J. exp. Biol. **140**, 89–105 (1988)
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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_{CO_2}, $[HCO_3^-]$ and the net movements of NH_4^+, HCO_3^- and H^+ between the fish and the water have been studied during exposure to ammonia stress induced either by infusion (NH_4Cl or NH_4HCO_3; 5 mmol kg^{-1}) or by external application (NH_4Cl; approx. 1 mmol l^{-1}).

Following NH_4Cl infusion, a rapid decrease in blood pH (0·36 units) and $[HCO_3^-]$ (2·38 mmol l^{-1}) was observed, and within 1 h about 40 % of the ammonia load had been excreted to the water. Analysis of NH_4^+ and HCO_3^- transfers revealed that the total ammonia (T_{Amm}) efflux was due to a loss of NH_3 and NH_4^+ in approximately equal proportions when an outwardly directed NH_3 diffusion gradient was established.

Infusion of NH_4HCO_3 induced only small changes in plasma pH, and the rate of net HCO_3^- excretion was some 90 % higher than that of NH_4^+ over 20 h. These data indicate a predominance of NH_3 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_{Amm} induced an initial uptake of NH_4^+, but after 4 h of exposure ammonia efflux resumed even though NH_3 diffusion gradients were negligible. Thus, in this seawater teleost, a role for the excretion of ammonia in the form of NH_4^+ is also likely.

Introduction

Teleost fish excrete nitrogenous waste products in the form of ammonia ($NH_3 + NH_4^+$). 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

Key words: marine teleost, ammonia, acid–base, gill, ion transfer.

freshwater trout (*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^+/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 (*Ictalurus punctatus*) can be maintained in the face of reversed NH_3 and NH_4^+ concentration gradients by activating an NH_4^+/H^+ exchange system. Using the isolated perfused head preparation, roles for the ionic diffusion of NH_4^+ as well as NH_4^+/Na^+ exchange have also been postulated for two seawater teleosts (*Opsanus beta* and *Myoxocephalus octodecimspinosus*; Goldstein *et al.* 1982; Claiborne *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 *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 (*Myoxocephalus octodecimspinosus* Mitchell) following exposure to infused ammonia loads (NH_4Cl or NH_4HCO_3) or a reversed external ammonia gradient.

Materials and methods

Long-horned sculpin (*Myoxocephalus octodecimspinosus*), 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 fibreglass 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 (1·3–2·0 l) 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 CO_2 concentration, T_{CO_2} (Capnicon-Con II; Cameron Instruments Inc.) and plasma total ammonia concentration (T_{Amm}; Sigma kit no. 170-UV). Plasma P_{CO_2} and $[HCO_3^-]$ were calculated from T_{CO_2} and pH using values for CO_2 solubility and pK′ derived from Boutilier *et al.* (1984). Water samples (20 ml) were collected periodically and analysed for T_{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 μl of acid (<1 %) in a 10 ml water sample, thus the overall resolution was approx. 25 μmol in a 1·3 l chamber. The ΔHCO_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 ΔHCO_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 NH_4Cl or NH_4HCO_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 NH_4Cl (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 $[T_{Amm}]$.

Calculations

ΔHCO_3^- and ΔNH_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 (ΔH^+) is therefore the difference between ΔNH_4^+ and ΔHCO_3^- (for details see Claiborne & Heisler, 1984, 1986; Heisler, 1984). 'Net Δ' 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 ΔH^+ during control conditions was due to a measured HCO_3^- uptake (20 %) and a more significant T_{Amm} excretion (80 %).

NH_4Cl infusion

As shown in Fig. 1A, intraperitoneal infusion of NH_4Cl induced an increase in plasma T_{Amm}, which rose to approx. 5400 μmol l^{-1} immediately following the infusion and then returned to levels near control values within 8 h (T_{Amm} control, 234 ± 29 μmol l^{-1}; T_{Amm} 8 h post-infusion, 314 ± 70 μmol 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 P_{CO_2} rose by

Table 1. *Control values for acid–base and ion transfer parameters in the long-horned sculpin*

Plasma ($N = 16$)	
pH	7·78 ± 0·01
T_{CO_2} (mmol l^{-1})	5·53 ± 0·29
P_{CO_2} (mmHg)	2·02 ± 0·09
$[HCO_3^-]$ (mmol l^{-1})	5·42 ± 0·29
T_{Amm} (mmol l^{-1})	0·25 ± 0·03
Cumulative ion transfer (mmol kg^{-1} h^{-1}; $N = 15$)	
ΔNH_4^+	0·29 ± 0·03
ΔHCO_3^-	−0·07 ± 0·03
ΔH^+	0·35 ± 0·03

X ± S.E., 'Δ' ion transfer rates represent the cumulative appearance of each ion in the surrounding water.

