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TRANSBRANCHIAL AMMONIA GRADIENTS AND ACID–BASE RESPONSES TO HIGH EXTERNAL AMMONIA CONCENTRATION IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) ACCLIMATED TO DIFFERENT SALINITIES

BY R. W. WILSON* AND E. W. TAYLOR

School of Biological Sciences, University of Birmingham, PO Box 363, Birmingham B15 2TT, UK and *ICI Environmental Laboratory, Brixham, Devon TQ5 8BA, UK*

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Summary

Transbranchial ammonia gradients and blood acid–base status have been examined in rainbow trout acclimated to fresh water (FW), 33 % sea water (33 % SW) and sea water (SW) and exposed to 1.0 mmol l^{-1} total ammonia (T_{Amm}) at pH 7.9 for 24 h. At all three salinities trout maintained large negative (inwardly directed) NH_3 and NH_4^+ gradients throughout the exposure, presumably by active excretion of NH_4^+ to counteract the passive inward diffusion of ammonia. Analysis of blood non-respiratory acid–base status (ΔH_m^+) revealed an acid load in FW trout and a base load in SW trout following 24 h of exposure. This indicates that active NH_4^+/H^+ exchange predominates in FW whereas NH_4^+/Na^+ is the principal exchange utilised in SW under these experimental conditions.

The plasma T_{Amm} load incurred during ammonia exposure increased with salinity. Compared to FW trout, plasma T_{Amm} values were 34 and 73 % higher in the 33 % SW and SW trout, respectively, after 24 h. This cannot be explained by differences in the prevailing transbranchial P_{NH_3} gradient because ambient P_{NH_3} was substantially lower at the higher salinities (due to higher pK′ and solubility values). We interpret the difference between FW and SW trout as an increased permeability to NH_4^+ in fish acclimated to the higher-salinity environments. Transbranchial diffusion of NH_4^+ is, therefore, probably more important as a route for ammonia excretion in SW than in FW trout, especially considering the favourable transepithelial potentials normally found in SW teleosts. In addition, increased NH_4^+ permeability implies that the toxicity of ammonia will be greater in seawater than in freshwater teleosts and should not simply be measured as a function of the unionised ammonia concentration when considering seawater-adapted species.

*Present address: Department of Biology, McMaster University, 1280 Main St West, Hamilton, Ontario, Canada L8S 4K1.

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Introduction

Teleost fish excrete the majority of their nitrogenous waste as ammonia (Smith, 1929). By far the largest proportion of this ammonia output occurs at the gills (Smith, 1929; Sayer and Davenport, 1987) and results from clearance of ammonia from the blood (Payan and Matty, 1975; Cameron and Heisler, 1983; Ogata and Murai, 1988) as it crosses the arterio-arterial circuit through the lamellae (Payan *et al.* 1984). Under normal conditions of low ambient [ammonia] the transbranchial ammonia gradients (ΔP_{NH_3} and ΔNH_4^+) are positive (from blood to water) and ammonia excretion can theoretically be achieved by a variety of mechanisms; transcellular and/or paracellular diffusion of NH_3, paracellular diffusion of NH_4^+, apical or basolateral NH_4^+/Na^+(or H^+) exchange, and basolateral $NH_4^+/Na^+/2Cl^-$ cotransport (see recent reviews by Cameron and Heisler, 1985; Evans and Cameron, 1986; Randall and Wright, 1987). Despite numerous attempts to quantify the importance of each of these transport mechanisms, the subject of branchial ammonia transfer across fish gills remains controversial.

Transbranchial ammonia gradients can be experimentally reversed by exposure to elevated external ammonia concentrations. When ΔP_{NH_3} and ΔNH_4^+ are reversed both freshwater and marine teleosts are able to maintain the total ammonia concentration ($T_{Amm}=NH_4^++NH_3$) in their extracellular fluid below that of the external environment (Cameron and Heisler, 1983; Cameron, 1986; Claiborne and Evans, 1988). These authors attributed this to active exchange of NH_4^+ for an external counterion (Na^+ or H^+), which is sufficient to balance the passive inward diffusion of ammonia.

