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ACID–BASE REGULATION, BRANCHIAL TRANSFERS AND RENAL OUTPUT IN A MARINE TELEOST FISH (THE LONG-HORNED SCULPIN *MYOXOCEPHALUS OCTODECIMSPINOSUS*) DURING EXPOSURE TO LOW SALINITIES

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Summary

A number of studies have implied a linkage between acid–base and ion exchanges in both freshwater and seawater fish, although little is known about the branchial and renal acid–base transfers involved as the animals move between different salinities. To investigate the role of these transfers in a marine teleost fish as it is exposed to a dilute environment, we measured plasma acid–base values and net movements from fish to water of NH_4^+, HCO_3^- and H^+ in long-horned sculpin (*Myoxocephalus octodecimspinosus*) placed in 100%, 20%, 8% or 4% sea water for 24–48h. Renal excretion of H^+ was also monitored in fish exposed to 4% sea water.

Sculpin proved to be somewhat euryhaline for they were able to maintain plasma ion and acid–base transfers in hypo-osmotic (20%) sea water, but could not tolerate greater dilutions for more than several days. Plasma pH and carbon dioxide concentration (C_{CO_2}) increased in the 20% and 8% dilution groups, with C_{CO_2} nearly doubling (control, $4.56 mmol\,l^{-1}$; 8% group, $8.56 mmol\,l^{-1}$) as a result of a combined increase in the partial pressure of plasma CO_2 (P_{CO_2}) and [HCO_3^-]. During a 44–46h exposure, HCO_3^- transfers increased progressively in the most dilute water, with animals in the 8% and 4% groups exhibiting a net H^+ loss that was smaller than that of seawater fish (control, $5.1 mmol\,kg^{-1}$; 8%, $0.9 mmol\,kg^{-1}$; 4%, $-2.9 mmol\,kg^{-1}$). Animals exposed to 4% sea water for 24h and then returned to normal sea water had a variable plasma pH, an elevated C_{CO_2} and a net efflux of H^+ that effectively stopped (control, $0.10 mmol\,kg^{-1}\,h^{-1}$; 4%, $0.02 mmol\,kg^{-1}\,h^{-1}$; seawater recovery, $0.20 mmol\,kg^{-1}\,h^{-1}$) during the low-salinity period. Renal acid excretion remained relatively constant throughout the experiment but only made up a significant portion (approximately 40%) of the total acid transfers during the 4% dilution period (control rate approximately $3\,\mu mol\,kg^{-1}\,h^{-1}$: 3% of branchial rate).

We postulate that the increase in plasma C_{CO_2} during exposure to low salinity may be due to mobilization of base from the intracellular bone compartment. The decrease in external salinity could induce base loss by alteration of gill ion exchanges (Na^+/H^+,

Key words: ammonia, pH balance, H^+ excretion, salinity, stenohaline, euryhaline, *Myoxocephalus octodecimspinosus.*

Cl^-/HCO_3^-) and/or changes in branchial HCO_3^- permeability. For the first time, we have shown that the effects of a dilute environment on acid–base transfers may be an important limitation to the survival of a euryhaline species in brackish or fresh water.

Introduction

When a marine euryhaline teleost is exposed to lower ambient salinities, several adaptive modifications in ion- and osmoregulatory processes are required. As the external milieu is diluted past the iso-osmotic point, the animal must begin to excrete a more dilute urine (Hickman and Trump, 1969; Lahlou *et al.* 1969) and limit the osmotic influx of water (Isaia *et al.* 1979). Internal salt conservation must also take place when the fish is in the dilute environment, and this is assisted by decreases in unidirectional efflux (Lahlou and Sawyer, 1969; see review by Evans, 1984) resulting from a reduction in branchial permeability (Zadunaisky, 1984). Long-term adaptations are probably driven by increased levels of circulating prolactin (reviewed by Loretz and Bern, 1982). Likewise, salt extrusion by branchial chloride cells (Silva *et al.* 1977) must be reduced in fresh water, and the net branchial uptake of NaCl must begin. Evidence for the uptake of NaCl by Na^+/NH_4^+, Na^+/H^+ and Cl^-/HCO_3^- exchange mechanisms has been found in a number of freshwater species (Maetz and Garcia-Romeu, 1964; Cameron, 1976; Claiborne and Heisler, 1984; Wood *et al.* 1984; Iwama and Heisler, 1991). Some seawater and euryhaline species may also possess the necessary freshwater gill exchange systems even when adapted to sea water (Evans, 1977; Claiborne *et al.* 1982; McDonald *et al.* 1982; reviewed by Evans, 1993) as these exchanges are thought to assist the animal in maintaining acid–base balance (Cameron, 1976; Claiborne and Evans, 1988). That seawater species excrete acid–base equivalents in exchange for the uptake of NaCl could be an indication of the importance of maintaining acid–base regulation (even though these exchanges would exacerbate the ionic load already faced by the hypo-osmotic fish; Evans, 1980).

If the immediate need to regulate internal pH can induce ion fluxes that amplify ionic/osmotic stresses, the converse should also be true: the lack of appropriate counter-exchange ions may inhibit acid–base adjustments. Indeed, compensatory H^+ excretion (or HCO_3^- uptake) during external hypercapnia in the marine gulf toadfish is approximately six times faster than in the freshwater carp (Evans, 1982; Claiborne and Heisler, 1986). Tang *et al.* (1989) showed that seawater-adapted rainbow trout could excrete an acid load (induced by exercise) approximately five times faster than freshwater-adapted conspecifics. Likewise, Iwama and Heisler (1991) recently demonstrated that recovery of plasma pH during hypercapnia in the trout could be enhanced when fish were adapted to higher external water salinities. The time course of hypercapnic compensation in the freshwater trout is also much longer (3 days *versus* 22h) in animals exposed to very low ambient ion concentrations (Janssen and Randall, 1975; Eddy *et al.* 1977; Heisler, 1982).