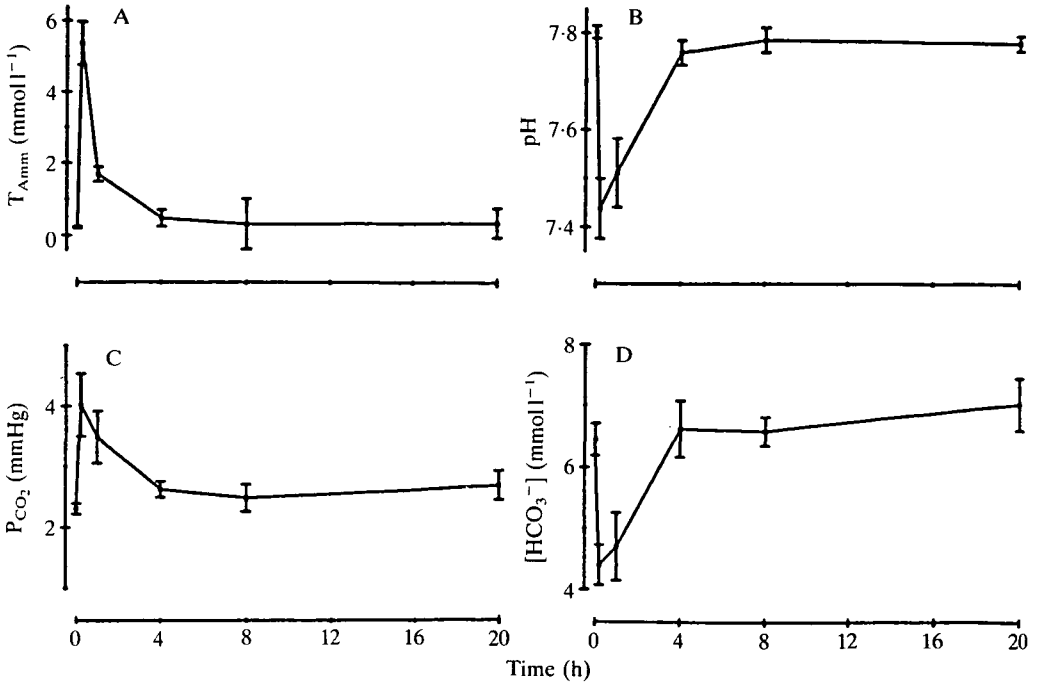

Fig. 1. Plasma total ammonia (A), pH (B), P_{CO_2} (C) and $[HCO_3^-]$ (D) in the sculpin following NH_4Cl infusion. Time zero points represent pre-infusion control values for each parameter (mean ± S.E., $N = 5$).

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_4Cl 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 $0{\cdot}29 \pm 0{\cdot}06$ mmol kg^{-1} h^{-1} to $2{\cdot}15 \pm 0{\cdot}08$ mmol kg^{-1} h^{-1} ($P < 0{\cdot}001$, $N = 4$). 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 $3{\cdot}57 \pm 0{\cdot}25$ mmol kg^{-1} at hour 4 and totalled $4{\cdot}61 \pm 0{\cdot}54$ 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 $0{\cdot}11 \pm 0{\cdot}02$ mmol kg^{-1} h^{-1} was altered to a net efflux of $1{\cdot}06 \pm 0{\cdot}28$ mmol kg^{-1} h^{-1} during the first hour after NH_4Cl application. HCO_3^- efflux was maintained up to

