EFFECT OF SALINITY ALTERATIONS AND RESPIRATORY ACIDOSIS ON ACID-BASE TRANSFERS IN THE MUMMICHOG (*FUNDULUS HETEROCLITUS*)

Jill C. Weakley[1], Samuel Sligh[2], Alison I. Morrison-Shetlar[1] and James B. Claiborne[1]
[1]Department of Biology, Georgia Southern University, Statesboro, GA 30460
[2]Hillside High School, Durham, NC

An elevation in the levels of ambient CO_2 rapidly induces respiratory acidosis in fish (Claiborne, J.B. In: *The Physiology of Fishes*. 2nd Edition, ed.: D.H. Evans, CRC Press, 179-200, 1998). While nonbicarbonate buffering plays a role in the restoration of plasma pH, gill transepithelial exchange of acid-base relevant molecules has been shown to be the major mechanism during compensation in most species (Claiborne, J.B., et al., J. Exp. Zool. 279: 509-520, 1997). These transfers involve Na^+/H^+ and Cl^-/HCO_3^- exchanges with the surrounding media. In freshwater adapted fish, H+ excretion (thought to be *via* H+-ATPase; Perry, S.F., et al., J. Exp. Biol. 203: 459-470, 2000) linked to Na^+ uptake would be optimal for both ion uptake as well as compensation of acid-base stress, but Na^+/H^+ exchange would cause an increase in the ionic and osmotic stresses already faced by fish adapted to seawater. The mummichog (*Fundulus heteroclitus*) is a euryhaline species which inhabits salinities ranging from freshwater to full-strength seawater. Little is known about the gill mechanisms of acid-base balance in this species, but the gill regulatory strategies utilized by freshwater adapted mummichogs appear to be very different from other freshwater fishes to date (Patrick, M.L. and Wood, C.M., Comp. Biochem. Physiol. 122: 445-456, 1999). We have recently shown that proteins immunologically similar to mammalian NHE-3 are expressed at higher levels in seawater adapted mummichogs exposed to external hypercapnia (Wall, B., et al., Bull. Mt. Desert Is. Biol. Lab. 40: 58-59, 2001) and the expression may be dependant on the salinity to which the animals are adapted (Edwards, S.L., et al., FASEB Journal (Abstracts) 15: supplement, pg. 11, LB43, 2001). The aim of this study was to collect baseline data towards understanding the net H^+ transport across the gills in this species adapted to various salinities and subjected to a respiratory acidosis.

Fundulus heteroclitus (3.86 ± 0.36 g; mean ± S.E., n=31) were caught in minnow traps in Northeast Creek, on Mount Desert Island, and acclimated in 10 gallon tanks for seven to 10 days. Fish were placed in one of three acclimation salinities: freshwater (FW: dechlorinated tap water + 1% seawater; ~10 mOsm), isoosmotic "brackish" water (BW: seawater diluted with dechlorinated tap water; ~325mOsm), or seawater (SW: ~930 mOsm). Aeration was accomplished from bubbling and filtering of the tanks, except the seawater tank which received running seawater. Following acclimation, animals were weighed, placed in individual plastic experimental chambers (total volume 150 mls) and a 13 hr control period with normal aeration was begun. Water samples (8 ml) were collected at the start and end of the control period. The water was then flushed with a fresh 150 ml volume of the same salinity and the air bubbled into the chamber was either maintained (normocapnia series) or modified to a 1% CO_2 in air mixture (hypercapnia series) from a GF-2 Gas Mixing Flowmeter (Cameron Instruments). In most cases, gases were bubbled into the fish chamber through small plastic pipette tips to avoid potential leaching of buffers from air stones and the confounding effect on water titration analysis which this caused (unpublished data). Over the 23 hour experimental period (water was flushed at hour 8 to prevent high ammonia levels (Claiborne, J.B., et. al., J. Exp. Biol. 193: 79-95, 1994) the media was periodically sampled (hr 0, 1, 2, 4, 8, 23). Volumetric titration of 2 ml portions of the water samples to a pH of 4.00 (FW samples: 0.025N

HCl; BW and SW samples: 0.1N HCl), analysis for total ammonia, and the calculation of net H^+ transfers between the fish and the water (ΔH^+ in mmol kg^{-1} h^{-1}) were calculated as described by Claiborne et al. (*op. cit.*).