The blood acid–base changes and the ammonia load induced by ammonia exposure will depend upon the relative permeability of the fish to NH_3 and NH_4^+ and the mechanism used in the active excretion of NH_4^+. In freshwater teleosts, when transbranchial ammonia gradients are reversed, the passive uptake of ammonia occurs predominantly *via* the inward diffusion of NH_3 (Fromm and Gillette, 1968; Cameron and Heisler, 1983; Cameron, 1986; Claiborne and Evans, 1988; Avella and Bornancin, 1989). This has generally been explained by the high diffusibility of lipid-soluble, gaseous NH_3 through cell membranes (Jacobs, 1940; Pitts, 1973) in contrast to the relatively low permeability of cell membranes and 'tight' epithelia (such as freshwater fish gills; Sardet, 1980; Pisam *et al.* 1987) to the hydrated, charged and poorly lipid-soluble ammonium ion. However, the gills of seawater-adapted teleosts have a higher ionic permeability than those of freshwater teleosts (by a factor of 10 or more; Girard and Payan, 1980; Evans, 1979, 1984), which may include an increased permeability to NH_4^+ (Goldstein *et al.* 1982; Claiborne and Evans, 1988; Evans *et al.* 1989). In seawater-adapted fish the uptake of ammonia when gradients are reversed may, therefore, be augmented by a significant inward diffusion of NH_4^+. To test this hypothesis we have compared the ammonia loading and blood acid–base response during exposure to high external ammonia concentration in rainbow trout acclimated to different salinities ranging from fresh water to full-strength sea water (35 ‰). Thus, for the first time,

the influence of salinity on branchial ammonia transfer has been investigated within a single euryhaline species.

Materials and methods

Animals

Rainbow trout, *Oncorhynchus mykiss* (Walbaum) (300–800 g), were obtained from Zeals Fish Farm, Wiltshire. Following transportation to the ICI Environmental Laboratory, Brixham, Devon, they were transferred to 600 l grey fibreglass tanks and maintained in continuously flowing, dechlorinated Brixham tapwater ($[Na^+]\approx0.6$, $[Cl^-]\approx0.5$, $[Ca^{2+}]\approx0.8$ mequiv l^{-1}; pH=7.0–7.8; T=10–18°C). Fish were kept on a maintenance diet (1 % body weight day^{-1}) of commercial trout pellets but were starved for 4 days prior to surgery (to avoid post-prandial changes in metabolic rate and excretion of nitrogenous waste during experiments; Jobling, 1981).

Freshwater trout

Trout used for freshwater experiments were transferred to 600 l acclimation tanks. Water temperature was adjusted to 15 °C in steps of 2 °C per day and then maintained at that temperature for at least 2 weeks prior to experiments.

33 % seawater-acclimated trout

A continuous supply of 33 % sea water was obtained by mixing dechlorinated tapwater and Torbay sea water at a ratio of 2:1 in a flow-through system. Freshwater trout were transferred to acclimation tanks and the salinity was raised to 12 ‰ ($[Na^+]\approx170$, $[Cl^-]\approx200$ mequiv l^{-1}) over a period of 24 h. The temperature of the acclimation water was adjusted to 15 °C in steps of 2 °C per day, and the fish were allowed a minimum acclimation period of 2 weeks once the desired salinity and temperature had been reached.

Seawater trout

Fish were transferred to acclimation tanks and the salinity of the incoming water was increased in steps to full-strength Torbay sea water (35 ‰, $[Na^+]\approx460$, $[Cl^-]\approx550$ mequiv l^{-1}) over a period of 10 days. During this time, water temperature was adjusted to 12 °C and the trout were then given a minimum of 4 weeks to acclimate to this salinity and temperature before use in experiments (the seawater temperature could not be maintained at 15 °C as in the previous two regimes, and 12 °C proved to be the highest constant temperature attainable). Daily water samples were taken for monitoring of salinity (Kent Industrial Instruments Salinometer; model MC5).

Experimental protocol

To enable repeated blood sampling, fish were anaesthetised with a 100 mg l^{-1}

solution of MS222 (Sigma) dissolved in a medium of the appropriate salinity (buffered to approximately pH 7.5 with $NaHCO_3$ in fresh water). Once anaesthetised, fish were fitted with a chronic indwelling dorsal aortic catheter (Soivio *et al.* 1972) whilst the gills were irrigated with a lower concentration of oxygenated anaesthetic (60 mg l^{-1} MS222). Following surgery, catheters were filled with sodium-heparinised trout saline (Perry *et al.* 1984; heparin=50 i.u. ml^{-1}) and fish were transferred to individual, darkened, Perspex respirometer tubes (40 cm×10 cm) supplied with aerated water ($P_{O_2} \geqslant 19.3$ kPa) of the appropriate salinity and allowed to recover for a minimum of 36 h.