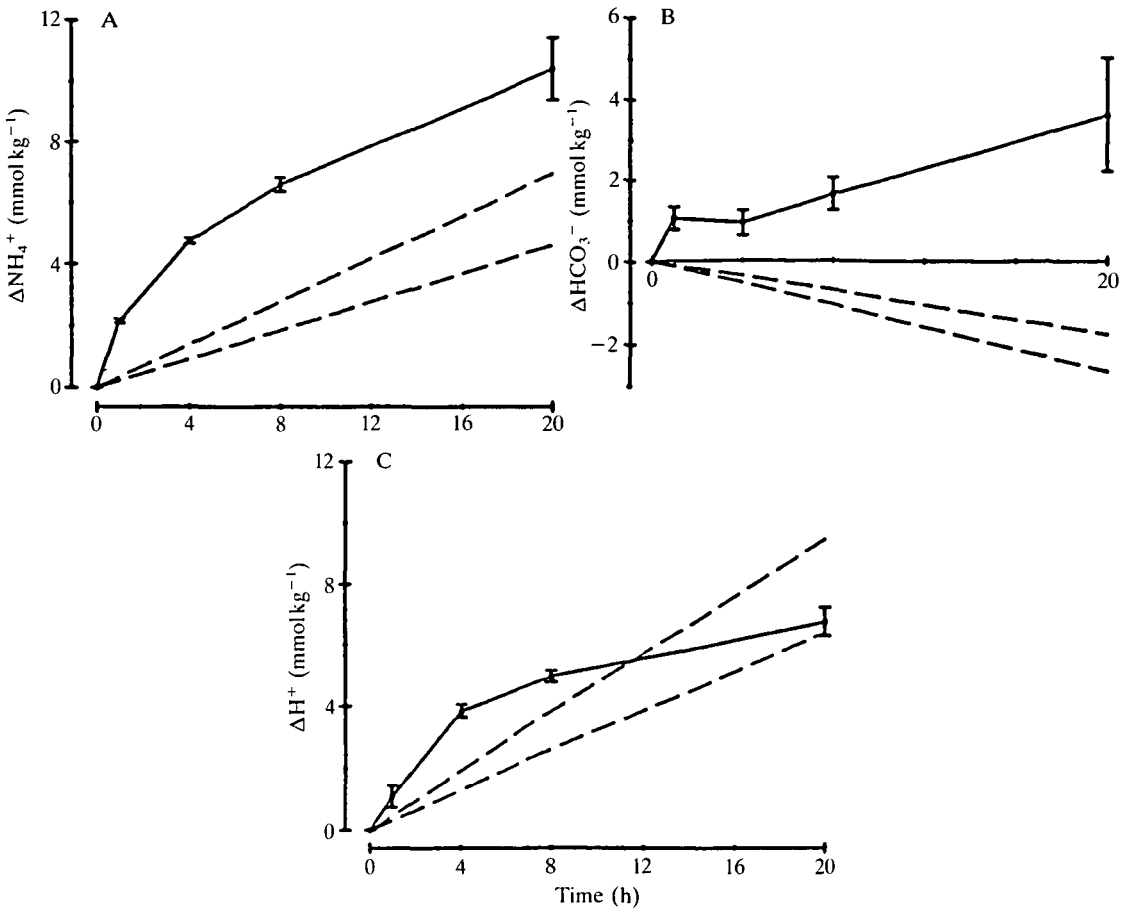

Fig. 2. Changes in cumulative NH_4^+ (A), HCO_3^- (B) and H^+ (C) transfers following NH_4Cl infusion (mean ± S.E., $N = 4$). Control lines (dashed) represent the measured control rate of efflux (±S.E.) extended as a reference over the subsequent experimental period.

and during hour 20 (0·14 ± 0·08 mmol kg^{-1} h^{-1}, hours 1–20). Net HCO_3^- 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.

NH_4HCO_3 infusion

Infusion of NH_4HCO_3 (5 mmol kg^{-1}) caused a rapid and significant increase in