As shown in Table 1., a significant increase in ΔH^+ excretion was measured in hypercapnic fish adapted to both FW and BW (measured over the first 8 hours of the experiment) when compared to control fish in the same salinities. High variability in the control SW fish clouded the resolution of the SW group, but paired analysis of the pre-hypercapnic control period (-0.31 ± 0.29 mmol kg^{-1} h^{-1}) with the post-hypercapnic rates (0.67 ± 0.28 mmol kg^{-1} h^{-1}), indicated a significant increase in ΔH^+ excretion was induced by hypercapnia in this group as well (paired t-test, one-tailed; $p<0.05$).

Table 1. ΔH^+ transfer rates between the fish and the water (over 8 hours) in *Fundulus heteroclitus* adapted to freshwater (FW), isoosmotic water (BW) and seawater (SW). The Net ΔH^+ is calculated as the mean difference between control and hypercapnic ΔH^+ rates for each group and represents the net increase in acid transfers from the animals during hypercapnia. Mean ± S.E. (N). One-tailed, unpaired t-test between groups. * indicates $p < 0.05$.

	Normocapnia (mmol kg^{-1} h^{-1})	Hypercapnia (mmol kg^{-1} h^{-1})	Net ΔH^+ (mmol kg^{-1} h^{-1})
FW	-0.47 ± 0.26 (5)	0.22 ± 0.08 (5)	0.70 *
BW	0.12 ± 0.07 (5)	0.52 ± 0.05 (5)	0.40 *
SW	0.17 ± 0.63 (6)	0.67 ± 0.28 (5)	0.50 (ns)

As has been demonstrated in several marine and freshwater species, hypercapnia induces a net increase in H^+ efflux in *Fundulus* adapted to all three salinities. This allows compensation of internal acidosis during the hypercapnic exposure (see review by Claiborne, op. cit.). Because of the control ΔH^+ uptake (or HCO_3^- loss) in the FW animals, the net ΔH^+ rate induced by hypercapnia was the highest in this group. At the same time, the magnitude of ΔH^+ transfers was the lowest in the FW while the BW and SW rates were similar (both increasing by ~4x over control), perhaps indicating the impact of external counter ion availability on the absolute rate of transport (Claiborne, op. cit.). Interestingly, the normocapnic FW fish lost ~9.15 mmol kg^{-1} of HCO_3^- over the 23 hour period. We also noted high rates of net HCO_3^- loss in long-horned sculpin (*Myoxocephalus octodecimspinosus*) and the gulf toadfish (*Opsanus tau*) following transfer to low salinities (Claiborne, et al., op. cit.; Claiborne, J.B., et al., J. Fish Biol. 56: 1539-1544, 2000), though the present values are more than double that of the euryhaline toadfish. Similar net H^+ uptake rates have also previously been reported in another study on this species (Patrick and Wood, *op. cit.*). It is unclear as to the root cause of the acid uptake, but the imbalance does imply that the mummichog is not in acid-base homeostasis when exposed to the most dilute salinity used in this study.

Immunological detection of Na^+/H^+ exchangers in the gills of this species has shown that NHE expression increases within one hour of 1% hypercapnic exposure (Wall et. al., op. cit.). Clearly, the mummichog is able to dramatically increase H^+ transfer to the water following hypercapnia. We speculate that NHE driven transfers are responsible for the acid-base compensation observed *in vivo*. This research supported by NSF IBN-9808141 and IBN-0111073 to J.B.C. and A.I.M.S, NSF REU DBI-9820400 to JCW and a Hancock County Fellowship to SS.