AMMONIA AND ACID–BASE BALANCE DURING HIGH AMMONIA EXPOSURE IN A MARINE TELEOST
(MYOXOCEPHALUS OCTODECIMSPINOSUS)

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Accepted 3 May 1988

Summary

For the first time in a marine teleost (the long-horned sculpin; Myoxocephalus octodecimspinosus), the maintenance of blood pH, P<sub>CO2</sub>, [HCO<sub>3</sub>–] and the net movements of NH<sub>4</sub><sup>+</sup>, HCO<sub>3</sub>– and H<sup>+</sup> between the fish and the water have been studied during exposure to ammonia stress induced either by infusion (NH<sub>4</sub>Cl or NH<sub>4</sub>HCO<sub>3</sub>; 5 mmol kg<sup>–1</sup>) or by external application (NH<sub>4</sub>Cl; approx. 1 mmol l<sup>–1</sup>).

Following NH<sub>4</sub>Cl infusion, a rapid decrease in blood pH (0.36 units) and [HCO<sub>3</sub>–] (2.38 mmol l<sup>–1</sup>) was observed, and within 1 h about 40% of the ammonia load had been excreted to the water. Analysis of NH<sub>4</sub><sup>+</sup> and HCO<sub>3</sub>– transfers revealed that the total ammonia (T<sub>Amm</sub>) efflux was due to a loss of NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup> in approximately equal proportions when an outwardly directed NH<sub>3</sub> diffusion gradient was established.

Infusion of NH<sub>4</sub>HCO<sub>3</sub> induced only small changes in plasma pH, and the rate of net HCO<sub>3</sub>– excretion was some 90% higher than that of NH<sub>4</sub><sup>+</sup> over 20 h. These data indicate a predominance of NH<sub>3</sub> as the form of ammonia lost. In both infusion experiments, a presumed intracellular buffering of a majority of the ammonia load was noted.

High external T<sub>Amm</sub> induced an initial uptake of NH<sub>4</sub><sup>+</sup>, but after 4 h of exposure ammonia efflux resumed even though NH<sub>3</sub> diffusion gradients were negligible. Thus, in this seawater teleost, a role for the excretion of ammonia in the form of NH<sub>4</sub><sup>+</sup> is also likely.

Introduction

Teleost fish excrete nitrogenous waste products in the form of ammonia (NH<sub>3</sub> + NH<sub>4</sub><sup>+</sup>). Although it is well accepted that the gills are the major site of this excretion (Smith, 1929; Evans, 1982; see review by Kormanik & Cameron, 1981; Evans & Cameron, 1986), the mechanism of ammonia transfer across the gill epithelium and the form(s) of the transferred ammonia are not as clear. In the
freshwater trout \textit{(Salmo gairdneri)}, ammonia loss under control conditions can be accounted for principally by simple diffusive loss of NH$_3$ (Cameron & Heisler, 1983). It has been theorized that branchial transfers such as NH$_4^+$/$\text{Na}^+$ exchange are only necessary when the animal is exposed to an abnormally high ambient [ammonia] (Cameron & Heisler, 1983). Recently, Cameron (1986) has hypothesized that ammonia excretion in the freshwater catfish \textit{(Ictalurus punctatus)} can be maintained in the face of reversed NH$_3$ and NH$_4^+$ concentration gradients by activating an NH$_4^+$/$\text{H}^+$ exchange system. Using the isolated perfused head preparation, roles for the ionic diffusion of NH$_4^+$ as well as NH$_4^+$/$\text{Na}^+$ exchange have also been postulated for two seawater teleosts \textit{(Opsanus beta} and \textit{Myoxocephalus octodecimspinosis}; Goldstein \textit{et al.} 1982; Claiborne \textit{et al.} 1982).

The ratio of NH$_3$ to NH$_4^+$ diffusion across the gills is dependent on pH, pK', temperature and the ionic strength of both the blood and the external environment, as well as the relative gill permeabilities of NH$_3$ and NH$_4^+$ (Cameron & Heisler, 1983; Boutilier \textit{et al.} 1984). Cameron (1986) has calculated that the ratio of NH$_3$ to NH$_4^+$ transferred may range from 32:1 to 1:9, depending on the relative permeabilities and blood/water parameters utilized. Though it is likely that diffusive loss of NH$_3$ can account for the majority of ammonia transfer in freshwater teleosts under normal conditions, the sparse data for seawater varieties indicate a role for some of the additional pathways described above. For example, since it is generally thought that the gill ion permeability of seawater-adapted teleosts is more than 10 times that of freshwater fish (Evans, 1984), ionic diffusion of NH$_4^+$ could be much more significant in seawater animals. To gain further insight into the relative roles of these transfers in a marine species, we monitored internal acid–base regulation and gill ammonia excretion in the long-horned sculpin \textit{(Myoxocephalus octodecimspinosis} Mitchell) following exposure to infused ammonia loads (NH$_4$Cl or NH$_4$HCO$_3$) or a reversed external ammonia gradient.

