ACID-BASE BALANCE IN A MARINE TELEOST: (Myoxocephalus Octodecimspinosus) THE EFFECT OF NH_4Cl INFUSION

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Teleost fish excrete nitrogenous waste products in the form of ammonia (NH_3 + NH_4^+). The majority of ammonia transfer to the surrounding water is via the gills. It is not known whether ammonia is excreted by non-ionic diffusion of NH_3, the electroneutral exchange of serosal NH_4^+ for external Na^+, an ionic diffusive efflux of NH_4^+, or some combination of these mechanisms. In the freshwater adapted trout (Salmo gairdneri) diffusive loss of NH_3 has been shown to be the principle route of ammonia excretion under normal conditions (Cameron & Heisler, J. Exp. Biol. 105:107-125, 1983). Na^+/NH_4^+ exchange and NH_4^+ diffusion are thought to play a role in ammonia efflux in seawater species (Claiborne et al., J. Exp. Biol. 96:431-434, 1982; Goldstein et al., J. Exp. Zool. 219:395-398, 1982). As demonstrated by Cameron and Kormanik (J. Exp. Biol. 99:143-160, 1982) and Cameron and Heisler (1983, Ibid.), the infusion of an ammonium salt (NH_4Cl or $(NH_4)_2SO_4$) into either the freshwater catfish (Ictalurus punctatus) or the S. gairdneri induces a pronounced plasma acidosis. The authors ascribe this effect to the rapid loss of the infused ammonia as NH_3, with a more gradual equimolar excretion or intracellular buffering of the remaining H^+, thus inducing the rapid decrease and secondary recovery of the observed plasma pH. To gain additional insight into the possible role of NH_3 diffusion in a marine teleost, it was of interest to duplicate these experiments by subjecting a stenohaline marine species to an infusion of NH_4Cl while monitoring extracellular acid-base status and ammonia excretion.

Long-horned sculpin (Myoxocephalus octodecimspinosus, mass = 182 ± 6 grams (n = 9); mean ± S.E.) were anesthetized (ms-222, 1:10,000) and cannulated in a manner similar to that described for the measurement of ventral aortic blood pressure (Claiborne & Evans, Mar. Biol. Lett. 2:123-130, 1981). In the present study, the cut tip of a 26 gauge needle connected to short length of heparinized, Ringer's-filled cannula (PE-50) was inserted into the afferent artery of the third branchial arch and secured in place with two sutures. An additional Ringer's-filled cannula (PE-50) was inserted through the skin and peritoneal musculature into the peritoneal cavity. The sculpin were then placed in a darkened plexiglass box (vol = 2 l) and allowed to recover for 24-48 hours. During this period, fresh running seawater (12-15 °C) was directed through the experimental chamber. Just prior to the start of the experimental period, duplicate control blood samples (0.5 mls) were collected via the aortic cannula. The experiment was begun when NH_4Cl (5 mM/kg, a 4-5 ml bolus of 200 mM NH_4Cl) was infused intraperitoneally into the animal over several minutes. After a 5 minute equilibration, a time zero blood sample was drawn, and additional samples were obtained at hours 1, 2, 4, 8, and 20 post-infusion. Blood pH and TCO_2 were measured utilizing a blood-gas analyzer (I.L. model 213) and a TCO_2 detection system (Capni-Con II; Cameron Instruments Inc.). Plasma HCO_3^- and pCO_2 were calculated from pH and TCO_2 using values for αCO_2 and pK' at 15 °C calculated from those for human plasma reported by Severinghaus (In "Handbook of Physiology", Section 3, Respiration, American Physiological Society, 1965). Plasma total ammonia (NH_3 + NH_4^+) was measured enzymatically (Sigma kit #170-UV). Three-four hours prior to the beginning of an experiment, the running seawater was disconnected so that changes in the bath concentration of total

ammonia [T_{amm}] could be monitored. Water samples (15 mls) were collected prior to, and during the experiment, and analyzed for [T_{amm}] using the phenolhypochlorite method. Four or eight hours post-infusion, the water in the fish chamber was briefly flushed with fresh seawater to limit the accumulation of external ammonia. [T_{amm}] excretion (mM/kg·hr) was calculated from the changes in water [T_{amm}] after adjusting for volume changes, the mass of the animal, and time between samples.