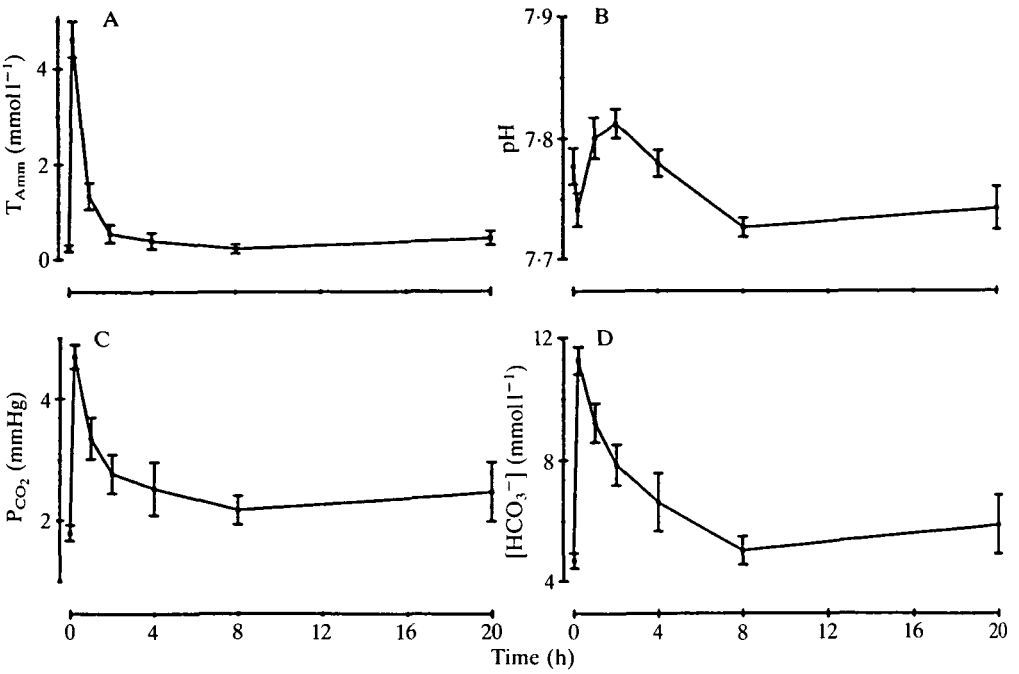

Fig. 3. Plasma total (A), pH (B), P_{CO_2} (C) and $[HCO_3^-]$ (D) following NH_4HCO_3 infusion. Time zero points represent pre-infusion control values for each parameter (mean ± S.E., $N = 5$).

both plasma T_{Amm} and $[HCO_3^-]$ (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 $[HCO_3^-]$ reached a maximum of 11·25 ± 0·45 mmol l^{-1} after NH_4HCO_3 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_{CO_2} (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 NH_4^+ excretion (Fig. 4A) increased by 12-fold during the first hour following the NH_4HCO_3 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 ΔNH_4^+ was 3·76 mmol kg^{-1} and reached a maximum of 4·42 mmol kg^{-1} at hour 8. ΔHCO_3^- 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, HCO_3^- loss continued through hours 4–8 (0·33 ± 0·06 mmol kg^{-1} h^{-1}, $P < 0·01$), resulting

