ACID-BASE REGULATION IN A STENOHALINE MARINE TELEOST (MYOXOCEPHALUS OCTODECIMSPINOSUS) DURING EXPOSURE TO DILUTE SALINITIES

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Both marine and fresh water fish are faced with ion- and osmoregulatory stresses keyed to the specific environment in which they live. Thus a fish (with a blood [NaCl] of ~150 mM) living in seawater (450-500 mM) must deal with a net diffusive gain of NaCl and an osmotic loss of H_2O. Homer Smith (Am. J. Physiol. 93:480-505, 1930) proposed that marine teleosts drink seawater to compensate for osmotic water loss, and at the same time, these animals actively extrude NaCl across the branchial epithelium (reviewed by Evans, Am. J. Physiol. 283:R224-R230, 1980). In contrast, fish living in fresh water (1 mM [NaCl]) must actively take up NaCl from the environment to compensate for diffusive salt loss, and usually excrete a dilute urine to balance osmotic water influx (Hickman & Trump, in "Fish Physiology", eds. W.S. Hoar & D.J. Randall, Vol I, pp. 91-239, 1969). Even more intriguing is the fact that several euryhaline families of fish (Salmonidae, Anguillidae, Mugilidae; see Evans, in "Fish Physiology", eds. W.S. Hoar & D.J. Randall, Vol Xb, pp. 239-283, 1984) are capable of living in waters of varying salinities and thus must modify transbranchial salt and water transfers to match the osmoregulatory demands of the new environment. Even some 'stenohaline' species may be capable of adjusting to changes in external salinity during variations in fresh water run-off or periodic migrations into estuarine systems (Wu & Woo, Aquaculture 32:175-181, 1983).

In order to inhabit brackish or fresh water environments, a seawater adapted fish must be capable of 'turning on' NaCl uptake systems. Several studies have now shown that at least some seawater and euryhaline species possess the necessary fresh water gill exchange systems, even when adapted to seawater (see Evans, 1984, ibid.). Transbranchial Na^+/NH_4^+, Na^+/H^+ and Cl^-/HCO_3^- exchanges are thought to be utilized by teleosts, not only for salt regulation, but for acid-base regulation as well. That several seawater species have been shown to excrete acid-base equivalents in exchange for the uptake of NaCl, is an indication of the importance of acid-base regulation to the animal, since in this case, these transfers would exacerbate the ionic load already faced by the hypo-osmotic fish (Evans, ibid., 1980). Thus, the immediate need to regulate internal pH may induce ion fluxes which amplify ionic/osmotic stresses. The converse may also be true: if marine teleosts do possess the branchial exchange mechanisms necessary for the uptake of NaCl, perhaps the linked transfer of acid-base equivalents becomes a limiting factor when some species enter brackish or fresh water. To explore this possibility, this study examines the role of acid-base regulation during the transition from seawater to lower salinities, in the stenohaline sculpin.

Sculpin (Myoxocephalus octodecimspinosus; mass = 161 $\pm$ 16 grams, mean $\pm$ S.E., n=5) were cannulated, placed in darkened plexiglas experimental chambers with fresh running seawater (15-17°C), and allowed to recover for 20-48 hours (as described previously: Claiborne & Evans, Bull. MDIBL 24:24-25, 1984). Prior to the start of the experiment, the running seawater was disconnected and continuous aeration of the chambers was begun. Control and experimental blood samples (0.2-0.4 ml) were drawn through the aortic cannula

and analyzed for $[Na^+]$, pH and Tco_2 from which plasma Pco_2 and $[HCO_3^-]$ could then be calculated. Water samples were periodically collected and the amounts of NH_4^+, HCO_3^-, and net H^+ transferred between the fish and the water were also determined (see Claiborne & Evans, 1984, ibid. and Claiborne & Evans, this volume, for more details). Following a control period, the sculpin were subjected to three experimental periods, each lasting 2 hours, during which the water within the fish chamber was slowly diluted to a new salinity by the addition of a well aerated and temperature equilibrated volume of tap water to one end of the box while draining from the other end (so as to provide minimal disturbance to the fish). At the completion of these periods, a fourth (and sometimes fifth) dilution of the water was made, and the fish were maintained at the new salinity for up to 70 hours. Death in these animals (defined as a cessation of ventilatory gill movements) was observed 13-61 hours subsequent to exposure at the lowest salinity. During each dilution period, the blood and water parameters described above, as well as the external osmolality of the water, was measured. Two dilution regimes were used: 100% seawater to 69%, 46%, 27%, and 14% seawater ('slow', n=3) versus 100% to 41%, 20%, 14%, and 8% ('fast', n=2).