The effect of NH_4Cl infusion on internal acid-base status in the sculpin is shown in Table 1. As would be expected, NH_4Cl infused into the peritoneal cavity induced a large increase in the [T_{amm}] of the blood which persisted for 1-2 hours. A rapid plasma pH depression of more than 0.4 units was observed immediately following the NH_4Cl infusion. Plasma pH then returned to control values within 4 hours post-infusion. The drop in pH was due to a decrease in serosal [HCO_3^-] (or an increase in [H^+]) and an elevation in PCO_2. After 20 hours, plasma [HCO_3^-] had increased by ≃50% over control measurements, thus compensating for the additional blood CO_2. Net T_{amm} efflux from the fish to the water was increased by ≃8x during the first 2 hours post-infusion.

It is clear that NH_4Cl infusion elicits pronounced internal acid-base effects. Indeed, the alterations in extracellular pH, [HCO_3^-], and PCO_2 are similar to those found following an acid infusion (Cameron & Kormanik, 1982, Op. Cit.). During the first two hours after the infusion, the ΔT_{amm} efflux (the net experimental rate - the net control rate) was 3.33 mM/kg. In other words, 2/3 of the infused ammonia was lost to the water concurrent to the observed plasma pH depression. This is a good indication that a significant portion of the T_{amm} infused as NH_4Cl was transferred to the water as NH_3. The remaining protons thus caused the observed acid-base effects. It is likely that over the next few hours the additional H^+ was excreted to the water (via Na^+/H^+ exchange, see Evans, J. Exp. Biol. 97:289-299, 1982), compensated by an uptake of HCO_3^- from the water by Cl^-/HCO_3^- exchange (Claiborne & Heisler, J. Exp. Biol. 108:25-43, 1984), or buffered intracellularly. Goldstein et al. (1982, Op. Cit.) found that a 10x increase in the Ringer's [NH_3] did not stimulate the T_{amm} efflux from the isolated, perfused head of the sculpin, thus indicating a low transbranchial NH_3 permeability in this preparation. The reason for the discordance between these *in vitro* results and our data is unknown at present. It also remains to be seen whether sculpin excrete NH_3 during "control" conditions, but it is clear that at least one species of marine teleost is capable of NH_3 excretion when faced with the stress imposed by high internal T_{amm}. (Funded by NSF PCM 83-02621 to DHE and an intramural grant from Georgia Southern College to JBC.)

Table 1. The effect of ammonium salt infusion on extracellular acid-base status and ammonia efflux in *M. octodecimspinosus*.

Period	[ammonia] (mM/l)	pH	[HCO_3^-] (mM/l)	pCO_2 (torr)	J^{amm} (mM/kg·hr)
control:	1.86 ± 0.54 (N = 6)	7.835 ± 0.048 (n = 7)	4.80 ± 0.54 (n = 7)	1.93 ± 0.33 (n = 7)	0.24 ± 0.03 (n = 8)
post-infusion: (hours)					
0	4.91 ± 0.94 *	7.423 ± 0.102 **	2.98 ± 0.55 **	3.08 ± 0.38 *	
1	1.77 ± 0.34	7.515 ± 0.072 **	3.29 ± 0.42 **	3.13 ± 0.60 *	2.68 ± 0.18 *
2	1.09 ± 0.07	7.682 ± 0.040 **	5.24 ± 0.63	2.98 ± 0.35 *	1.13 ± 0.11 *
4	0.51 ± 0.10	7.809 ± 0.028	6.54 ± 0.88 *	2.63 ± 0.29 *	0.32 ± 0.06
8	0.82 ± 0.25	7.767 ± 0.030	5.88 ± 0.69 *	2.64 ± 0.25 *	0.48 ± 0.06 *
20	1.36 ± 0.42	7.787 ± 0.025	7.34 ± 0.92 *	3.21 ± 0.38 *	0.20 ± 0.04

Mean ± S.E. test of significance by paired t-test, $p < 0.05$, two tailed: * = significant increase from control values; ** = significant decrease from control values. J^{amm} = net ammonia efflux calculated for each sampling interval.