Several studies have explored the effects of salinity changes on internal acid–base balance and ion-regulatory status (Milne and Randall, 1976; Bath and Eddy, 1979). Perry and Heming (1981) observed that, following transfer of freshwater trout (*Oncorhynchus mykiss*) to sea water, blood pH was increased significantly because of an elevation in

plasma [HCO_3^-]. They suggested that this was due to an increase in the coupled influx of HCO_3^- for the excretion of Cl^- (an adaptive ion-regulatory maneuver for a fish entering sea water). Likewise, seawater-adapted salmon (*Salmo salar*) and flounder (*Platichthys flesus*) exhibit long term plasma acid–base and ventilatory changes when exposed to fresh water (Maxime *et al.* 1990; Nonnotte and Truchot, 1990). The inherent relationship between acid–base and ion movements is therefore found in animals exposed to either hypo- or hyperionic conditions. Little is known about the acid–base transfers between the fish and the water that may occur during these salinity changes.

A significant capacity for renal acid–base excretion following acid–base disturbances has also been shown in several species of freshwater teleost (McDonald and Wood, 1981; Wheatly *et al.* 1984). In the freshwater catfish, renal output accounts for 16–18% of the total measured transfer (Cameron and Kormanik, 1982). Less is known about the role of the kidney of seawater teleosts in acid–base regulation. In contrast to freshwater fish, the kidneys of marine animals are generally thought to be involved with the conservation of water and the excretion of divalent cations (for a review, see Sullivan, 1986). Hodler *et al.* (1955) suggested that the pH of urine in marine teleosts is fixed at 5.8, but Hickman and Trump (1969) demonstrated that urine pH (in the southern flounder) can range from 5.68 to 8.24. McDonald *et al.* (1982) found little involvement of the kidney during recovery from acid infusion in the seawater lemon sole (*Parophrys vetulus*). In contrast, Maren *et al.* (1992) have recently shown a role for limited net H^+ excretion by the kidney (28% of total infused acid load) in the marine long-horned sculpin and that the rate of transfer is not dependent on renal carbonic anhydrase. It is likely, therefore, that, when marine fish are exposed to lower ambient salinities, both branchial and renal acid–base regulation are affected. It was the objective of the present study to examine the effects of acute exposure to hypo-osmotic salinities on acid–base regulation, branchial exchanges and renal excretion in a stenohaline marine teleost, the long-horned sculpin.

Materials and methods

Long-horned sculpin (*Myoxocephalus octodecimspinosus* Mitchill) were collected by a commercial fisherman in Frenchman Bay, Maine USA. They were maintained in running seawater tanks at 13–15°C for 2–5 days before the experiment began. Fish were anesthetized with tricaine methane sulfonate (MS-222, 1:10000), placed in a moist tray and cannulated through a branchial artery according to the methods of Claiborne and Evans (1988). Animals were placed in darkened 2.0–2.5l acrylic boxes containing fresh running sea water (14–16°C) and allowed to recover from anesthesia for 12–36h. During a subsequent control flux period of 10–12h, the chamber was closed and the sea water within the box was continuously aerated. When a dilution experiment was to begin, control blood samples (250–300 μl) were collected and the chamber was then flushed several times with water of the desired salinity and finally filled with this water. This change was completed within 3–5min. External concentrations tested were: 20, 8 or 4% of the normal seawater concentration (measured as [Cl^-]; natural sea water contained approximately 500mmol l^{-1} Cl^-). All dilutions were hypo-osmotic to the animals: ambient [Cl^-] in the three solutions was approximately 62.5, 25 and 12.5% of control

plasma [Cl^-] (typically 160mmol l^{-1} in seawater-adapted animals). A control group was allowed to remain in 100% sea water for the length of the experiment (normally 48–72h). Preliminary studies had shown that acute exposure to the 4 and 8% dilutions for 48–60 h was often lethal, so these series were usually limited to 24 and 36h, respectively. In some experiments, fish were exposed to 4% water for 24h, followed by a 24h recovery period in sea water.

During the period of exposure to low salinity (LSE), plasma pH (pH_{pl}), total CO_2 (C_{CO_2}) and plasma [Cl^-] ($[Cl^-]_{pl}$) were regularly measured. Likewise, 20ml water samples were collected for the subsequent analysis of total ammonia (T_{amm}) and titratable base. Water [Cl^-] was periodically monitored during the experiment and the chambers were intermittently flushed with water (every 8–12h) to maintain the desired concentration and to prevent the accumulation of external ammonia.

pH_{pl} was measured in a small-volume pH electrode (30–50 μl; Instrumentation Laboratory Inc.) connected to an Orion Research EA 920 expandable ion analyzer. The electrode was mounted in a custom-built acrylic water jacket, which contained a reference electrode (Corning X-EL) and a KCl bridge, around which water was circulated at 15°C (Neslab Endocal RTE-9B). After centrifugation in heparinized capillary tubes, duplicate plasma samples were conductometrically analyzed for C_{CO_2} (Capni-con 3a; Cameron Instrument Company). Plasma P_{CO_2} and [HCO_3^-] were then calculated from pH and C_{CO_2} using values for CO_2 solubility at 15°C taken from Heisler (1984) and for pK′ (taken from Boutilier *et al.* 1984; from original data of Albers and Pleschka, 1967). Duplicate 20 μl plasma samples were also utilized to measure $[Cl^-]_{pl}$ by coulometric titration (Haake Buchler chloridometer).

Water T_{amm} was measured according to the methods of Solorzano (1969). Net titratable base was determined by duplicate volumetric titrations of 8ml water samples to pH3.800 as described by Claiborne and Evans (1988), based on the methods of Cameron and Kormanik (1982). Combination pH electrodes (Orion Research, Inc.) were calibrated in standard KH_2PO_4/Na_2HPO_4 buffers at normal concentrations or adjusted to approximate seawater [NaCl] (450mmol l^{-1}). The titration accuracy and span of the electrode in salinities ranging from sea water to 4% sea water were tested by titrating water samples to which aliquots of acid had been added. Only electrodes which exhibited a low selectivity and response to changes in ionic strength were chosen for titratable base determinations. The net change in HCO_3^- (ΔHCO_3^-, mmol kg^{-1}) was calculated as the difference between the titratable base at the beginning and end of each time interval after multiplying by the volume of the experimental bath and adjusting for the volume changes caused by water sampling and the mass of the animal. ΔNH_4^+ was determined in the same manner, from the changes in T_{amm} between periods. ΔH^+ was calculated as the difference between ΔNH_4^+ and ΔHCO_3^- (see Claiborne and Heisler, 1986; Heisler, 1986).