Surprisingly, plasma $[Na^+]$ in most animals remained at ~160 mM even though the surrounding water had been diluted to 35-60 mM by the end of the experiment; perhaps an unexpected response from a 'stenohaline' marine teleost such as the sculpin. At the same time, animals in the 'slow' dilution group maintained plasma pH near control values (7.80; after an initial 0.15 unit decrease during the first two dilutions), while fish exposed to a more rapid dilution sequence exhibited a more drastic pH depression (from ~7.80 to ~7.60 over 22 hours). The observed pH depressions were due to a 0.5 - 2.0 torr increase in the plasma Pco_2 (control: ~2.0 torr). This respiratory acidosis was less dramatic and returned to near-normal values in the 'slow' animals (over 19 hours) while 'fast' fish became more hypercapnic over time. The hypercapnic acidosis in both groups was compensated by a ~1 to 4 mM increase in plasma $[HCO_3^-]$ (increased from a pre-experimental value of 5.0 mM) as has been observed in other species (see Claiborne & Evans, this volume). It should be noted that though the 'slow' animals did not exhibit the same time course of acid-base effects that was seen in the 'fast' fish, all blood acid-base parameters measured in one 'slow' animal subsequent to an additional 25 hours in 8% seawater, approached that of the 'fast' fish. It remains to be seen if the respiratory acidosis and compensatory pH adjustment observed here actually impinge of the survival of the sculpin in low salinities, however, a further stress imposed by this exposure becomes apparent when the transfers of acid-base equivalents between the fish and the water are examined. While only a minor increase in ΔNH_4^+ excretion (~0.7 mMole/kg in the majority of animals) was noted, a large net loss of HCO_3^- to the water (7x the ammonia loss) occurred as sculpin were exposed to the lower salinities. This increase began sooner in the 'fast' fish and is summarized in Figure 1. ΔH^+ is calculated as the difference between ΔNH_4^+ and ΔHCO_3^-. Net ΔH^+ values are the difference between control and experimental ΔH^+ at any time. Upon examination of Figure 1, it is evident that the animals are gaining H^+ from (or losing HCO_3^- to) the dilute water. After exposure to the lowest salinity for ~13-17 hours, the net amount of H^+ taken up averaged 5.57 $\pm$ 0.67 mMole/kg, an acid load comparable to that observed in the spotted dogfish (Scyliorhinus stellaris) during strenuous exercise (Holeton & Heisler, J. Exp. Biol. 103:31-46, 1983). Though we have no direct evidence, intracellular compartments must have contributed to the measured transfer, since an increase in plasma $[HCO_3^-]$ was observed concurrent to the large net

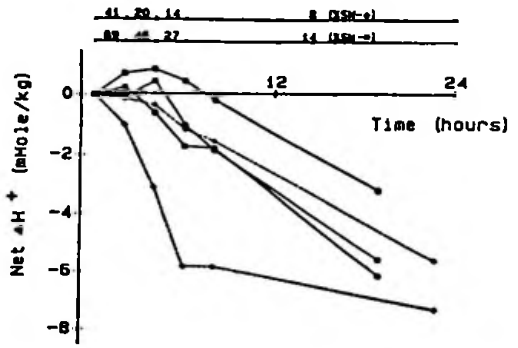

Figure 1. Net ΔH^+ transfers by 5 sculpin during several seawater dilutions (% seawater shown in top axis). Data for fish exposed to the faster dilution sequence (- - -) are depicted with: ◆; animals subjected to slower dilutions (——) are described by: ■. A negative net ΔH^+ indicates a net uptake of H^+.

HCO_3^- efflux. In all fish tested, the net loss of HCO_3^- began as the animal was exposed to the 41-46% seawater period. It may be important that 41% seawater is approximately iso-osmotic with sculpin blood. Further dilution of the media to hypo-osmotic levels elicited increased rates of HCO_3^- loss.

Clearly, a test of more animals is required before statistical validity of these observations can be made, nevertheless, several questions present themselves. First, why do these animals lose HCO_3^- (or gain H^+) as they are exposed to water of decreasing salinities? Were these transfers associated with ion regulatory exchanges? That plasma [Na^+] did not decrease during the experiment, could be an indication that Na^+/H^+ exchange was not responsible for the apparent H^+ influx. As noted above, a fish entering dilute water must begin to actively take up NaCl from the environment, thus, a Cl^-/HCO_3^- transfer would be appropriate for ion-regulation. Though we presently have no data on plasma [Cl^-] changes, it would seem maladaptive for sculpin to jeopardize intracellular acid-base equilibrium solely for the utilization of a counter-exchange ion (HCO_3^-) to permit the uptake of Cl^-. Perhaps the rapid net loss of HCO_3^- (or gain of H^+) was a cause of the final expiration of the animals after exposure to the lowest salinities. Though the sculpin are not as strictly 'stenohaline' as we would predict, they were not able to adjust to the low salinities and the time course utilized here. One could hypothesize that the rapid loss of intracellular HCO_3^- pools contributed to the death of these fish. Interestingly, the exposure of a single individual of a known euryhaline species (the toadfish, Opsanus tau) to the same salinity regime as the 'fast' fish, also resulted in a large net ΔHCO_3^- loss to the water (more than 19 mMole/kg over 72 hours), though the fish was maintained (and survived) in 5% seawater for more than 96 hours when the experiment was finally terminated (Claiborne & Evans, unpublished observations). In contrast to the sculpin, the rate of net ΔHCO_3^- loss from the toadfish was slowly ameliorated over the length of the low salinity exposure so that even though the average rate of transfer was similar in both species (0.25 mMole kg^{-1} h^{-1}), the measured net Δ loss in the toadfish ranged from a high of 0.73 mMole kg^{-1} h^{-1} after 2 hours to a low of 0.16 mMole kg^{-1} h^{-1} after 72 hours - thus approaching the control rate of transfer again. Though only a single observation, these results cloud the simplicity of the above hypothesis. That the increase in acid-base transfers also occurred in a euryhaline fish, may be an indication of the adaptive linkage between acid-base and ion exchanges which were employed to cope with the dilution stress. Perhaps it is the secondary limitation in the rate of HCO_3^- loss after several days in hypo-osmotic media (via modification of gill ion exchange systems or a reduction in overall branchial permeability; eg., Evans, op. cit., 1984) which allows the toadfish (and other euryhaline) species to survive in dilute brackish and fresh water. This issue will be addressed in future studies. (Funded by a Faculty Res. Grant from GSC to JBC and NSF PCM 83-02621 to DHE).