\textbf{Materials and methods}

Long-horned sculpin \textit{(Myoxocephalus octodecimspinosis)}, mass = 165.1 ± 10.4 g ($N=16$) (mean ± s.e.) were caught by local fishermen in Frenchman’s Bay, Maine near the Mount Desert Island Biological Laboratory. The animals were maintained in large wooden or fiberglass tanks and supplied with running sea water (13–15°C). Before use, specimens were held for 2–6 days without feeding. To facilitate blood sampling in these relatively small animals, the fish were cannulated in a manner similar to that described for the measurement of ventral aortic blood pressure (Claiborne & Evans, 1981). Each fish was anaesthetized (MS-222, 1:10 000), placed in a moist tray, and periodically ventilated with aerated sea water during the 5–10 min operative procedure. The cut tip of a 23 gauge needle, connected to a short length of heparinized, Ringer-filled cannula (PE-50) was inserted into the afferent artery of the third branchial arch and secured in place with a suture. In some animals, an additional Ringer-filled cannula (PE-50) was inserted through the skin and peritoneal musculature into the peritoneal
cavity, and secured with a suture through the skin. The animals were then placed in darkened Plexiglas boxes (1·3–2·01) and allowed to recover for 20–48 h. During this period, fresh running sea water was directed through the experimental chamber. Several hours prior to the start of each experimental series, the running sea water was disconnected so that control net ion and ammonia fluxes (see below) could be measured. Duplicate control blood samples were also drawn during this period.

Each blood sample (0·2–0·4 ml) was analysed for pH (I.L. model 213 or Radiometer ‘gun’ electrode with an Orion 701A pH meter), total \( \text{CO}_2 \) concentration, \( T_{\text{CO}_2} \) (Capnicon-Con II; Cameron Instruments Inc.) and plasma total ammonia concentration (\( T_{\text{Amm}} \); Sigma kit no. 170-UV). Plasma \( P_{\text{CO}_2} \) and \([\text{HCO}_3^-]\) were calculated from \( T_{\text{CO}_2} \) and pH using values for \( \text{CO}_2 \) solubility and \( pK' \) derived from Boutilier et al. (1984). Water samples (20 ml) were collected periodically and analysed for \( T_{\text{Amm}} \) using the phenolhypochlorite method (Solorzano, 1969). The net titratable base was determined by volumetric titration of a portion of the sample to a pH of 3·800 or 3·700 with 0·1 mol l\(^{-1}\) HCl using a syringe micrometer burette (model SB2, Micro Metric Instrument Co.) according to the methods of Cameron & Kormanik (1982). This method was typically repeatable within ±1 \( \mu \)l of acid (<1 %) in a 10 ml water sample, thus the overall resolution was approx. 25 \( \mu \)mol in a 1·3 l chamber. The \( \Delta \text{HCO}_3^- \) was then calculated as the difference between the titratable base at the beginning and end of each time interval. Mucus, proteins and other buffers excreted by the animal would cause an overestimation of \( \Delta \text{HCO}_3^- \). In some cases, seawater pH was also recorded before acid was added.

**Ammonia infusion series**

After all control measurements had been made, animals were infused intraperitoneally with either \( \text{NH}_4\text{Cl} \) or \( \text{NH}_4\text{HCO}_3 \) (5 mmol kg\(^{-1}\), a 3–5 ml bolus of a 200 mmol l\(^{-1}\) stock solution infused over about 2 min). After a 5-min equilibration, time zero blood and water samples were collected, and additional samples were obtained at 1, 2, 4, 8 and 20 h post-infusion. At 4 and 8 h of the experimental period, the water within the box was flushed with sea water to limit the accumulation of external ammonia.

**High external ammonia series**

Following the control period, the ammonia concentration within the experimental chamber was increased to approx. 1 mmol l\(^{-1}\) by the addition of \( \text{NH}_4\text{Cl} \) (approx. 6 ml of 200 mmol l\(^{-1}\) stock). After a 6-h exposure, the high ammonia bath was replaced by normal sea water for an additional 15 h. Blood was drawn at 0·5, 1, 2, 4 and 6 h of the high external ammonia (HEA) exposure, and at 1 and 15 h of the recovery period. External bath samples were also collected during all perturbations and the water within the box was flushed at 4 h of the recovery period to maintain a relatively low external [\( T_{\text{Amm}} \)].
Calculations

$\Delta$HCO$_3^-$ and $\Delta$NH$_4^+$ (mmol kg$^{-1}$) were calculated for all time periods by multiplying the measured concentration of each ion by the volume of the experimental bath, and adjusting for volume changes due to sampling and the mass of the animal ($T_{Amm}$ is effectively [NH$_4^+$] in sea water since the $pK'$ of the NH$_3$/NH$_4^+$ equilibrium is about 9.6; Cameron & Heisler, 1983). The total amount of H$^+$ transferred between the sculpin and the water ($\Delta$H$^+$) is therefore the difference between $\Delta$NH$_4^+$ and $\Delta$HCO$_3^-$ (for details see Claiborne & Heisler, 1984, 1986; Heisler, 1984). ‘Net $\Delta$’ values are the differences between the experimental and control rate of transfers for each time period. Data analysis was performed on a microcomputer (Franklin 1200 or Apple IIe) and Student’s $t$-test (one- or two-tailed) was applied where appropriate.