The experimental set-up was designed to allow exposure of fish to elevated levels of ammonia in a pH-statted flow-through system. Respirometers received water at a rate of 500–1000 ml min^{-1} during experiments (according to fish size). When required, a stock solution of 250 mmol l^{-1} ammonium sulphate was dosed into mixing cells situated immediately upstream from the respirometers using peristaltic pumps (Watson–Marlow) at 1/500 of the water flow rate to produce the desired water concentration of 1.0 mmol l^{-1} total ammonia. Water pH was nominally maintained at 7.9 throughout all regimes by controlled addition of either 3 mol l^{-1} HCl (to sea water) or 2 mol l^{-1} NaOH (to fresh water). Water pH was monitored using Corning pH and reference electrodes in conjunction with a Kent Industrial pH meter. The output from the meter controlled the speed of a programmable peristaltic pump (Watson–Marlow, 202U/AA), which dosed the acid or base. In addition to this control system, water pH was monitored in the mixing cells (Corning electrodes and meter) to give more accurate readings for the pH of water entering the respirometers.

Catheterised trout were exposed to 1.0 mmol l^{-1} [T_{Amm}] at pH 7.9 for a single 24-h period, in fresh water, 33 % sea water (both at 15°C) and full-strength sea water (12°C). Approximately 1 h before exposure to high ammonia concentrations a single 'control' blood sample was taken. To follow the changes in acid–base status, plasma ammonia and ions, blood samples were subsequently taken after 1, 5 and 24 h of exposure to high ammonia.

Analytical techniques

Arterial blood samples (1.0 ml) were anaerobically drawn into chilled Hamilton gas-tight syringes. A 400 μl subsample was immediately centrifuged for 3 min at 13 500 *g* (MSE Microcentaur) for later plasma T_{Amm} and ion analysis. The remainder of the sample was used for the determination of whole-blood Pa_{O_2}, haematocrit (Hct), haemoglobin content ([Hb]), and the pH and total CO_2 (T_{CO_2}) of both whole blood and plasma. The blood used for Pa_{O_2} measurement (approximately 250 μl) was then returned to the animal followed by infusion of enough saline to replace the net blood volume removed (approximately 750 μl). Whole-blood and true plasma pH were measured using a Radiometer G279/G2 glass capillary electrode coupled with a K497 calomel reference electrode. Both electrodes were thermostatted to the experimental temperature (15 or 12°C) and used in conjunction with a pH/blood gas monitor (Radiometer PHM73). Total

CO_2 was measured on 50 μl subsamples by the method of Cameron (1971) using a P_{CO_2} electrode (Radiometer E5037) connected to the same pH/blood gas monitor as above. For both plasma pH and T_{CO_2} measurements, plasma was taken from blood centrifuged anaerobically in microhaematocrit tubes used for Hct determination (see below). Arterial blood oxygen tension (Pa_{O_2}) and water P_{O_2} were measured using oxygen electrodes (Radiometer E5046), both thermostatted to experimental temperature, and oxygen meters (Strathkelvin). For each blood sample Hct was determined in duplicate, using 80 μl sodium-heparinised microhaematocrit tubes centrifuged at 12 000 *g* in a haematocrit centrifuge (Hawksley) for 2 min.

[Hb] was determined in duplicate on 20 μl samples of blood using the cyanomethaemoglobin method (Sigma kit no. 525). Absorbance of the sample plus reagent solution was measured on a dual-beam spectrophotometer at 540 nm (Kontron Uvikon 860). Plasma [T_{Amm}] was measured on 200 μl subsamples using a specific enzymatic assay (GlDH/NADH; Sigma 170-UV at 340 nm) and a dual-beam spectrophotometer (Kontron Uvikon 860). Another 40 μl subsample of plasma was immediately diluted 100 times in deionised water and frozen (−20°C) for later measurement of the plasma cations Na^+, K^+, Ca^{2+} and Mg^{2+} by atomic absorption spectrophotometry (Pye Unicam SP9). Plasma [Cl^-] was measured by amperometric titration on 50 μl samples of undiluted plasma (Aminco–Cotlove automatic chloride titrator).