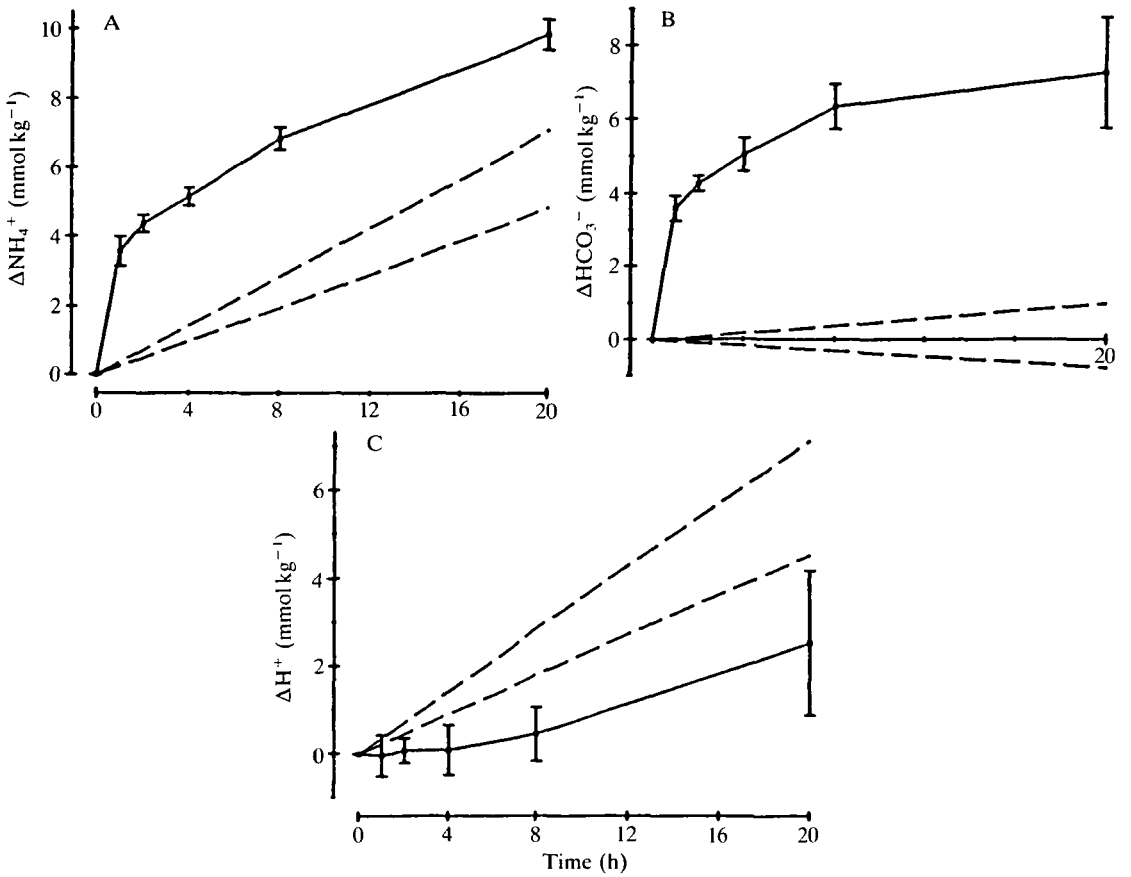

Fig. 4. Changes in cumulative NH_4^+ (A), HCO_3^- (B) and H^+ (C) transfers following NH_4HCO_3 infusion (mean ± S.E., $N = 5$). Control lines (dashed) represent the measured control rate of efflux (±S.E.) extended as a reference over the subsequent experimental period.

in a net ΔHCO_3^- 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 T_{CO_2} 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

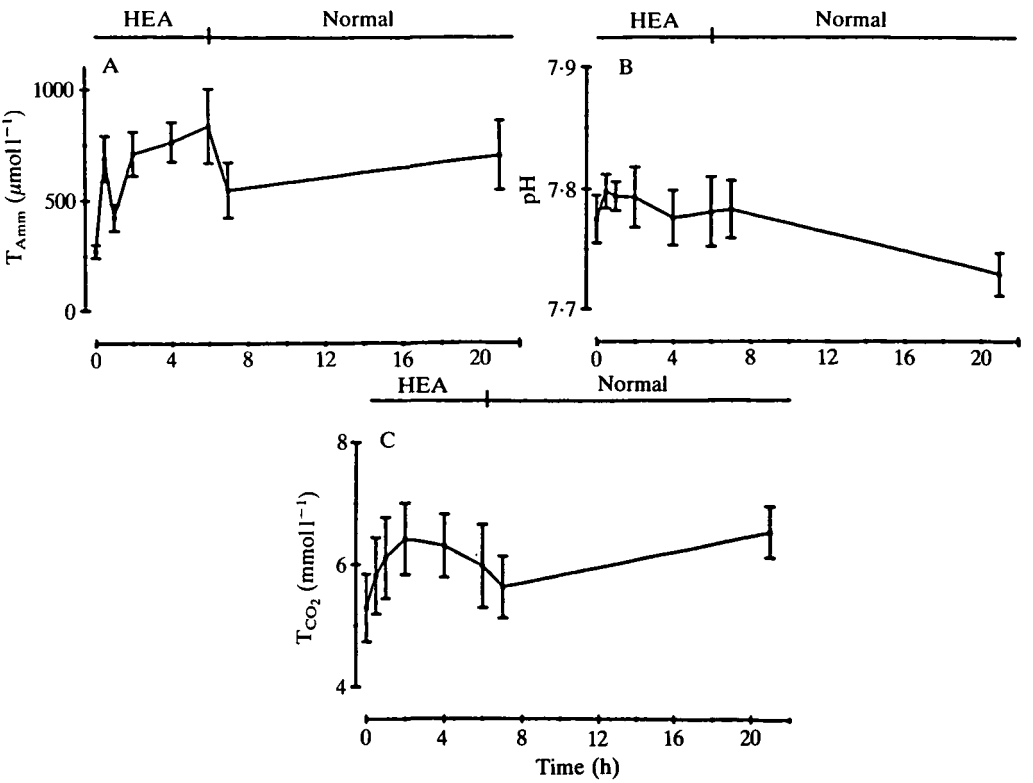

Fig. 5. Plasma total ammonia (A), pH (B) and T_{CO_2} (C) during and after exposure to high external ammonia concentration (HEA; approx. 1 mmol l^{-1} NH_4Cl). Time zero points represent pre-exposure control values for each parameter (mean ± S.E., $N = 6$).

from a control of 230 ± 30 to 690 ± 103 μmol l^{-1}. T_{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 ΔNH_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, ΔNH_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. ΔHCO_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, ΔHCO_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