Results

Control measurements

Pooled control values for acid–base parameters and ion transfers are listed in Table 1. The calculated $\Delta$H$^+$ during control conditions was due to a measured HCO$_3^-$ uptake (20%) and a more significant $T_{Amm}$ excretion (80%).

NH$_4$Cl infusion

As shown in Fig. 1A, intraperitoneal infusion of NH$_4$Cl induced an increase in plasma $T_{Amm}$, which rose to approx. 5400 $\mu$mol l$^{-1}$ immediately following the infusion and then returned to levels near control values within 8 h ($T_{Amm}$ control, 234 ± 29 $\mu$mol l$^{-1}$; $T_{Amm}$ 8 h post-infusion, 314 ± 70 $\mu$mol l$^{-1}$; mean ± S.E., $N=5$). Fig. 1B–D depicts the concurrent changes in blood acid–base status following the ammonia infusion. Plasma pH decreased from 7.80 ± 0.01 to 7.44 ± 0.06 ($P<0.01$, $N=5$) and then regained control levels at hour 4. Plasma PCO$_2$ rose by

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<th>Table 1. Control values for acid–base and ion transfer parameters in the long-horned sculpin</th>
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<td>Plasma ($N=16$)</td>
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<td>$T_{CO_2}$ (mmol l$^{-1}$)</td>
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<td>$P_{CO_2}$ (mmHg)</td>
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<td>[HCO$_3^-$] (mmol l$^{-1}$)</td>
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<td>$T_{Amm}$ (mmol l$^{-1}$)</td>
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<td>Cumulative ion transfer (mmol kg$^{-1}$ h$^{-1}$; $N=15$)</td>
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<td>$\Delta$NH$_4^+$</td>
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<td>$\Delta$HCO$_3^-$</td>
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X ± S.E., ‘$\Delta$’ ion transfer rates represent the cumulative appearance of each ion in the surrounding water.
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Fig. 1. Plasma total ammonia (A), pH (B), $P_{CO_2}$ (C) and $[HCO_3^-]$ (D) in the sculpin following NH$_4$Cl infusion. Time zero points represent pre-infusion control values for each parameter (mean ± s.e., $N = 5$).

74% from 2.3 to 4.0 mmHg (1 mmHg = 133.3 Pa) and remained significantly above pre-infusion values until hour 8, while $[HCO_3^-]$ was depressed by 2.0 mmol l$^{-1}$ (approx. 32%) after the infusion but had returned to normal at the 4-h sample.

The effects of the infusion on transfers of NH$_4^+$, HCO$_3^-$ and H$^+$ between the fish and the external bath are shown in Fig. 2A–C, respectively. In these and all following ion transfer figures, control lines represent mean ± s.e. of the preliminary control flux period extrapolated over the length of the subsequent experimental period(s). NH$_4$Cl infusion induced a large increase in ammonia efflux from the animal (Fig. 2A). Over the first hour after infusion, $T_{Amm}$ loss increased by about sevenfold from a control rate of $0.29 ± 0.06$ mmol kg$^{-1}$ h$^{-1}$ to $2.15 ± 0.08$ mmol kg$^{-1}$ h$^{-1}$ ($P < 0.001$, $N = 4$). The rate of ammonia transfer remained above the control rate until the final sampling period (hours 8–20). The ‘net’ loss of ammonia (the experimental rate minus the extrapolated control rate) was equal to $3.57 ± 0.25$ mmol kg$^{-1}$ at hour 4 and totalled $4.61 ± 0.54$ mmol kg$^{-1}$ at hour 20. Apparent HCO$_3^-$ movements between the animal and the water (Fig. 2B) also changed following the infusion: the control uptake rate of $0.11 ± 0.02$ mmol kg$^{-1}$ h$^{-1}$ was altered to a net efflux of $1.06 ± 0.28$ mmol kg$^{-1}$ h$^{-1}$ during the first hour after NH$_4$Cl application. HCO$_3^-$ efflux was maintained up to
and during hour 20 (0.14 ± 0.08 mmol kg⁻¹ h⁻¹, hours 1–20). Net HCO₃⁻ loss was 1.39 ± 0.38 mmol kg⁻¹ at hour 4 and 5.82 ± 1.64 mmol kg⁻¹ after 20 h (P < 0.05, N = 4). The calculated H⁺ transfer (see Materials and methods) from fish to environment (Fig. 2C) increased significantly over the first 4 h after infusion (control, 0.40 ± 0.08 mmol kg⁻¹ h⁻¹; at hour 4, 0.90 ± 0.05 mmol kg⁻¹, P < 0.02, N = 4), and then decreased 60% below the control ΔH⁺ by the end of the experiment (0.15 ± 0.03 mmol kg⁻¹ h⁻¹, P < 0.05). Net ΔH⁺ reached a maximum of 2.18 ± 0.48 mmol kg⁻¹ at hour 8, but was not significantly different from 0 by hour 20.