The water [T_{Amm}] was measured using an ammonia electrode (Philips IS 570-NH3 connected to an Orion specific ion meter, model 407A) after alkalisation of 50 ml samples with 1 ml of 10 mol l^{-1} NaOH to convert all ammonia to the free base form (NH_3). High-salinity samples were first treated with 1.5 mol l^{-1} EDTA (tetrasodium salt; 1 ml added to each 50 ml sample) to chelate metal ions that would otherwise form hydroxide precipitates on addition of NaOH.

Calculation of derived variables

Blood P_{CO_2} and plasma bicarbonate values were calculated from measurements of plasma T_{CO_2} and whole-blood pH using a rearrangement of the Henderson–Hasselbalch equation and values for CO_2 solubility and pK′ derived from Boutilier *et al.* (1984).

The P_{NH_3} and NH_4^+ in plasma and fresh water were similarly calculated from their respective pH and T_{Amm} values using the rearranged Henderson–Hasselbalch equation and values of pK′ and solubility determined by Cameron and Heisler (1983). For the 33 % sea water and full sea water media, values for pK′ were calculated from their NaCl concentrations and experimental temperatures (15 and 12°C, respectively) using the nomogram of Cameron and Heisler (1983) (9.605 for 33 % sea water and 9.776 for 100 % sea water), which both agree well with the values obtained from the data of Whitfield (1974) for salinities of 12 ‰ (at 15°C) and 35 ‰ (at 12°C).

It is important to note that the pK′ and solubility values of ammonia both increase as salinity increases and temperature decreases (Thurston *et al.* 1979). As

a result, the fraction of ammonia present as NH_3 was progressively lower at the higher salinities, despite constant water pH and T_{Amm} (see Table 1).

The transbranchial P_{NH_3} and $[NH_4^+]$ gradients (ΔP_{NH_3} and $\Delta[NH_4^+]$) were calculated by simple subtraction:

$$\text{e.g. } \Delta P_{NH_3} = P_{NH_3} \text{ in plasma} - P_{NH_3} \text{ in water},$$

where the plasma is from the dorsal aorta and, therefore, postbranchial in origin. Ideally, the mean plasma concentration in blood passing through the gill, ([arterial]+[venous])/2, should be used to determine the transbranchial gradients. Under normal conditions this can be predicted from the arterial plasma concentration alone, assuming a constant ratio for the $[T_{Amm}]$ in pre- and postbranchial blood (from 1.66 to 1.81; Cameron and Heisler, 1983; Wright and Wood, 1985; Ogata and Murai, 1988). However, this ratio does not remain constant under conditions where the ambient $[T_{Amm}]$ is raised (see Cameron and Heisler, 1983) and, hence, the mean plasma concentration cannot be estimated unless simultaneous dorsal and ventral aortic cannulations are made. In the present study, transbranchial gradients were simply calculated from dorsal aortic blood plasma measurements, which leads to an underestimate of ΔP_{NH_3} and ΔNH_4^+ during control conditions. However, this will have a much smaller influence on the calculated gradients during ammonia exposure because the arterial–venous T_{Amm} difference is much reduced (venous/arterial $[T_{Amm}]$ ratio approximately equal to 1.05 according to the data of Cameron and Heisler, 1983).

The concentration of metabolic (or non-respiratory) protons added to or removed from the blood plasma during the interval between any two successive blood samples was calculated according to the formula of McDonald *et al.* (1980) using non-bicarbonate buffer values estimated from the blood [Hb] and the regression relationship of Wood *et al.* (1982). The net load of acidic equivalents in blood plasma at any sample time was then calculated by summing the ΔH_m^+ values, signs considered, for each period from the control sample onwards.

Values are expressed as mean±one standard error (N) throughout the text. Time-dependent changes during each exposure regime were tested against individual pre-exposure control values, using a Student's two-tailed t-test (paired) at 5 % and 1 % levels of significance. The mean values for 33 % seawater and seawater trout, at each sample time, were also compared with the corresponding mean values from freshwater trout using a Student's unpaired t-test, again at 5 % and 1 % levels of significance.

