REGULATION OF ACID-BASE BALANCE IN THE LONG-HORNED SCULPIN (MYOXOCEPHALUS OCTODECIMSPINOSUS) FOLLOWING ACID INFUSION: EFFECT OF AMBIENT SALINITY

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We have previously shown that acid-base transfers in the long horned sculpin (Myoxocephalus octodecimspinosus) are impaired when the fish is exposed to dilutions of the ambient water (Walton & Claiborne, Bull MDIBL 27:4-5, 1988; Claiborne & Perry, Bull MDIBL 30:60-61, 1991). When in seawater, the sculpin is able to excrete an administered NH_4^+, HCO_3^- (Claiborne & Evans, J. Exp. Biol 140:89-105, 1988) or H^+ (Maren & Fine, Bull MDIBL 30:60-61, 1991) load mainly via the gills. Thus, while this species may possess the branchial mechanisms for acid-base regulation, (Na^+/NH_4^+, Na^+/H^+, and/or Cl^-/HCO_3^- exchange; Evans, in "Fish Physiology", eds. W. S. Hoar & D. J. Randall, Vol Xb, pp. 239-283, 1984), low external salt concentrations should alter the ability of the animal to maintain normal H^+ excretion (Claiborne & Perry, ibid.). The purpose of the present study was two-fold: (1) to measure the time course of plasma acid-base balance and net transfers between the fish and water following an acid infusion (2 meq kg^{-1} HCl), and (2) to test the effects of low salinity exposure on these parameters subsequent to the acid load.

Long-horned sculpin (Myoxocephalus octodecimspinosus) were cannulated and placed in experimental chambers according to the methods described by Walton & Claiborne (ibid.). In addition, an intraperitoneal cannula (PE-50) was introduced into the animal (Claiborne & Evans, ibid.) to allow the infusion of acid (0.1 N HCl; 2 meq kg^{-1} in teleost Ringers). Following a recovery period of 8 or more hours, and an 11-12 hour control flux period, the animals were infused with acid over a 5 minute period. After a one hour equilibration period, fish were either maintained in MDIBL seawater (~500 mM NaCl) or the external water was changed to 20% seawater (~100 mM NaCl; measured as Cl^-), or 4% seawater (~20 mM NaCl). During the control and post-infusion periods, water NH_4^+ and HCO_3^- were measured so that cumulative transfers of H^+ between the fish and the water could be calculated (Claiborne & Evans, ibid.). Likewise, blood samples (30-50 µl) were taken regularly throughout the experiment for the determination of plasma pH and total CO_2 and the calculation of plasma P_{CO_2} and [HCO_3^-] (for details see Claiborne and Evans, ibid.).

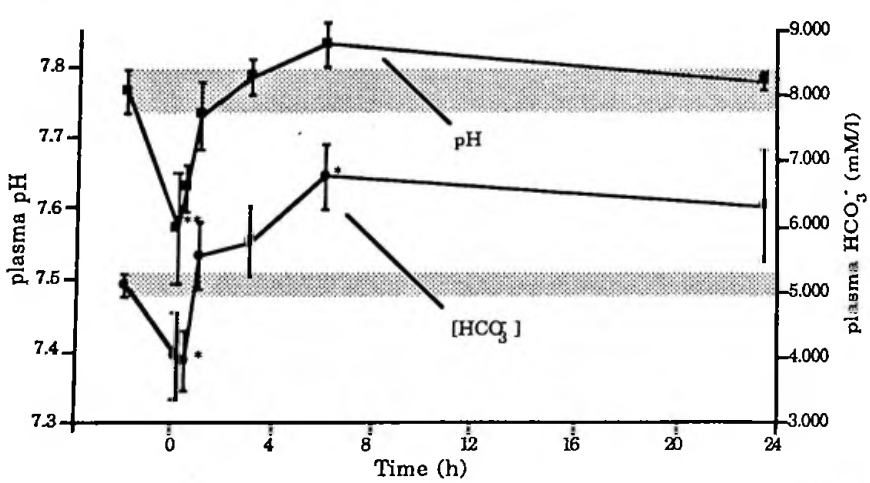

Figure 1. Plasma pH and [HCO_3^-] in 5 seawater animals following acid infusion. Shaded bars represent pre-infusion control values. Infusion at hour 0. * = significant increase, ** = decrease, mean ± S.E.