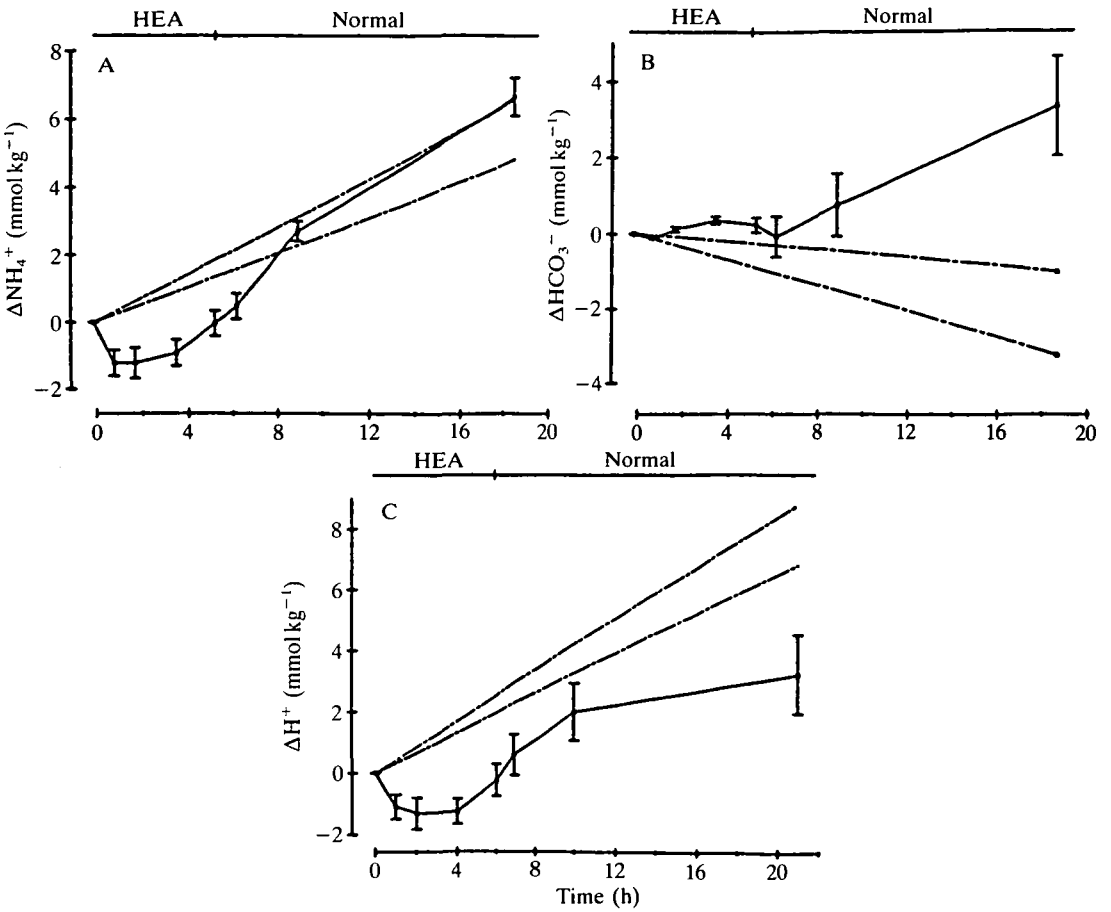

Fig. 6. Changes in cumulative NH_4^+ (A), HCO_3^- (B) and H^+ (C) transfers during and after exposure to high external ammonia concentration (HEA) (mean ± S.E., $N = 6$). Control lines (dashed) represent the measured control rate of efflux (±S.E.) extended as a reference over the subsequent experimental periods.

recovery period. The measured NH_4^+ and HCO_3^- transfers resulted in a negative net ΔH^+ (a net base loss; Fig. 6C) of $2{\cdot}74 \pm 0{\cdot}58$ mmol kg^{-1} from hours 1 to 4 of HEA. ΔH^+ then resumed rates similar to or slightly higher than the control ΔH^+ measurement ($0{\cdot}37 \pm 0{\cdot}05$ mmol kg^{-1} h^{-1}) from hours 4 to 10, such that during this interval a net H^+ efflux of 1·00 mmol kg^{-1} occurred. During the last sampling period of the recovery, ΔH^+ again decreased to $0{\cdot}12 \pm 0{\cdot}04$ mmol kg^{-1} h^{-1}.

