

ACID-BASE TRANSFERS IN THE LONG-HORNED SCULPIN (MYOXOCEPHALUS
OCTODECIMSPINOSUS) FOLLOWING
ACID INFUSION: EFFECT OF LOW EXTERNAL SODIUM

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Long horned sculpin (Myoxocephalus octodecimspinosus) can excrete an infused acid load over 12-24 h when in seawater or dilute (20%) seawater, but the excretion is impaired in very dilute water (4%). The H⁺ excretion rate in seawater is also ~2x higher than in animals acutely exposed to the 20% dilution (Claiborne & Perry, Bull. MDIBL 31:54-56, 1992). Thus, presumed acid-base transfers across the gills (Na⁺/NH₄⁺, Na⁺/H⁺, and/or Cl⁻/HCO₃⁻ exchange; Evans, in "Fish Physiology", eds. W. S. Hoar & D. J. Randall, Vol Xb, pp. 239-283, 1984) are influenced by external counter ion concentrations. Preliminary evidence (Claiborne & Perry, Bull. MDIBL 30:107-108, 1991) indicated that external Na⁺ may be required for acid excretion to be maintained. To further delineate the transfer mechanisms in these fish, we have studied the effect of acid infusion (2 meq kg⁻¹ HCl) in animals pre-adapted to 20% seawater (~100 mM NaCl), and how exposure to low external [Na⁺] (<2% of normal seawater) following acid infusion may alter the pattern of regulation in these animals.

Long-horned sculpin (Myoxocephalus octodecimspinosus) were pre-adapted for 10-14 days in 20% seawater in an aerated box (10 liters) immersed in a running seawater tank (to maintain temperature). The water was changed every 24-48 h to prevent the accumulation of external NH₄⁺. After pre-adaptation, fish were cannulated and placed in experimental chambers according to the methods of Claiborne & Evans (J. Exp. Biol. 140:89-105, 1988) and Claiborne & Perry (1992, *ibid.*). Following a 10-12 h pre-infusion control flux period, the animals were infused with the acid load and either maintained in 20% seawater or the water was rapidly flushed with a pre-mixed and temperature equilibrated solution of 100 mM N-methyl-D-glucamine chloride (final [Na⁺]: 2.5 ± 0.8 mM, mean ± S.E., n=5). After a 4 h low Na⁺ post-infusion exposure, the water was returned to 20% seawater for a subsequent 20-20.5 h recovery period. During the pre-infusion, post-infusion, and recovery periods, water NH₄⁺ and HCO₃⁻ were measured so that cumulative transfers of H⁺ between the fish and the water could be calculated (Claiborne & Evans, *ibid.*). Blood samples were also taken throughout the experiment to monitor plasma acid-base status (not reported here).

Figure 1 shows the net transfer rates of NH₄⁺, HCO₃⁻, and H⁺ between the fish and the ambient water measured over the pre-infusion, post-infusion (calculated from either the 4 h low Na⁺ exposure or the first 3 h post-infusion in the group which remained in 20% seawater), and recovery (the subsequent 7.5-8 h) periods. Following 20% pre-adaptation, sculpin were able to significantly increase both NH₄⁺ excretion, and HCO₃⁻ uptake (or excretion of H⁺) after the acid infusion. 100% of the load had been excreted within 10.5 h, and in a fashion similar to seawater adapted animals (Claiborne & Perry, 1992, *ibid.*), an over-compensation by 1.9x of the administered load was transferred to the water by 23.3 h. Net H⁺ excretion in fish pre-adapted to 20% seawater was also 2x higher than that measured in

animals exposed acutely to the 20% dilution after an acid infusion (0.16 ± 0.04 in the present study versus 0.08 ± 0.05 $\text{mmol kg}^{-1} \text{hr}^{-1}$ from Claiborne & Perry, 1992, *ibid.*).