In order to measure the effect of acute LSE (4% sea water) on renal output, sculpin (205–444g, N=9) were anesthetized as above, and the opening of the urinary papilla was catheterized with PE-50 tubing. The perforated end of the catheter was pushed well within the bladder (2–4cm) before the papilla of the fish was ligated. The animals were then placed in the experimental chambers as described above. The external end of each urine catheter was placed in a pre-weighed vial attached to the outside of the darkened

box. Each vial was pre-treated with 10 μl of streptomycin solution (0.25 g l^{-1}) to inhibit bacterial growth (Cala, 1977). Urine was then collected over a 12–24h seawater period, a 24h LSE, and another 12–24h period in normal sea water. At the beginning of the LSE, the chamber was closed and flushed several times, and then filled with water that had been pre-diluted to 4% sea water and equilibrated to 15°C. Throughout the experiment, collection vials were periodically changed and weighed and the urine was then decanted for analysis of pH, $[Na^+]$, $[Cl^-]$ and $[NH_4^+]$. Urine pH was measured either in a small-volume pH electrode (as above for pH_{pl}) or in a small test tube (200–400 μl) with a narrow tip combination electrode and a model 901 ion analyzer (both from Orion Research). $[Na^+]$ was analyzed using flame photometry (Instrumentation Laboratories model 943); $[Cl^-]$ and T_{amm} were analyzed as above. To measure titratable acidity, a portion of the urine (0.5–1.0ml) was titrated with 100mmol l^{-1} NaOH using a syringe micrometer burette (model SB2, Micro Metric Instrument Co.) to a pH of 7.80 (as modified from Cameron and Kormanik, 1982). Net ion and H^+ excretion rates were calculated as the differences between individual ion concentrations (or, for H^+, titratable acidity) between each urine sample after accounting for the collection volume, time and mass of the animal.

All calculations were performed using spreadsheet software (Excel 3.0) on a Macintosh II computer (Apple Computer). Student's *t*-tests for paired or unpaired data (one- or two-tailed) were applied where appropriate. A control for overall comparison error rate in repeated-measures data was made by using the Bonferroni procedure (overall protection level, 0.05 calculated as α/k; where α is the individual critical level and k is the number of comparisons; Miller, 1985). *Post-hoc* Tukey HSD tests were used to evaluate differences between experimental periods in repeated-measures data (see Figs 4, 5).

Results

Changes in plasma $[Cl^-]$ during exposure to sea water and to 20, 8 or 4% sea water are shown in Fig. 1. $[Cl^-]_{pl}$ values of animals in 20% sea water were significantly lower than those of the control group at 24h (control, 158±1mmol l^{-1}; 20%, 148±1.5mmol l^{-1}; mean ± S.E.M.) and 48h, but had returned to normal values by 72h. $[Cl^-]_{pl}$ of fish in 8% sea water fell to 141mmol l^{-1} by 10h and continued to drop throughout the experiment. Exposure to the lowest salinity (4% sea water) induced a rapid drop in $[Cl^-]_{pl}$, which fell to 105±4mmol l^{-1} within 24h.

Plasma pH (pH_{pl}) was somewhat variable but significantly different from control values in at least one sampling time for all three dilution groups (Fig. 2A). pH_{pl} in the 20% seawater fish was significantly above the control groups at 8h and again at 48h. At 24h, pH_{pl} in the 8% LSE group had also increased significantly, while pH_{pl} in the 4% group had fallen below controls. Plasma C_{CO_2} (Fig. 2B) also increased above control levels in both the 8% and 20% groups from 35 to 72h.

Exposure to decreased salinities induced a change in the net transfers of NH_4^+ and HCO_3^- with a resulting alteration in H^+ excretion (Fig. 3). In general, the greatest effect on the measured transfers was observed in animals in the lowest salinities. Fish in 4% sea water excreted 12.9mmol kg^{-1} of NH_4^+ over 44h of LSE, while NH_4^+ transfers from

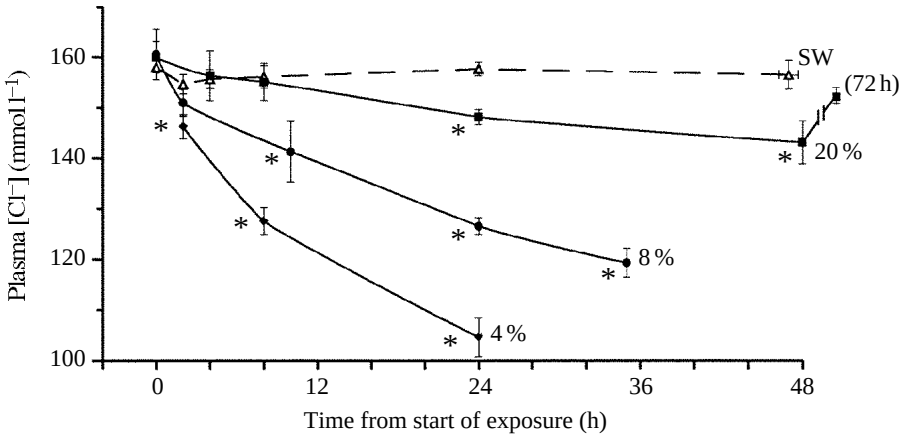

Fig. 1. The effect of decreasing external salinity on plasma [Cl^-]. Four groups of fish were exposed to sea water (SW: *N*=6) and diluted sea water (20%, *N*=4; 8%, *N*=4; 4%, *N*=3; mean ± S.E.M.). Asterisks represent values that are significantly different from the seawater groups. The point labeled 72h represents a sample taken after 72h of exposure to 20% sea water. Horizontal error bars for the rightmost SW point represent the S.E.M. of the mean time of sample collection for that sample.

control fish amounted to only 8.5mmolkg^{-1} over 46h (P<0.05; Fig. 3A). Cumulative HCO_3^- transfer increased significantly in all groups exposed to lower salinities (Fig. 3B). At the end of 46h, control fish had lost 3.4mmolkg^{-1} of HCO_3^- to the water, while the 20%, 8% and 4% groups had transferred 7.6mmolkg^{-1}, 9.7mmolkg^{-1} (44h) and 15.8mmolkg^{-1} (44h) respectively. ΔH^+ transfer (calculated as the difference between ΔNH_4^+ and ΔHCO_3^-) in the 8% and 4% LSE groups was below that observed in seawater controls. Control ΔH^+ amounted to excretion of 5.1mmolkg^{-1} at the end of the 46h, whereas at 44h the 8% and 4% groups had lost 0.9 and −2.9mmolkg^{-1}, respectively (a negative ΔH^+ indicates a net gain of H^+ from the water).