To study the gradients driving the movement of NH_3 across the gills (Fig. 7), we utilized the measured plasma and water pH and T_{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) NH_3 diffusion gradient of about 60 nmHg was measured. Within 1 h after exposure to the HEA, however, plasma P_{NH_3} was not significantly different from

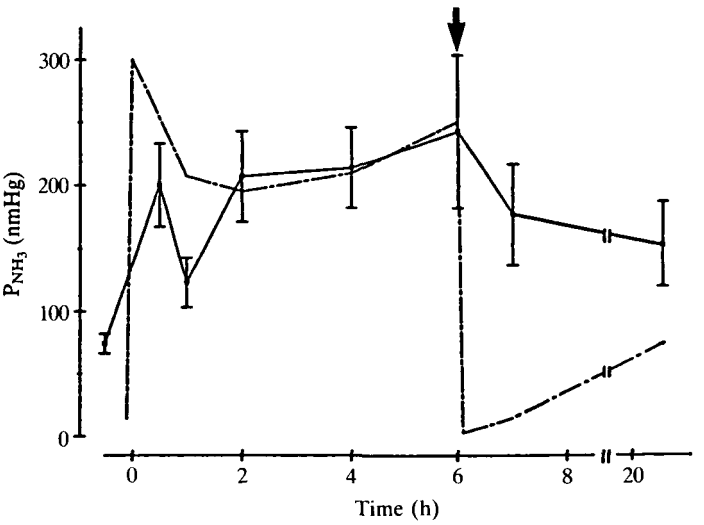

Fig. 7. Plasma and water (dashed line; mean ± S.E., $N = 6$) P_{NH_3} during and after high external ammonia concentration. Control values are those prior to time zero. The bath was flushed with normal sea water at the time shown by the arrow.

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_{CO_2} and P_{O_2} indicated that these parameters remained normal over the length of the experiment (J. B. Claiborne & D. H. Evans, unpublished observations).

NH_4Cl infusion

In many aspects, the present data confirm those presented by Cameron & Kormanik (1982) and Cameron & Heisler (1983) for two freshwater-adapted teleosts. We found that infusion of NH_4Cl induced a rapid decrease in plasma pH and $[HCO_3^-]$ and an increase in P_{CO_2}. The responses are similar to those expected following a metabolic acid load or infusion (Holeton *et al.* 1983; Turner *et al.* 1983; Cameron & Kormanik, 1982). The above authors ascribe these effects to the rapid loss of the infused ammonia as NH_3 (into the water), with a more gradual equimolar excretion or intracellular buffering of the remaining H^+. An alternative explanation for the observed extracellular acidosis could be that the infused ammonia enters metabolic pathways and is thus buffered intracellularly as NH_3 (for example with glutamate; L. Goldstein, personal communication) thus again liberating equimolar amounts of H^+. The NH_3 may then be slowly shuttled back into the blood where it could be extruded along with the remaining protons (as $NH_3 + H^+$ and/or NH_4^+).

By examining the transfers of NH_4^+, HCO_3^- and net H^+ 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 NH_4^+ excretion of 2·1 mmol kg^{-1} in combination with a ΔHCO_3^- of approx. 1·0 mmol kg^{-1} resulted in a net H^+ loss of approx. 1·1 mmol kg^{-1}. Since any NH_3 leaving the animal would create an equivalent amount of HCO_3^- in the water (the majority of NH_3 lost to the sea water would be immediately protonated to NH_4^+), it appears that, during this first hour, about 48 % of the total ammonia excretion was due to the loss of NH_3, and the remainder was *via* the combined loss of NH_3 and H^+, the direct transfer of NH_4^+ along with Cl^-, or the exchange of NH_4^+ with external Na^+ (see review by Evans & Cameron, 1986). Unfortunately, the transfer of $NH_3 + H^+$ is indistinguishable from the movement of NH_4^+ using present analytical methods. When the control rate of ammonia excretion is taken into account, the calculated net NH_4^+ efflux is 1·9 mmol kg^{-1}. Thus, about 40 % of the infused ammonia had appeared in the water within 1 h. At the same time, a net H^+ loss of 0·7 mmol kg^{-1} amounted to about 15 % of the infused load, again an indication of the direct loss of NH_3.