**NH₄HCO₃ infusion**

Infusion of NH₄HCO₃ (5 mmol kg⁻¹) caused a rapid and significant increase in
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![Graphs showing plasma total (A), pH (B), P CO₂ (C) and [HCO₃⁻] (D) following NH₄HCO₃ infusion.](image)

Fig. 3. Plasma total (A), pH (B), P CO₂ (C) and [HCO₃⁻] (D) following NH₄HCO₃ infusion. Time zero points represent pre-infusion control values for each parameter (mean ± s.e., N = 5).

Both plasma T Amm and [HCO₃⁻] (Fig. 3A, D). Plasma T Amm increased from a control value of 0.24 ± 0.07 to 4.62 ± 0.38 mmol l⁻¹ (mean ± s.e., N = 5) immediately after the infusion but had returned to control levels by hour 2 after infusion. Plasma [HCO₃⁻] reached a maximum of 11.25 ± 0.45 mmol l⁻¹ after NH₄HCO₃ application, and then returned to values not different from the pre-infusion control of 4.69 ± 0.26 mmol l⁻¹ by the hour 4 sample. Fig. 3B shows the effect of the infusion on blood pH. The pH increased slightly but significantly at hour 2 (control, 7.78 ± 0.02; hour 2, 7.81 ± 0.01, P < 0.05, N = 5), dropped below control at hour 8 (7.73 ± 0.01), and then approached pre-infusion levels by the end of the experiment. Plasma P CO₂ (Fig. 3C) increased from 1.80 ± 0.13 to 4.69 ± 0.20 mmHg immediately after infusion. This parameter then slowly decreased to control levels by hour 4.

The rate of NH₄⁺ excretion (Fig. 4A) increased by 12-fold during the first hour following the NH₄HCO₃ load (control, 0.30 ± 0.06 mmol kg⁻¹ h⁻¹; hours 0–1, 3.55 ± 0.43 mmol kg⁻¹ h⁻¹, N = 5) but regained control rates by hour 2. By hour 2, the net ΔNH₄⁺ was 3.76 mmol kg⁻¹ and reached a maximum of 4.42 mmol kg⁻¹ at hour 8. ΔHCO₃⁻ also increased rapidly during the first hour post-infusion (Fig. 4B), increasing from a control rate of 0.01 ± 0.04 mmol kg⁻¹ h⁻¹ to 3.60 ± 0.35 mmol kg⁻¹ h⁻¹. Though much reduced from the initial hour, HCO₃⁻ loss continued through hours 4–8 (0.33 ± 0.06 mmol kg⁻¹ h⁻¹, P < 0.01), resulting
in a net ΔHCO₃⁻ of 6.29 mmol kg⁻¹ at the end of this period and 7.11 mmol kg⁻¹ after 20 h. ΔH⁺ transfers between the animal and the water are shown in Fig. 4C. Though variable, ΔH⁺ remained near zero throughout the experiment. When compared with a control excretion rate of 0.29 ± 0.06 mmol kg⁻¹ h⁻¹, the low experimental values resulted in a significant net H⁺ uptake of 3.40 mmol kg⁻¹ (P < 0.05) over the 20 h post-infusion period.

**High external ammonia concentration**

High external ammonia concentration (HEA) induced several effects on acid–base balance and ion transfers in the sculpin (N = 6). Plasma pH and TCO₂ appeared to increase slightly, but did not vary significantly from the control measurements of 7.78 ± 0.02 and 5.29 ± 0.55 mmol l⁻¹, respectively (Fig. 5B,C). After 30 min in HEA, plasma T_Amm (Fig. 5A) had increased by nearly threefold.
from a control of 230 ± 30 to 690 ± 103 μmol l⁻¹. $T_{\text{Amm}}$ reached a maximum of 839 ± 157 μmol l⁻¹ at hour 6 (but remained below that of the external bath: 1175 ± 43 μmol l⁻¹), and then decreased to 552 μmol l⁻¹ 1 h after normal sea water was reinstated.