Figs 4 and 5 illustrate results from a group of fish in which the changes in pH_{pl}, C_{CO_2} and acid–base transfers were measured during a 24h exposure to 4% sea water (LSE) and then a return to normal sea water. pH_{pl} (Fig. 4) varied around control values (7.85±0.03, *N*=7) but decreased significantly between 8 and 24h (*N*=6, P<0.05) and then increased above the control value by the end of the recovery period (*N*=3, P<0.05). Plasma C_{CO_2} increased significantly by 24h and returned to pre-LSE levels by 46h. Plasma P_{CO_2} and [HCO_3^-] were also elevated by the end of the 24h exposure (P_{CO_2}: control, 0.21±0.01kPa; 24h, 0.38±0.04kPa. [HCO_3^-]: control, 5.06±0.41mmol l^{-1}; 24h, 7.65±0.52mmol l^{-1}; P<0.01).

LSE induced an increase in NH_4^+ efflux (Fig. 5A) during the first 12h (control, 0.19mmolkg^{-1}h^{-1}; peak at 4–8h, 0.37mmolkg^{-1}h^{-1}). Likewise, HCO_3^- efflux (Fig. 5B) increased during the first 12h of the LSE period, and the peak rate of efflux was observed at 4–8h, when the animals lost 0.34mmolkg^{-1}h^{-1} (control rate, 0.09mmolkg^{-1}h^{-1}). The resulting H^+ efflux (Fig. 5C) effectively stopped during the

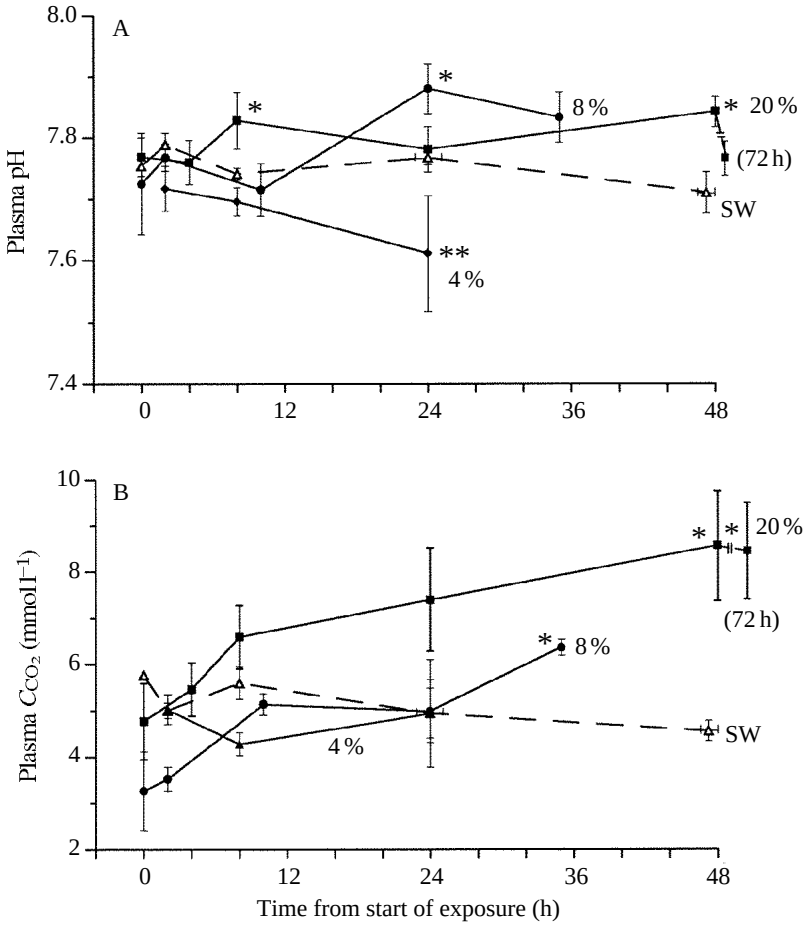

Fig. 2. The effect of decreasing external salinity on plasma pH (A) and total CO_2 (C_{CO_2}; B). Treatment groups are the same as in Fig. 1. Values are mean ± S.E.M. Single asterisks represent values that are significantly above seawater means; double asterisks represent values that are significantly below the value for the seawater group. For clarity, error bars for the SW and 8% groups are not shown at 24h, but are 0.54 and 0.68mmol l^{-1}, respectively. Horizontal error bars at 24h and 48h for SW represent the S.E.M. of the mean time of sample collection for those samples.

final 16h of LSE (compared with a control rate of 0.10mmolkg^{-1}h^{-1}). When the animals were returned to normal sea water, NH_4^+ excretion again increased significantly for the first 10h, while HCO_3^- efflux decreased to control values. These changes resulted in a significant increase in H^+ efflux above control values during the first 2h after the return to sea water (0.40mmolkg^{-1}h^{-1}). During the first 10h following the return to sea water, average H^+ efflux was also 0.16mmolkg^{-1}h^{-1} higher than that measured in the last 20h of LSE.