Following acid infusion, sculpin in seawater exhibited a rapid decrease and then recovery of plasma pH and [HCO_3^-] which was complete within 1 hour post-infusion (Fig. 1). By hour 6, plasma pH had increased slightly (from 7.77 ± 0.03 to 7.83 ± 0.03) and [HCO_3^-] was ~32% higher than pre-infusion control (6.74 ± 0.50 versus 5.09 ± 0.18 mM; $p<0.05$, mean ± S.E., n=5). In animals exposed to 20% seawater following acid infusion, both plasma pH and [HCO_3^-] were well above control 7 hours post-infusion (Fig. 2, pH: 7.72 ± 0.05 → 7.90 ± 0.02, $p< 0.05$; [HCO_3^-]: 5.56 ± 0.04 → 7.80 ± 0.34 mM, $p<0.02$). 4% seawater induced a significant fall in plasma pH at hour 4 (Fig. 2; 7.77 ± 0.03 → 7.68 ± 0.01) and an extended decrease in [HCO_3^-] through hour 7 (5.03 ± 0.43 → 4.25 ± 0.39 mM, $p<0.02$).

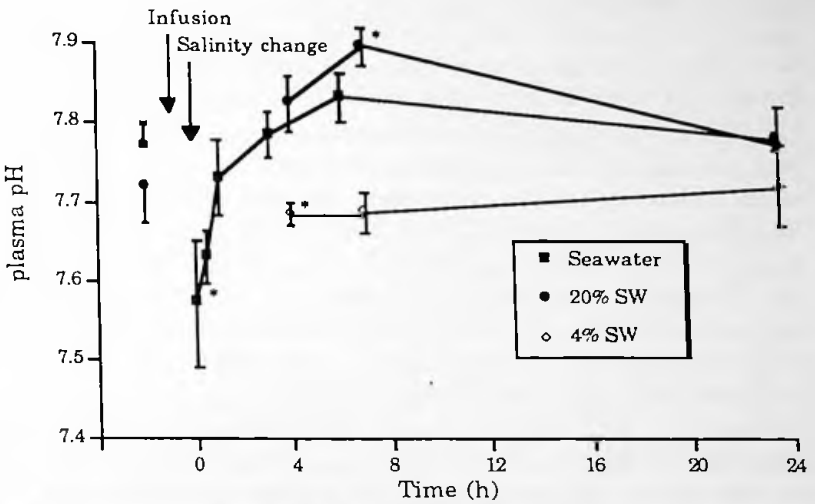

Figure 2. Plasma pH in fish exposed to seawater (n=5), 20% seawater (n=5), or 4% seawater (n=6) after acid infusion. Initial points are pre-infusion control values.

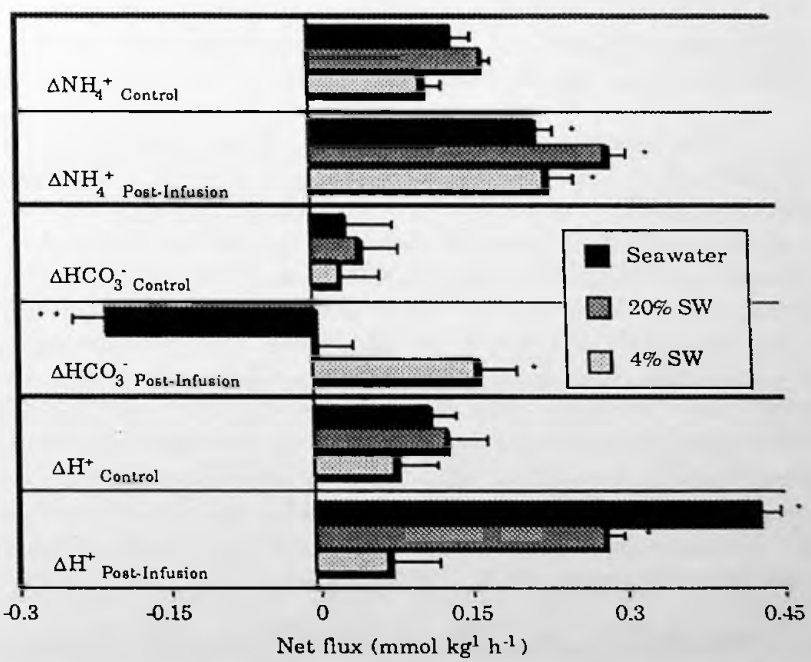

Figure 3. Net transfer rates of NH_4^+, HCO_3^-, and H^+ (mmol kg^{-1} h^{-1}) before (Control) and after (Post-Infusion) acid infusion in three groups of fish exposed to various salinities one hour after the infusion. Post-infusion flux calculated over 10.5 h for seawater group and 12 hours for 20% and 4% seawater series.