A control ammonia efflux (0.271 ± 0.042 mmol kg⁻¹ h⁻¹) was reversed to an influx as a $\Delta NH_4^+$ of $-1.216 ± 0.390$ mmol kg⁻¹ was observed during the first hour of HEA (Fig. 6A). This uptake resulted in a calculated net $NH_4^+$ gain of approx. 2.0 mmol kg⁻¹ after 4 h. From hours 4 to 6, $\Delta NH_4^+$ returned to an efflux once again (0.442 ± 0.088 mmol kg⁻¹ h⁻¹, $P < 0.01$). During the first 3 h of the recovery period, a large net $NH_4^+$ loss was observed which equalled the ammonia gained in the preceding period. The rate of $NH_4^+$ efflux remained significantly higher than the control (approx. 30 %) until the end of the experiment. $\Delta HCO_3^−$ (Fig. 6B) was reversed from a control uptake of 0.10 ± 0.05 mmol kg⁻¹ h⁻¹ to an excretion of 0.22 ± 0.08 mmol kg⁻¹ h⁻¹ at hour 2 of HEA. During the recovery period, $\Delta HCO_3^−$ remained near zero until the last sampling interval when an efflux of 0.24 ± 0.06 mmol kg⁻¹ h⁻¹ was observed. A large but variable net $HCO_3^−$ loss totalling 4.66 ± 1.14 mmol kg⁻¹ ($P < 0.01$, $N = 6$) was observed during the 15-h
recovery period. The measured \( \text{NH}_4^+ \) and \( \text{HCO}_3^- \) transfers resulted in a negative net \( \Delta \text{H}^+ \) (a net base loss; Fig. 6C) of 2.74 ± 0.58 mmol kg\(^{-1}\) from hours 1 to 4 of HEA. \( \Delta \text{H}^+ \) then resumed rates similar to or slightly higher than the control \( \Delta \text{H}^+ \) measurement (0.37 ± 0.05 mmol kg\(^{-1}\) h\(^{-1}\)) from hours 4 to 10, such that during this interval a net \( \text{H}^+ \) efflux of 1.00 mmol kg\(^{-1}\) occurred. During the last sampling period of the recovery, \( \Delta \text{H}^+ \) again decreased to 0.12 ± 0.04 mmol kg\(^{-1}\) h\(^{-1}\).

To study the gradients driving the movement of \( \text{NH}_3 \) across the gills (Fig. 7), we utilized the measured plasma and water pH and \( T_{\text{Amm}} \) values as well as appropriate solubility and pK’ constants derived for trout plasma and sea water (Cameron & Heisler, 1983). Under control conditions, a positive (from fish to water) \( \text{NH}_3 \) diffusion gradient of about 60 nmHg was measured. Within 1 h after exposure to the HEA, however, plasma \( P_{\text{NH}_3} \) was not significantly different from
that of the surrounding sea water. When the external bath was flushed with normal sea water, plasma $P_{NH_3}$ decreased (but remained above the control average), and a positive diffusion gradient was established once more. Sea water $[NH_3]$ decreased during the first 2 h of HEA as ammonia entered the fish, and then significantly increased again during hours 2–6.

**Discussion**

As seen in Table 1, acid–base parameters for the sculpin are similar to those for other teleosts (Heisler, 1984). Ion transfer rates also appear to be 'normal' for a seawater species (Wood et al. 1977; Turner et al. 1983). Although we have no data on the branchial versus renal partitioning of the observed ion movements, it is likely that a large portion of the net transfers were transbranchial (Kormanik & Cameron, 1981; Heisler, 1984). It should be noted that owing to the relatively small size and the particular vascular structure of these animals, we have found it very difficult to cannulate the dorsal aorta using the usual methods (e.g. Soivio et al. 1972; Claiborne & Heisler, 1986) but have utilized a method which we previously developed for this species (Claiborne & Evans, 1981). In contrast to dorsal aortic cannulation, it is likely that mixed venous blood was drawn from the animal when utilizing the present method. Possible disadvantages of our procedure may include disruption of proper water flow through the branchial cavity and ischaemia of the cannulated gill arch. However, the lack of any undue struggling or perturbations in the acid–base status of the animal during control conditions demonstrates that this procedure is acceptable for this species. In about
80% of the cannulations, afferent blood flow did not appear to be impeded (upon visual examination of the gill arch at the termination of an experiment) and blood acid–base balance was maintained even by animals in which gill flow was blocked. In several fish returned to large holding tanks, the cannula remained patent for up to 2 weeks and periodic sampling of blood pH, P\textsubscript{CO}_2, and P\textsubscript{O}_2 indicated that these parameters remained normal over the length of the experiment (J. B. Claiborne & D. H. Evans, unpublished observations).

\textit{NH}_4\textit{Cl} infusion

In many aspects, the present data confirm those presented by Cameron & Kormanik (1982) and Cameron & Heilsler (1983) for two freshwater-adapted teleosts. We found that infusion of \textit{NH}_4\textit{Cl} induced a rapid decrease in plasma pH and [HCO\textsubscript{3}~] and an increase in P\textsubscript{CO}_2. The responses are similar to those expected following a metabolic acid load or infusion (Holeton \textit{et al.} 1983; Turner \textit{et al.} 1983; Cameron & Kormanik, 1982). The above authors ascribe these effects to the rapid loss of the infused ammonia as NH\textsubscript{3} (into the water), with a more gradual equimolar excretion or intracellular buffering of the remaining H\textsuperscript{+}. An alternative explanation for the observed extracellular acidosis could be that the infused ammonia enters metabolic pathways and is thus buffered intracellularly as NH\textsubscript{3} (for example with glutamate; L. Goldstein, personal communication) thus again liberating equimolar amounts of H\textsuperscript{+}. The NH\textsubscript{3} may then be slowly shuttled back into the blood where it could be extruded along with the remaining protons (as NH\textsubscript{3} + H\textsuperscript{+} and/or NH\textsubscript{4}\textsuperscript{+}).