Changes in selected renal variables in response to LSE are shown in Table 1. Urine pH values ranged from 6.21 to 7.61 during the control period, and there were no significant

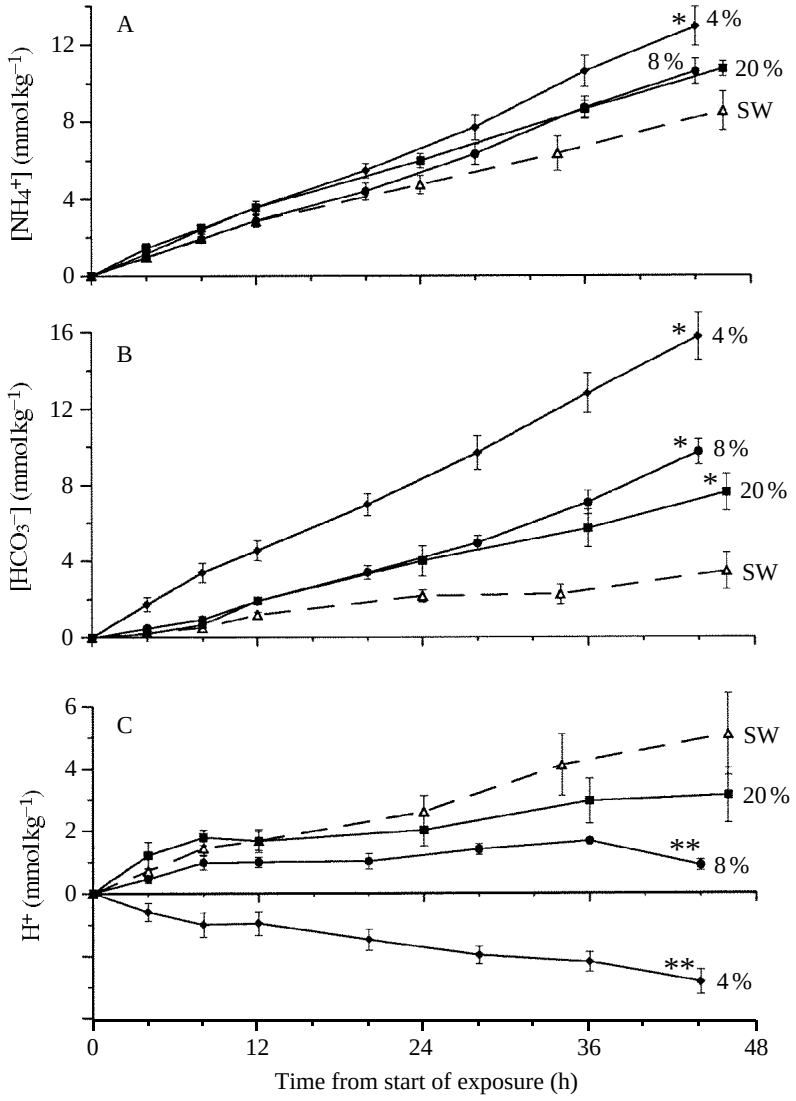

Fig. 3. Cumulative transfers of NH_4^+ (A), HCO_3^- (B) and H^+ (C) between the fish and the ambient water during exposure to different salinities. Positive values represent a net efflux. Fish were exposed to sea water (SW, *N*=6 up to 24h, *N*=4 after 24h) or diluted sea water (20%, *N*=4; 8%, *N*=4; 4%, *N*=6 up to 20h, *N*=3 after 20h). Values are mean ± S.E.M. Asterisks represent values that are significantly above seawater means; double asterisks represent values that are significantly below the value for the seawater group.

changes induced by the LSE. Urine flow rate doubled following LSE exposure and remained elevated when the animals were returned to sea water. Na^+ and NH_4^+ loss also increased significantly, while net H^+ and Cl^- excretion in the urine did not change significantly during or after the LSE.

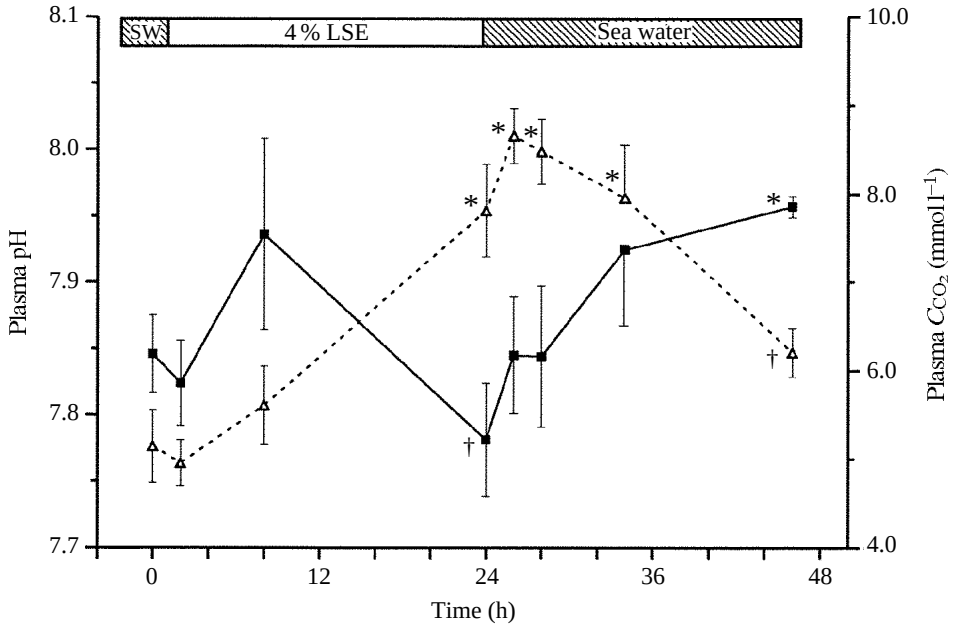

Fig. 4. Plasma pH (■) and C_{CO_2} (△) in sculpin during and after exposure to 4% sea water (4% LSE; *N*=7 up to 8h, *N*=6 at 24h, *N*=5 at 26–34h, *N*=3 at 46h). Values are mean ± S.E.M. Asterisks indicate a significant increase from pre-exposure values. Daggers represent values that have changed significantly from the previous measurement period.