Figure 3 shows the net transfer rates of NH_4^+, HCO_3^-, and H^+ between the animal and the water measured over the pre-infusion control period and 10.5-13 hours post-infusion. In seawater, sculpin rapidly excreted 160% of the infused load. ΔH^+ efflux increased by 3.9 times (from 0.11 ±

0.03 to 0.43 ± 0.02 mmol kg^{-1} h^{-1}; $p<0.001$, n=5) in the first 10.5 hours following the infusion. The elevation in ΔH^+ was due to a significant increase in the rate of NH_4^+ loss and the net uptake of HCO_3^- (or excretion of H^+). Sculpin in 20% seawater excreted ~100% of the acid load in the first 12 hours as ΔH^+ increased from 0.13 ± 0.04 to 0.28 ± 0.04, mainly due to a 70% elevation in ΔNH_4^+ efflux. In contrast, ΔH^+ remained unchanged in animals exposed to 4% seawater, and they did not excrete the infused load. ΔNH_4^+ and ΔHCO_3^- increased in parallel fashion during this period (a net increase of ~12 mmol kg^{-1} h^{-1} over control rates; $p<0.02$), which produced little change in net H^+ loss. The patterns of efflux described for all experimental groups continued through hour 21.5-23.5 post-infusion.

Clearly, sculpin were able to rapidly compensate for the infused load when in seawater. Plasma pH and $[HCO_3^-]$ were near normal within 1 hour, and were above controls by hour 6 (Fig. 1). The minimal and short-lived pH plasma depression immediately following the infusion was probably due to a slow uptake of the acid load from the intraperitoneal cavity (when compared to intravenous injection) and a rapid excretion of acid both branchially (~85%) and renally (~15%; calculated from Maren and Fine, ibid). Indeed, the fish exhibited an over-excretion of net H^+ to the water of 3.2 mmol kg^{-1} in the first 10.5 hours (Fig. 3), and the rate of excretion was still above control over the first 21.5 hours (resulting in a net H^+ loss of 4.3 mmol kg^{-1} when only 2 meq kg^{-1} had been infused). About 75% of the increase in ΔH^+ was due to a reversal of normal ΔHCO_3^- loss to a net uptake (or excretion of H^+, these are indistinguishable using the present methods; see Claiborne & Evans, ibid.). The remainder was driven by an elevation in total ammonia efflux. Similarly, a transbranchial over-compensation to acid infusion in the marine lemon sole (Parophrys vetulus; McDonald et al., J. Exp. Biol. 98:403-414, 1982) and the seawater adapted rainbow trout (Salmo gairdneri; Tang et al., J. exp. Biol. 134:297-312, 1988) have also been demonstrated. Thus, it appears that once the appropriate gill exchange mechanisms (see above) have been activated, net H^+ excretion continues well past the amount required for a compensation equivalent to the infused load.

When sculpin were acid loaded and subsequently exposed to decreased ambient salinities, the pattern of acid-base transfers changed. Fish in 20% seawater were able to regain normal (and above normal) plasma pH (Fig. 2) and $[HCO_3^-]$, though the net acid excretion was mainly due to an increase in ΔNH_4^+ loss while ΔHCO_3^- was negligible (Fig. 3). This is supported by our finding that non-infused sculpin can also maintain near normal ΔH^+ transfers in 20% seawater (Claiborne & Perry, ibid.). In contrast, following exposure to 4% seawater, plasma pH and $[HCO_3^-]$ remained below control for 4-7 hours, and the infused load was not excreted. A large net HCO_3^- loss at these low salinities (also observed in animals which were not acid loaded; Walton & Claiborne, ibid.) nullified an increase in net NH_4^+ excretion. We have hypothesized previously (Claiborne & Perry, ibid.) that changes in external $[Na^+]$ may be responsible for the apparent HCO_3^- or H^+ transfer imbalances at these low salinities. The present data indicate that even when potential acid excretory mechanisms (eg., Na^+/H^+ exchange) should have been stimulated by increased internal H^+, low external [NaCl] may still limit the degree of acid-base compensation which can be achieved by these animals.

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