By examining the transfers of \textit{NH}_4\textsuperscript{+}, HCO\textsubscript{3}~ and net H\textsuperscript{+} between the fish and the water, one can attempt to quantify the relative amounts of acid–base exchanges occurring across the gills following the ammonium infusion. As seen in Fig. 2, during the first hour after infusion, a net \textit{NH}_4\textsuperscript{+} excretion of 2-1 mmol kg\textsuperscript{-1} in combination with a \(\Delta\text{HCO}_3\~\) of approx. 1-0 mmol kg\textsuperscript{-1} resulted in a net H\textsuperscript{+} loss of approx. 1-1 mmol kg\textsuperscript{-1}. Since any NH\textsubscript{3} leaving the animal would create an equivalent amount of HCO\textsubscript{3}~ in the water (the majority of NH\textsubscript{3} lost to the sea water would be immediately protonated to NH\textsubscript{4}\textsuperscript{+}), it appears that, during this first hour, about 48% of the total ammonia excretion was due to the loss of NH\textsubscript{3}, and the remainder was \textit{via} the combined loss of NH\textsubscript{3} and H\textsuperscript{+}, the direct transfer of \textit{NH}_4\textsuperscript{+} along with Cl\textsuperscript{−}, or the exchange of \textit{NH}_4\textsuperscript{+} with external Na\textsuperscript{+} (see review by Evans & Cameron, 1986). Unfortunately, the transfer of NH\textsubscript{3} + H\textsuperscript{+} is indistinguishable from the movement of \textit{NH}_4\textsuperscript{+} using present analytical methods. When the control rate of ammonia excretion is taken into account, the calculated net \textit{NH}_4\textsuperscript{+} efflux is 1-9 mmol kg\textsuperscript{-1}. Thus, about 40% of the infused ammonia had appeared in the water within 1 h. At the same time, a net H\textsuperscript{+} loss of 0-7 mmol kg\textsuperscript{-1} amounted to about 15% of the infused load, again an indication of the direct loss of NH\textsubscript{3}.

Using 1-0 mmol kg\textsuperscript{-1} as an estimate of the NH\textsubscript{3} leaving the fish during the first hour after infusion (and thus the proton load remaining within the animal), an extracellular space estimate of 20% (Cameron, 1980) and a blood buffer value of
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−12 mequiv pH unit−1 (Cameron & Heisler, 1983), if all the proton load were to remain in the extracellular fluids during this period, the plasma pH would have decreased by approximately 0.4 units; in fact, the pH had increased slightly after 1 h (Fig. 1B). This is clear, if indirect, evidence that the H⁺ liberated from the NH₄Cl was removed from the extracellular space and probably buffered intracellularly. As calculated by Heisler (1980) and Cameron & Kormanik (1982), the relatively large volume and buffering capacity of the intracellular space could absorb this excess H⁺ without a drastic effect on intracellular pH.

From hours 1 to 4, a shift in the mode of ammonia and H⁺ transfer is apparent. During this period, the net H⁺ loss was driven almost completely by the NH₄⁺ (or NH₃ + H⁺) efflux (the net ΔHCO₃⁻ was near 0). 1.5 mmol kg⁻¹ (cumulative total, 40%) of H⁺ was cleared from the animal during this period. Paralleling these transfers was a recovery of plasma pH that was complete by hour 4.

Over the subsequent 16 h of the experiment, a cumulative NH₄⁺ loss of 5.7 mmol kg⁻¹ and a ΔHCO₃⁻ of 2.8 mmol kg⁻¹ indicate that about 47% of the total ammonia excretion was again due to NH₃ while the remainder must have resulted from the transfer of NH₄⁺ (or NH₃ + H⁺). The net NH₂⁺ lost after 20 h was 4.61 ± 0.54 mmol kg⁻¹, or 92% of the infused load. In contrast, from hours 4 to 20, net ΔH⁺ became negative. Owing to this reversal, the total H⁺ excreted over the entire 20 h was not different from that expected from control fish. In other words, the proton load induced by the infusion of NH₄Cl and any non-ionic elimination of NH₃ had not been excreted by the animal after 20 h. Paradoxically, 40% of the infusion had been lost by hour 4, but the animals developed a net uptake of H⁺ (or an efflux of HCO₃⁻) during the next 16 h (Fig. 2C). Cameron & Kormanik (1982) found that after an infusion of NH₄Cl the freshwater catfish (Ictalurus punctatus) excreted only a portion of the total ammonia (65%) and H⁺ (48%) load. They theorized that the remainder of these components was either excreted slowly by the animal or retained in the intracellular compartment. The plasma pH of the sculpin had returned to normal within 4 h post-infusion and remained at this level for the next 16 h. Since plasma pH could be maintained even while the animal was not excreting the infused H⁺, it is again likely that the net H⁺ gain was sequestered intracellularly. Though we have no data past hour 20, it is possible that the sculpin also excrete the sequestered H⁺ load over the next few days. If the reversal in H⁺ transfer is a maladaptive response, perhaps it is due to the artificially high concentrations of internal ammonia to which the animals were exposed following the infusion (about 23 times control). It remains to be seen whether an ammonia ‘overload’ alters the metabolic homeostasis of amino acid catabolism and synthesis of NH₃ and H⁺ within the gill epithelium (Goldstein et al. 1964; Cameron & Heisler, 1983), and thereby indirectly affects intra- and extracellular H⁺ and HCO₃⁻ balance.