Table 1. *Urine pH and flow rate and ion excretion rates in the long-horned sculpin during and after exposure to dilute salinities*

	Seawater I	LSE	Seawater II
Urine pH	7.18±0.14 (9)	6.93±0.18 (5)	6.71±0.18 (4)
Urine flow rate (ml kg^{-1} h^{-1})	0.26±0.04 (9)	0.58±0.23 (5)*	0.50±0.12 (4)*
Na^+ (μmol kg^{-1} h^{-1})	18.1±6.9 (9)	45.1±20.8 (5)	45.6±13.1 (4)*
Cl^- (μmol kg^{-1} h^{-1})	19.7±8.9 (6)	61.1±27.3 (3)	40.2±9.2 (4)
H^+ (μmol kg^{-1} h^{-1})	3.4±1.3 (5)	6.6±3.4 (5)	5.0±1.7 (4)
NH_4^+ (nmol kg^{-1} h^{-1})	18±9 (9)	32±10 (5)	51±17 (4)*

Means ± S.E.M. (*N*).

Asterisks indicate significant increases ($P<0.05$) from the first seawater period. The first seawater exposure (Seawater I) was for 12–24h. This was followed by 24h of exposure to lowered salinity (4%) and a 12–24h period of recovery (Seawater II).

Discussion

It is clear that sculpin are somewhat 'euryhaline', as they can maintain normal $[Cl^-]_{pl}$ (Fig. 1) and H^+ transfers (Fig. 3C) and survive well in 20% ambient sea water for several weeks (Claiborne *et al.* 1993; Oikari, 1980). 20% sea water (approximately 100mmol l^{-1} NaCl) is hypo-osmotic to the plasma, but further dilutions, to 8% or 4%, induced

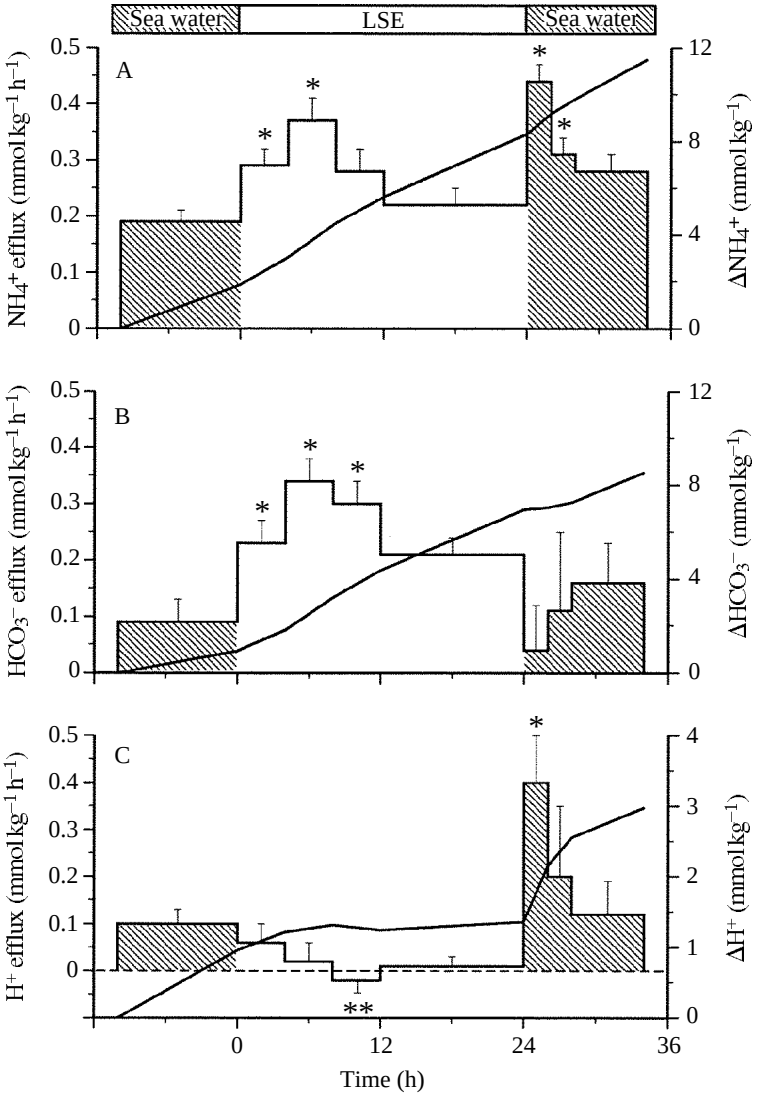

Fig. 5. Net efflux rate (histograms) and cumulative transfers (solid lines) of NH_4^+ (A), HCO_3^- (B) and H^+ (C) between the fish and the ambient water during and after exposure to 4% sea water (LSE). Positive values represent transfers from the animal to the water. *N*=10; values are mean ± S.E.M. Asterisks represent efflux rates that are significantly above pre-exposure means; double asterisks represent values that are significantly below the pre-exposure averages.

significant plasma ion loss and fatalities in fish exposed to these concentrations for more than 24–48h. Plasma pH increased significantly in the 20% and 8% groups but, because of an increase in plasma P_{CO_2}, decreased in fish exposed to the most dilute sea water tested (Fig. 2A). The 20% and 8% groups exhibited an approximately twofold increase

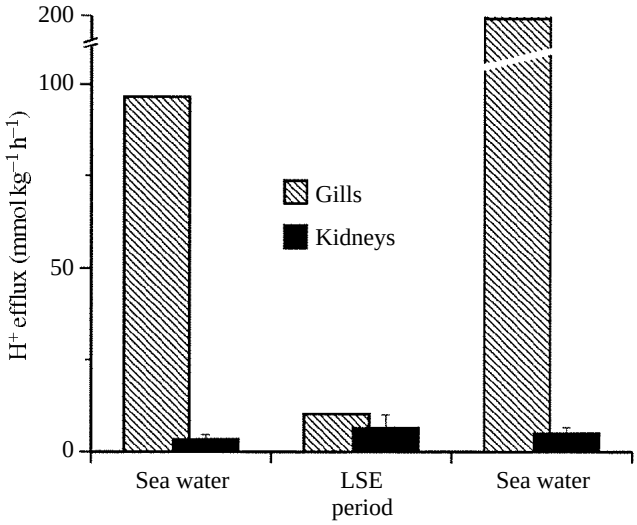

Fig. 6. Partitioning of H^+ efflux between urine and gills during and after exposure to 4% sea water (LSE). Gill rates are calculated as the difference between 'whole-body' rates (Fig. 5) and urine H^+ output measured in a separate group of fish (N=5).

in plasma C_{CO_2} (Fig. 2B), and this was presumably responsible for the observed elevations in pH. Thus, LSE induced variable changes in internal acid–base status, with plasma pH varying by approximately 0.1unit from control values and C_{CO_2} increasing in two of the three dilution groups.