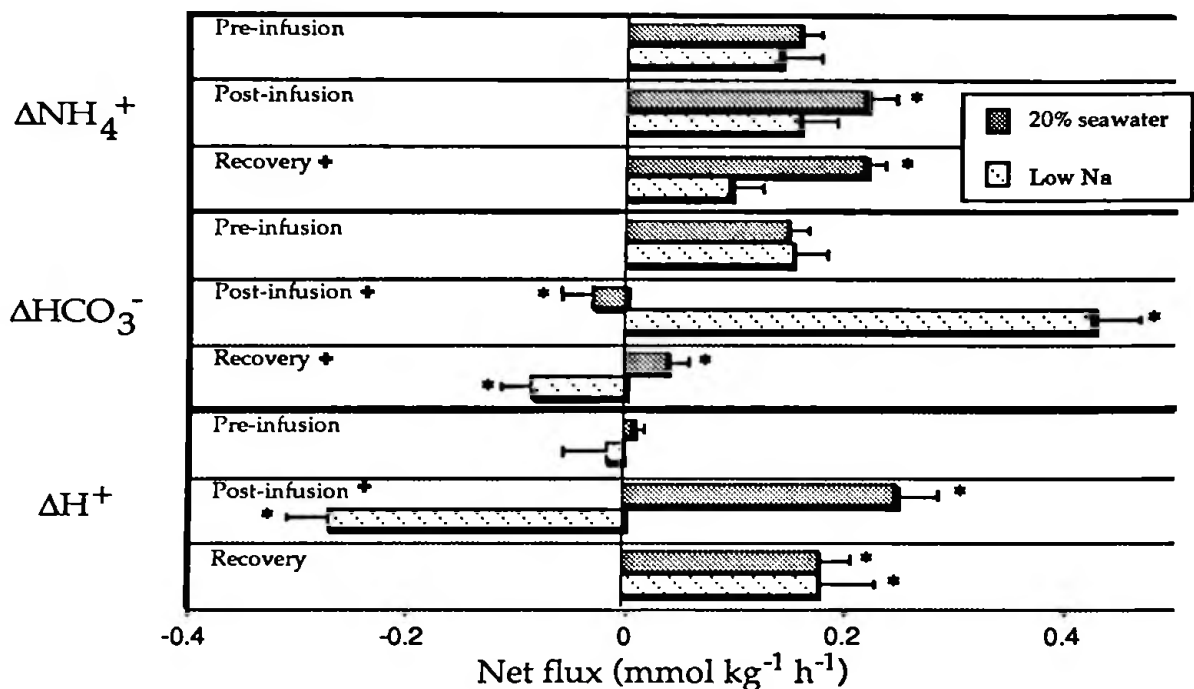


Figure 1. Net transfer rates of NH_4^+ , HCO_3^- , and H^+ ($\text{mmol kg}^{-1} \text{h}^{-1}$) before (Pre-infusion; 10.5-12 h), immediately after (Post-infusion; 3-4 h), and then 10.5-12 h after (Recovery; 7.5-8 h) an acid infusion in two groups of fish which were pre-adapted to 20% (~ 100 mM NaCl) seawater. During the Post-infusion period the low Na⁺ group was exposed to 100 mM NMDG-Cl. A positive net transfer rate indicates a net efflux from the animal. mean \pm S.E., $n=5$; +: 20% seawater group is significantly different from the low Na⁺ group during the indicated period; *: flux is significantly different from the Pre-infusion value for this group.

Sculpin placed in low Na⁺ water exhibited a very different pattern of acid-base transfers. ΔNH_4^+ did not change in this group following the acid infusion, and was significantly lower than the 20% seawater group during the recovery period. Also in contrast to the 20% group, net HCO_3^- loss increased by nearly 3x during the low Na⁺ period and then changed to a net HCO_3^- uptake during the recovery. The sum of these transfers resulted in a significant negative ΔH^+ (equivalent to a H^+ uptake) of -0.27 ± 0.04 $\text{mmol kg}^{-1} \text{hr}^{-1}$ during the post-infusion period. When the external Na⁺ was restored in the recovery period, ΔH^+ changed to an excretion of 0.18 ± 0.05 $\text{mmol kg}^{-1} \text{hr}^{-1}$, which was identical to the rate in the 20% seawater group. Thus, during the 4 h low Na⁺ exposure, the animals were unable to excrete any of the administered acid load (2 meq kg^{-1}), but surprisingly, "took up" an additional 1 meq kg^{-1} . When ambient Na⁺ was available, ~ 1.6 meq kg^{-1} of the acid was lost during the 8 h recovery period, and a total of 3.8 meq kg^{-1} was excreted over 20 h.

Adaptation to the hypo-ionic salinity (20% seawater) enabled the long-horned sculpin to compensate for an acid disturbance more effectively than animals acutely exposed to this dilution prior to acid infusion (Claiborne & Perry, 1992, *ibid.*). The pre-adapted animals were able to excrete the acid load at a rate similar to seawater fish. That this excretion was accomplished in the face of lower external ion concentrations implies that the osmoregulatory adjustments (e.g., alterations in gill exchanges and permeability) required to live in the dilute media have also presumably altered acid-base transfers across the gills of the fish. If a Na^+/H^+ exchange had been activated or amplified in the pre-adapted animals (to allow the uptake of Na^+ from the water; Evans, *ibid.*), this exchange would also permit the animal to more effectively excrete the administered acid load. Clearly, external Na^+ was necessary for appropriate transfers to take place. Furthermore, the increase in measured net HCO_3^- loss during the low Na^+ period (and following the acid infusion) may imply that a $\text{Cl}^-/\text{HCO}_3^-$ exchange was also operating and was only revealed when the Na^+/H^+ transfers were inhibited by the low Na^+ water. Preliminary experiments using low Cl^- media have indicated that this may be the case (Claiborne & Perry, 1991, *ibid.*) and this will be pursued in further studies.

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