Although the modes of ammonia excretion in these fish appear to include the transfer of both NH₄⁺ (or NH₃ + H⁺) and NH₃, the data indicate that the ratio of each of these components in the total ammonia lost may vary at different stages of the experiment. During the first hour post-infusion, and again after hour 4, about
50% of ammonia excretion was due to the excretion of NH₃, whereas from hours 1 to 4, NH₄⁺ or NH₃ + H⁺ transfer accounted for the entire ammonia lost. The question arises as to why these animals would switch from a passive non-ionic diffusion of NH₃ to a presumably carrier-mediated transport of NH₄⁺ or H⁺ during hours 1-4. A probable answer becomes clear when one considers the ammonia gradients between the fish and the water through the course of the experiment. The observed alterations in the mode of ammonia transfer may have been due to a build-up of external ammonia in the environmental water following the infusion. The bath was flushed with fresh sea water at hour 4 (and again at hour 8; see Materials and methods), but by hour 4 the external PNH₃ was approx. 95 nmHg and the plasma PNH₃ was approx. 125 nmHg [calculated using solubility and pK' derived for sea water and trout plasma by Cameron & Heisler, 1983, observed plasma pH values (this study), and a mean water pH of 7.5, J. B. Claiborne & D. H. Evans, unpublished]. Thus, only a small net PNH₃ gradient (30 nmHg) was available to drive diffusion of NH₃ during this period. In contrast, the gradient for NH₃ efflux immediately following the NH₄Cl infusion was approx. 640 nmHg (20 versus 660 nmHg, water and plasma, respectively). Subsequent to the seawater change at hour 4, the PNH₃ of the water decreased to approx. 9 nmHg, thus creating a larger gradient for the outward diffusion of NH₃ once more. That ammonia excretion could continue (Fig. 2A) when the PNH₃ gradient was relatively small, is an indication that these animals can utilize some active form of NH₄⁺ excretion when necessary. This finding led us to perform a separate series of experiments designed to assess further these transfers during high external ammonia exposure (see below).

**NH₄HCO₃ infusion**

The infusion of NH₄HCO₃ is different from that of NH₄Cl (described above), in that NH₄HCO₃ dissociation will result in the fish receiving equal amounts of an acid (NH₄⁺) and a base (HCO₃⁻). If the animal were to lose the infused ammonia as NH₄⁺, any HCO₃⁻ not concurrently excreted should cause an internal base excess and concomitant pH increase. In contrast, if ammonia were transferred as NH₃, the remaining H⁺ would be buffered by the additional HCO₃⁻ (leading to an increase in plasma PCO₂ due to the dehydration of HCO₃⁻ to CO₂ and H₂O), and no pH change should be apparent. The present data indicate that both processes may have occurred during the hours subsequent to the infusion. In the first 2 h, NH₄⁺ and HCO₃⁻ excretion rates were similar (ΔH⁺ ≈ 0). Plasma T_Amm rapidly increased and then returned to control levels. At the same time, extracellular [HCO₃⁻] was elevated but subsequently did not decrease to the same extent. Both plasma PCO₂ and pH increased significantly during this interval. Since plasma [HCO₃⁻] remained elevated and blood pH increased, one could hypothesize that a portion of the excreted ammonia was lost in the form of NH₄⁺ without a parallel loss of HCO₃⁻. Given the maximum observed pH increase (0.071 units; the difference between hours 0 and 2), an estimate for extracellular space of 20%, and a blood buffer value of -12 mequiv pH⁻¹ (see previous section), only about 4% of
the observed NH₄⁺ transfer could have been voided in this manner. Thus, the pH changes following the infusion were much less than one would expect if a significant proportion of the ammonia excretion were due to NH₄⁺ transport without the concurrent loss of HCO₃⁻. Likewise, had all ammonia lost during the first few minutes post-infusion been due to NH₄⁺ transfer, the resulting plasma [HCO₃⁻] of 11-25 mmol l⁻¹ (and no change in PCO₂) would have led to a metabolic alkalosis with serosal pH values of approx. 8-12 (calculated from using solubility and pK' values derived from Boutilier et al. 1984). That the pH did not increase during the initial period, concurrent with the observed rapid elevation in plasma PCO₂, is evidence for a diffusive NH₃ transfer component.