As shown previously (Claiborne and Evans, 1988), sculpin exhibit a slight positive excretion of H^+ under control conditions. In the present study, control ΔNH_4^+ was approximately 2.5-fold higher than ΔHCO_3^-, resulting in a net H^+ excretion of $0.11 mmol kg^{-1} h^{-1}$ over the 46h period (Fig. 3). These transfers were disrupted during the LSE. Ammonia excretion increased by approximately 50% in animals exposed to 4 % sea water (or to elevated external ammonia; see Claiborne and Evans, 1988). ΔHCO_3^- excretion increased progressively in all three LSE groups, with the 4% animals exhibiting an approximately 4.5-fold increase in total HCO_3^- loss. These predominant changes in ΔHCO_3^- resulted in a significant decrease in ΔH^+ in the 8% group between 36 and 44h, and a reversal to a negative ΔH^+ (an uptake of H^+) in the 4% animals from the start. The net ΔH^+ 'gain' (the difference between cumulative H^+ transferred in the seawater and each experimental group at the end of the experiment) was quite large; amounting to 4.2 and $8.0 mmol kg^{-1}$ over the 44h period (in the 8% and 4% groups, respectively). This amount was similar in magnitude to the acid load generated by the spotted dogfish (*Scyliorhinus stellaris*) during strenuous exercise (Holeton and Heisler, 1983) and clearly caused an acid–base imbalance in the sculpin.

To define the effects of exposure to very dilute water and subsequent recovery, a second group of animals was exposed to 4% sea water for 24h and then returned to normal sea water (Figs 4 and 5). Acute exposure to this extreme dilution again altered

plasma acid–base values and fish-to-water ion transfers, but the animals were able to recover when returned to normal sea water. pH_{pl} remained near pre-LSE values, but plasma C_{CO_2} increased by 52% during the LSE, then returned towards control values during the seawater recovery period. This plasma C_{CO_2} increase was similar to that observed in the flounder (*Platichthys flesus*) 24h after transferring the animal from sea water to fresh water (Nonnotte and Truchot, 1990). These authors attributed the increase in plasma [HCO_3^-] and P_{CO_2} to hypercapnia resulting from hypoventilation during the freshwater exposure. This may also be the case in the present study as both plasma P_{CO_2} and [HCO_3^-] increased (from 0.21 to 0.38kPa and from 5.06 to 7.66mmol l^{-1}, respectively) during the LSE. It is especially interesting that pH_{pl} remained relatively constant during the LSE whereas C_{CO_2} increased concurrently with the large net HCO_3^- loss (Fig. 5B). ΔH^+ was near zero from 8h to 24h even though plasma [HCO_3^-] continued to increase during this period. Upon return to sea water, an elevation in ΔNH_4^+ and a decrease in ΔHCO_3^- during the first few hours increased ΔH^+ once again. These changes resulted in a net H^+ gain to the animal of 1.9mmol kg^{-1} by the end of the LSE and a net loss of 0.94mmol kg^{-1} during the subsequent seawater period. Thus, approximately 50% of the net H^+ gained during the LSE was excreted in the first 10h in sea water. Given that the H^+ gain was equivalent to a HCO_3^- loss, then if the 1.9mmol kg^{-1} of HCO_3^- lost to the water during the LSE originated entirely from the extracellular space, plasma [HCO_3^-] would have been expected to decrease by more than 9 mmol l^{-1} (using an extracellular space estimate of 20%; Cameron, 1980). This value is quite implausible, considering that the control plasma C_{CO_2} was approximately 5 mmol l^{-1}. Indeed, plasma C_{CO_2} *increased* by 2.7mmol l^{-1} during the LSE and, when combined with the measured HCO_3^- loss to the water during this period, amounted to 2.4mmol kg^{-1} of base equivalents (the sum of the 1.9mmol kg^{-1} loss and 0.2 times the 2.7mmol l^{-1} plasma [HCO_3^-] increase) moving from an intracellular compartment into the plasma.

It would seem likely that the base loss originated from the muscle, since red and white muscle have been shown to contribute to intracellular buffering during acid–base disturbances under a number of conditions in several species (Heisler *et al.* 1976, 1982; Cameron and Kormanik, 1982; Claiborne and Heisler, 1986; Wright *et al.* 1988; for a review see Heisler, 1993). Surprisingly, preliminary evidence indicates that intracellular muscle pH in the sculpin does not change during 23h of 4% LSE. Intracellular pH of white muscle in both control and LSE fish was approximately 7.3, whereas pHi in moderately exercised fish was approximately 6.8 (J. B. Claiborne and L. Barber, unpublished observations, using the methods of Pörtner *et al.* 1991). This suggests another origin for the base that was lost to the water. Bone resorption and Ca^{2+} efflux are known to increase during acidosis in mice (Bushinsky *et al.* 1993). Acidosis-induced physiochemical bone dissolution also results in similar amounts of Na^+ release (Bushinsky *et al.* 1992). Presumably the loss of Ca^{2+} and Na^+ from the bone is accompanied by the transfer of phosphate and carbonates. In contrast, Cameron (1985) showed that Ca^{2+} and PO_4^{3-} were the two major mineral salts in the bone of catfish, but that carbonate mobilization from bone did not act as a source of acid compensation during hypercapnia in that species. It is interesting that, in the sculpin during 24h of 4%

seawater LSE, plasma [PO_4^{3-}] increased and a significant fraction of intracellular Ca^{2+} was released to the water (Claiborne *et al.* 1990). It remains to be seen whether demineralization of the bone compartment acts as a source of base during LSE in the sculpin.

