PLASMA ION AND ACID-BASE REGULATION IN THE LONG-HORNED SCULPIN (MYOXOCEPHALUS OCTODECIMSPINOSUS) DURING EXPOSURE TO LOW SALINITIES

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In order for a fish to make the transition from seawater to fresh water, a number of osmoregulatory adjustments are required (Evans, in "Fish Physiology", eds. W. S. Hoar & D. J. Randall, Vol Xb, pp. 239-283, 1984). Our previous reports (Walton & Claiborne, Bull MDIBL 27:4-5, 1988; Compton-McCullough et al., Bull MDIBL 28:44-45, 1989) have examined the role of both branchial and renal transfers in the long-horned sculpin during the adaptation to dilute salinities. We showed that the inability of this species to adjust to very dilute salinities (<10% seawater) may have been due to impaired transbranchial HCO_3^- and/or H^+ transfer. While a large net loss of HCO_3^- to the water was measured during low salinity exposure (LSE), it was unclear as to the both the origin and mechanism of this loss. In the present study we have determined the effects of LSE on plasma [ion], total osmolarity, acid-base status (plasma pH and T_{CO_2}), and net transfers of Ca^{++} between the fish and the water.

Long-horned sculpin (Myoxocephalus octodecimspinosus) were cannulated and placed in experimental chambers according to the methods described by Walton & Claiborne (ibid.). Following a seawater control period of 10 to 14 hours, the animals were subjected to a 24 hour LSE (to water which had been prediluted to ~20 mM NaCl, measured as $[Cl^-]$; a concentration equivalent to approximately 4% of standard seawater), and then a subsequent 24 hour recovery period in normal seawater once again. Blood samples (~350 µl) were collected periodically throughout the experiment, and after centrifugation, aliquots of plasma were analyzed for $[Na^+]$ (IL flame photometer), $[Cl^-]$ (Haake Buchler chloridometer, $[Ca^{++}]$ (Perkin-Elmer atomic absorption spectrophotometer), $[PO_4^{--}]$ (Sigma inorganic phosphorus assay) and total osmolarity (T_{osm}; Wescor osmometer). Plasma pH and T_{CO_2} was also determined for each sample. During the LSE period, water $[Ca^{++}]$ was measured so that net transfers between the fish and the water could be calculated (as per the methods for ΔHCO_3^-; Claiborne & Evans, Bull. MDIBL 25:32-34, 1985).

As can be seen in Table 1, exposure to dilute media induced a significant decrease in plasma T_{osm}, $[Na^+]$, $[Cl^-]$, and $[Ca^{++}]$ over 24 hours. $[Na^+]$ and $[Ca^{++}]$ regained control levels when the fish were returned to seawater. In contrast, $[PO_4^{--}]$ increased significantly both during and after the LSE. Plasma T_{CO_2} also increased (due to a rise in both P_{CO_2} and $[HCO_3^-]$ while plasma pH remained relatively stable). During an LSE of 20 to 24.5 hours, a 2.28 ± .34 mmol kg^{-1} (n=5) net loss of Ca^{++} to water was observed.

LSE induces a rapid loss of HCO_3^- to the water (Walton & Claiborne, op cit.) even though plasma pH remains stable and plasma T_{CO_2} increases by ~50% (Table 1). The present data may help elucidate the origin of this base loss. As expected when a (somewhat) stenohaline teleost is exposed to low salinities, plasma ion concentrations decreased by approximately 25% over 24 hours, due to the loss of these ions to the dilute medium (Evans, op. cit.). In contrast, plasma $[PO_4^{--}]$ concurrently increased. It is also likely that PO_4^{--} was being lost to the water during this time. We would propose that the origin of this PO_4^{--}, and the measured net loss of base to the water, is demineralization of the bone compartment. While plasma $[Ca^{++}]$ decreased by 0.4 mM during LSE, the appearance of 2.3 mmol kg^{-1} in the water indicates that a large fraction of the Ca^{++} was derived intracellularly. Indeed, had the Ca^{++} originated solely from the ECF, plasma $[Ca^{++}]$

Table 1. Plasma [ion] and acid-base changes during and after exposure to dilute seawater in the long-horned sculpin.							
Period	T_{osm} (mOsm)	$[Na^+]$ (mM)	$[Cl^-]$ (mM)	$[Ca^{++}]$ (mM)	$[PO_4^{--}]$ (mM)	TCO_2 (mM)	pH
Control	378 ± 27 (4)	178 ± 4 (12)	163 ± 5 (12)	2.2 ± 0.3 (9)	1.9 ± 0.1 (6)	5.1 ± 0.4 (7)	7.85 ± 0.03 (7)
LSE (24 hr)	242 ± 10 (4)*	133 ± 3 (12)*	117 ± 4 (12)*	1.8 ± 0.3 (9)*	2.2 ± 0.2 (6)**	7.8 ± 0.5 (6)**	7.78 ± 0.04 (6)•
Seawater (22-24 hr)	312 ± 10 (4)*	174 ± 7 (10)•	148 ± 4 (10)*	2.6 ± 0.4 (6)•	2.5 ± 0.2 (6)**	6.2 ± 0.3 (3)**	7.96 ± 0.01 (3)•
Mean ± s.e. (n); * : significantly below control values; **= significantly above control; •: not significantly different from control ($p<0.05$; Student's paired t-test, two-tailed).							

should have decreased by more than 11 mM (given an extracellular space of 20%; see similar calculation for proton loads by Claiborne & Evans, J. Exp. Biol. 140:89-105, 1988), a value quite implausible considering that control plasma $[Ca^{++}]$ was 2.2 mM. Cameron (J. Exp. Biol. 117:307-18, 1985) demonstrated that Ca^{++} and PO_4^{--} were the two major mineral salts in the bone of the catfish (Ictalurus punctatus). While Cameron (ibid.) found no contribution of the bone compartment to acid-base regulation during hypercapnia, our indirect evidence would suggest that bone demineralization is a source of the observed acid-base alterations during LSE. Whether the bone contribution is an adaptive response to the dilution stress, an effect secondary to the diffusive loss of other ions, or a pathological condition which ultimately leads to the expiration of the animal, remains to be determined. (Funded by NSF DCM 86-02905 to JBC).