As shown in the previous section, extracellular–intracellular transfers of the infused loads can play a role in the regulation of the observed exchanges. For example, the sum of the net excreted ammonia and that measured in the extracellular compartment after 1 h can only account for approx. 3-5 mmol (70%) of the infused load. The remainder was presumably sequestered intracellularly. In contrast, beginning at hour 1, net HCO₃⁻ transferred out of the animal plus the extracellular HCO₃⁻ increase was equal to or greater than the amount infused. Upon infusion of equal amounts of acid and base, the net H⁺ lost by the fish might be expected to remain near 0 (no different from control rates). Interestingly, net ΔH⁺ for the sculpin did not remain constant and, over the 20-h experiment, these animals exhibited a net H⁺ gain (or a net HCO₃⁻ loss) of 3-6 mmol kg⁻¹ due mainly to a 42% ‘overshoot’ in HCO₃⁻ loss (approx. 7-1 mmol kg⁻¹ over the 20-h experiment). The maintenance of the pre-infusion plasma [HCO₃⁻] vis-à-vis the net efflux of HCO₃⁻ is evidence for a contribution of the intracellular HCO₃⁻ pool to the observed net loss of this ion, and could again be due to the ‘ammonia stress’ imposed on the animal (see above).

High external ammonia concentration

When exposed to high external ammonia concentration (HEA) the plasma Tₐₐₚₚ of the sculpin increased threefold during the first 30 min of the exposure. This increase was due to a large net ammonia influx (approx 2-0 mmol kg⁻¹; Fig. 6A) which lasted for the first 4 h. Importantly, alterations in the rate of ammonia transfer accounted for about 90% of the calculated ΔH⁺ during the first few hours of HEA. This result, in combination with the negligible effects of the HEA on plasma pH, may suggest that a large portion of the Tₐₐₚₚ which entered the animal was in the form of NH₄⁺. Had NH₃ been the predominant form taken up by the animal, a reduction in ΔHCO₃⁻ and a blood alkalosis might be predicted (Cameron & Heisler, 1983; Cameron, 1986). These results, though in contrast to those described for freshwater trout and catfish, are not unexpected since the ionic permeability of the gills in marine species is thought to be relatively high (Evans, 1979), and these animals are capable of a more rapid recovery from acid–base disturbances (Toews et al. 1983; Claiborne & Heisler, 1984, 1986). Indeed, evidence for a role of ionic NH₄⁺ diffusion across the gills of the sculpin has been described previously (Goldstein et al. 1982). Interestingly, NH₄⁺ transfer changed
to an efflux from hours 4 to 6 of HEA and remained so for the entire recovery period such that net $\Delta NH_4^+$ returned to zero. Stated another way, the net ammonia gained during the first part of the HEA exposure was subsequently excreted again. Furthermore, the initial loss of ammonia was accomplished by the fish when the external [NH$_4$Cl] was still elevated to approx. 1·2 mmol l$^{-1}$. As can be seen in Fig. 7, this excretion was also measured at a time when the diffusion gradient for NH$_3$ between the fish and the water was nil. That a NH$_4^+$ efflux could be maintained during a period when NH$_3$ gradients between the animal and the water were effectively zero and NH$_4^+$ gradients were negative (from the water into the animal) suggests that the sculpin is capable of actively extruding ammonia (perhaps via Na$^+$/NH$_4^+$ or NH$_4^+$/$H^+$ exchange; see review by Evans & Cameron, 1986; Cameron, 1986) under these extreme conditions. However, sculpin (unlike the freshwater trout and catfish; Cameron & Heisler, 1983; Cameron, 1986, respectively) cannot maintain plasma $P_{NH_3}$ levels below that of an elevated external environment. When the high-ammonia water was flushed with normal sea water, plasma $T_{Amm}$ remained above the control level until hour 20. It is likely that ammonia taken up during the HEA was shuttled back out of intracellular compartments and, finally, into the water.

In conclusion, the maintenance of acid–base balance and the movements of NH$_4^+$ and HCO$_3^-$ have been studied in a marine teleost during exposure to either infused or externally induced ammonia stress. The present results demonstrate that the sculpin is permeable to both NH$_3$ and NH$_4^+$. Blood and ion-exchange data following ammonia infusion indicate that at least 50% of the infused load is excreted in the form of NH$_3$. In contrast, when external $T_{Amm}$ levels are elevated, ammonia appears to enter the animal as NH$_4^+$. A role for the excretion of ammonia in the form of NH$_4^+$ is also apparent (either via ionic diffusion of NH$_4^+$ or perhaps by some form of cation exchange), especially when outwardly directed diffusive NH$_3$ gradients are negligible. After each infusion, a majority of the ammonia and associated acid or base load is sequestered intracellularly and then presumably released to the extracellular fluid over time.

The authors would like to thank Drs James Cameron, Leon Goldstein and Gregg Kormanik as well as Ms Julie Walton for helpful discussions concerning this work. This research was supported by NSF DCM 86-02905 and Georgia Southern College Faculty Research Grants to JBC, and NSF PCM 83-02621 to DHE.

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