne & Evans, 1983, 1984, op. cit.) may demonstrate that the sculpin gill is permeable to both NH_3 and NH_4^+, and while excretion of ammonia is predominantly via NH_3 under normal conditions (an outwardly directed P_{NH3}), some form of NH_4^+ exchange may be utilized to maintain nitrogenous waste balance, even in the face of reversed ammonia gradients. (Funded by a Faculty Research Grant from GSC to JBC and NSF PCM 83-0261 to DHE).

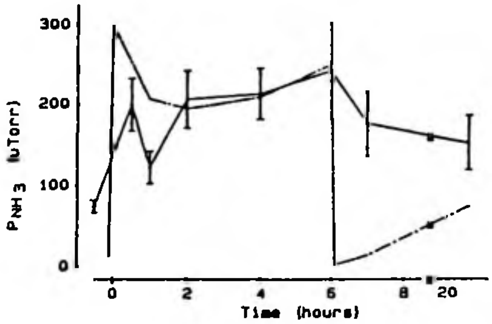

Figure 2. Plasma (mean ± S.E.) and water (hashed line) PNH_3 during and after HEA. Control values are those prior to time = 0.