Using 1·0 mmol kg^{-1} as an estimate of the NH_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

-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 NH_4Cl 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 NH_4^+ (or $NH_3 + H^+$) efflux (the net ΔHCO_3^- 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 NH_4^+ loss of 5·7 mmol kg^{-1} and a ΔHCO_3^- of 2·8 mmol kg^{-1} indicate that about 47 % of the total ammonia excretion was again due to NH_3 while the remainder must have resulted from the transfer of NH_4^+ (or $NH_3 + H^+$). The net NH_4^+ lost after 20 h was $4{\cdot}61 \pm 0{\cdot}54$ 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 NH_4Cl and any non-ionic elimination of NH_3 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 HCO_3^-) during the next 16 h (Fig. 2C). Cameron & Kormanik (1982) found that after an infusion of NH_4Cl 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 NH_3 and H^+ within the gill epithelium (Goldstein *et al.* 1964; Cameron & Heisler, 1983), and thereby indirectly affects intra- and extracellular H^+ and HCO_3^- balance.

Although the modes of ammonia excretion in these fish appear to include the transfer of both NH_4^+ (or $NH_3 + H^+$) and NH_3, 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 NH_3, whereas from hours 1 to 4, NH_4^+ or $NH_3 + H^+$ transfer accounted for the entire ammonia lost. The question arises as to why these animals would switch from a passive non-ionic diffusion of NH_3 to a presumably carrier-mediated transport of NH_4^+ 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 P_{NH_3} was approx. 95 nmHg and the plasma P_{NH_3} 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 P_{NH_3} gradient (30 nmHg) was available to drive diffusion of NH_3 during this period. In contrast, the gradient for NH_3 efflux immediately following the NH_4Cl infusion was approx. 640 nmHg (20 *versus* 660 nmHg, water and plasma, respectively). Subsequent to the seawater change at hour 4, the P_{NH_3} of the water decreased to approx. 9 nmHg, thus creating a larger gradient for the outward diffusion of NH_3 once more. That ammonia excretion could continue (Fig. 2A) when the P_{NH_3} gradient was relatively small, is an indication that these animals can utilize some active form of NH_4^+ 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).

NH_4HCO_3 infusion

The infusion of NH_4HCO_3 is different from that of NH_4Cl (described above), in that NH_4HCO_3 dissociation will result in the fish receiving equal amounts of an acid (NH_4^+) and a base (HCO_3^-). If the animal were to lose the infused ammonia as NH_4^+, any HCO_3^- not concurrently excreted should cause an internal base excess and concomitant pH increase. In contrast, if ammonia were transferred as NH_3, the remaining H^+ would be buffered by the additional HCO_3^- (leading to an increase in plasma P_{CO_2} due to the dehydration of HCO_3^- to CO_2 and H_2O), 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, NH_4^+ and HCO_3^- excretion rates were similar ($\Delta H^+ \approx 0$). Plasma T_{Amm} rapidly increased and then returned to control levels. At the same time, extracellular [HCO_3^-] was elevated but subsequently did not decrease to the same extent. Both plasma P_{CO_2} and pH increased significantly during this interval. Since plasma [HCO_3^-] remained elevated and blood pH increased, one could hypothesize that a portion of the excreted ammonia was lost in the form of NH_4^+ without a parallel loss of HCO_3^-. 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 NH_4^+ 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 NH_4^+ transport without the concurrent loss of HCO_3^-. Likewise, had all ammonia lost during the first few minutes post-infusion been due to NH_4^+ transfer, the resulting plasma $[HCO_3^-]$ of 11·25 mmol l^{-1} (and no change in P_{CO_2}) 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 P_{CO_2}, is evidence for a diffusive NH_3 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 HCO_3^- transferred out of the animal plus the extracellular HCO_3^- 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 HCO_3^- loss) of 3·6 mmol kg^{-1} due mainly to a 42 % 'overshoot' in HCO_3^- loss (approx. 7·1 mmol kg^{-1} over the 20-h experiment). The maintenance of the pre-infusion plasma $[HCO_3^-]$ *vis-à-vis* the net efflux of HCO_3^- is evidence for a contribution of the intracellular HCO_3^- 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 T_{Amm} 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 T_{Amm} which entered the animal was in the form of NH_4^+. Had NH_3 been the predominant form taken up by the animal, a reduction in ΔHCO_3^- 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 NH_4^+ diffusion across the gills of the sculpin has been described previously (Goldstein *et al.* 1982). Interestingly, NH_4^+ transfer changed

to an efflux from hours 4 to 6 of HEA and remained so for the entire recovery period such that net ΔNH_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_4Cl] 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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