Urine flow rate doubled following LSE exposure, remained elevated when the animals were returned to sea water and was always within the range measured in other benthic species (Table 1; Shannon, 1938). The variation in urine pH of control animals contrasts with early work on this and other species (Brull *et al.* 1953; Hodler *et al.* 1955; Fanelli and Nigrelli, 1963), which indicated that marine teleosts have a 'fixed' acidic pH (approximately 6.0). Our data (here and in Compton-McCullough *et al.* 1989) agree with the recent study of Maren *et al.* (1992), which demonstrated a variable urine pH in sculpin following acid–base disturbances such as base infusion or imidazole injection. Na^+ and NH_4^+ losses also increased significantly during the LSE but, along with Cl^- excretion, these losses amounted to less than 1% of the measured 'whole-body' (branchial) transfers for these ions (e.g. renal Na^+ efflux, approximately 18 $\mu mol kg^{-1} h^{-1}$ *versus* a total Na^+ efflux of approximately 15$mmol kg^{-1} h^{-1}$; Claiborne and Evans, 1981). These data are not surprising considering the negligible role for monovalent ion transport by the kidneys of marine teleosts (Hickman and Trump, 1969).

Net whole-body H^+ excretion (Fig. 5C) in the seawater sculpin was approximately 100 $\mu mol kg^{-1} h^{-1}$ and decreased to approximately 17 $\mu mol kg^{-1} h^{-1}$ when fish were subjected to 4% LSE for 24h. Net H^+ excretion in the urine measured under the same two conditions was 3.4 and 6.6 $\mu mol kg^{-1} h^{-1}$, respectively (Table 1, Fig. 6). Thus, renal H^+ loss during exposure to sea water made up only 3% of the measured whole-body H^+ excretion. In contrast, during the LSE, nearly 40% of the total measured 'whole-body' ΔH^+ was actually due to the continued renal H^+ efflux. Thus, the contribution of the kidney to pH regulation in these animals during the LSE is similar to that reported in freshwater species (McDonald and Wood, 1981; Cameron and Kormanik, 1982; Hobe *et al.* 1983). Nonetheless, the direct effect of LSE is on the branchial transfers of H^+, and the assistance provided by the kidney is not sufficient to ameliorate the acid–base imbalance (Fig. 6).

The decrease and then reversal of ΔH^+ across the gills during the LSE (Figs 3C, 5C) could have been driven by a number of mechanisms. Loss of intracellular HCO_3^- into the blood and then into the water by passive diffusion down its electrochemical gradient was possible, for the external water C_{CO_2} decreased during the LSE (from approximately 2.0 to 0.2$mmol l^{-1}$; J. B. Claiborne, unpublished observation) and the transepithelial potential of the sculpin becomes serosa-negative during the LSE (sea water, +22mV; 20% sea water, $-10 mmol l^{-1}$; 4% sea water, −39mV; Walton, 1991). However, the gradient is also outwardly directed when the animals are in 20% sea water, and they are able to maintain near normal ΔH^+ in this salinity. In addition, sculpin exposed to 4% sea water containing $5 mmol l^{-1}$ $NaHCO_3$ exhibited changes in ΔH^+ that were similar to those measured during LSE here (Walton, 1991). An elevation of external [Ca^{2+}] to seawater levels ($10 mmol l^{-1}$) during the LSE (which should decrease gill permeability; Carrier and Evans, 1976; for a review, see Evans, 1984) also had no effect on HCO_3^- loss to the water (Walton, 1991). Ammonia lost by the animal under the normal conditions of

a fish-to-water partial pressure gradient is thought to be mainly through the passive diffusion of NH_3 across the gills (Cameron and Heisler, 1983; Claiborne and Evans, 1988; Wright *et al.* 1988). If the changes in ΔNH_4^+ measured here were due to differences in NH_3 (rather than NH_4^+) production and loss, then the observed ΔH^+ was driven by the net movements of H^+ and/or HCO_3^- (OH^-) between the fish and the water (predominantly across the gills; see above). When the animal was transferred to lower salinities, less external NaCl would be available for branchial exchange through Na^+/H^+ or Cl^-/HCO_3^- mechanisms (Iwama and Heisler, 1991; Goss and Wood, 1991), but any electrogenic excretion of H^+ (Lin and Randall, 1991) would not be directly affected. It remains to be seen what the individual effect(s) of decreasing external [Na^+] and/or [Cl^-] are on the net acid excretion, but this might shed more light on the transbranchial processes involved. It is interesting to note that the euryhaline toadfish *Opsanus tau* also lost HCO_3^- to the water in net amounts similar to those measured in the present study when it was subjected to the same LSE but, after several days in the lowered salinity, its net excretion of H^+ had returned to seawater rates (J. B. Claiborne, unpublished results; Walton, 1991; Compton-McCullough, 1993). That an increase in ΔHCO_3^- of similar magnitude occurred in this truly euryhaline species (with no obvious deleterious effects to the fish) may imply that these transfers are employed to cope with the dilution stress. If the HCO_3^- lost to the water is a purely passive effect of the LSE, then perhaps it is the inability of the sculpin to alter the rate of HCO_3^- efflux (by modification of gill ion-exchange systems or a reduction in overall branchial permeability) that limits this species' survival in very dilute brackish and fresh water.

In conclusion, we have investigated the regulation of extracellular acid–base variables and transfers between the fish and the water when marine sculpin are exposed to sea water or 20%, 8% or 4% sea water for 24–48h. Sculpin were partially euryhaline: they maintained plasma ion and acid–base transfers in hypo-osmotic (20%) sea water, but could not tolerate greater dilutions for more than several days. Plasma pH and C_{CO_2} increased in the 20% and 8% dilution groups because of an increase in plasma P_{CO_2} and [HCO_3^-] (perhaps caused by a mobilization of base from the intracellular bone compartment). ΔHCO_3^- increased progressively in the 8% and 4% groups, and those animals lost H^+ at lower rates than did seawater fish. Animals that were exposed to 4% sea water for 24h and then returned to normal sea water had a variable plasma pH, elevated C_{CO_2} and a near zero net efflux of H^+ during the low-salinity period. Renal acid excretion remained unchanged and made up approximately 40% of the total acid transfers during the 4% dilution period but was only 3% of the branchial rate in sea water. Dilution of the ambient water could induce base loss by alteration of gill ion exchanges (Na^+/H^+, Cl^-/HCO_3^-) and/or changes in branchial HCO_3^- permeability. The effects of reduced salinities on acid–base transfers may therefore be additional evolutionary and environmental factors that restrict euryhalinity in some species of marine fish.

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