Results

Ammonia gradients and internal ammonia load

Control values for $[T_{Amm}]$ in arterial plasma averaged 43±5 (6) in FW trout, 85±20 (8) in 33 %SW trout and 86±12 (5) μmol l^{-1} in SW trout (Fig. 1). The twofold higher plasma $[T_{Amm}]$ from the 33 %SW and SW trout ($P<0.05$) coincided with the higher $[T_{Amm}]$ in Brixham sea water (10–30 μmol l^{-1} compared with less

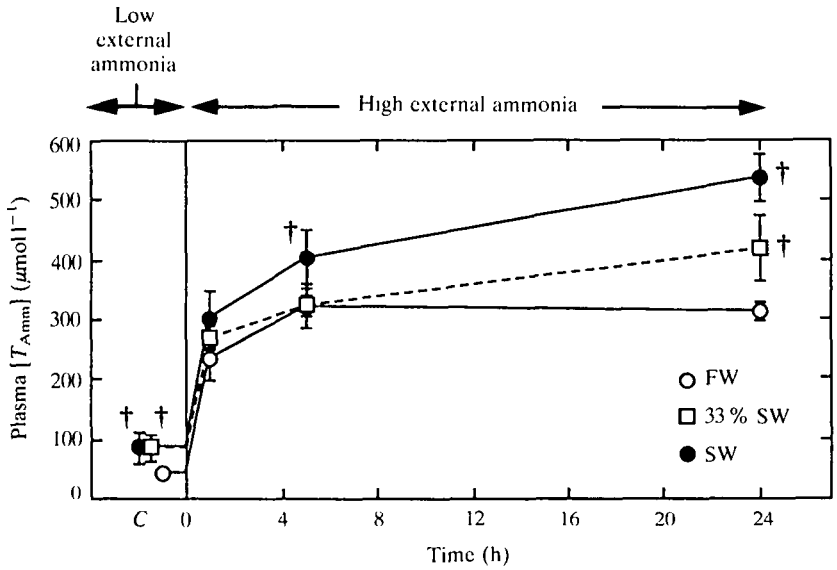

Fig. 1. Plasma total ammonia (T_{Amm}) in FW, 33 %SW and SW trout prior to and during 24 h of exposure to high external ammonia concentration (1000 μmol l^{-1} T_{Amm} at pH 7.9). External T_{Amm} during the control period (*C*) was less than 30 μmol l^{-1}. † denotes values significantly different when compared with the corresponding value from FW trout ($P<0.05$; Student's unpaired *t*-test). Mean values are shown ±1 S.E.M., $N=6$, 8 and 5 for FW, 33 %SW and SW trout, respectively.

than 10 μmol l^{-1} in fresh water). The resultant transbranchial ammonia gradients (ΔNH_4^+ and ΔP_{NH_3}) were always positive (from blood to water) during the control periods with no significant differences between the three groups (Fig. 2).

At the start of each exposure, water [T_{Amm}] was raised to a nominal value of 1000 μmol l^{-1} (995±9 in FW, 974±11 in 33 %SW and 989±25 μmol l^{-1} in SW). This caused an immediate reversal of both ΔP_{NH_3} and ΔNH_4^+ to large negative values. However, the magnitude of the initial ΔP_{NH_3} reversal was dependent on the ambient P_{NH_3} during high-ammonia treatments (Table 1). Accordingly, the initial reversals were 22 and 40 % smaller in 33 %SW and SW trout, respectively, when compared with FW trout. In contrast the $\Delta[NH_4^+]$ reversals were effectively the same in all three media (Fig. 2).

Reversal of the transbranchial ammonia gradients caused rapid and substantial increases in plasma T_{Amm} in all three groups within the first hour (Fig. 1). The general pattern of hyperammoniaemia was the same at all three salinities (a rapid initial rise over the first few hours followed by a stabilisation of plasma T_{Amm}). Although in all cases plasma T_{Amm} remained well below the external T_{Amm}, the magnitude of the resultant plasma ammonia load increased substantially with salinity. After 24 h the plasma T_{Amm} levels in FW, 33 %SW and SW trout were 311±15, 418±56 and 537±17 μmol l^{-1}, respectively. Both the 33 %SW and SW

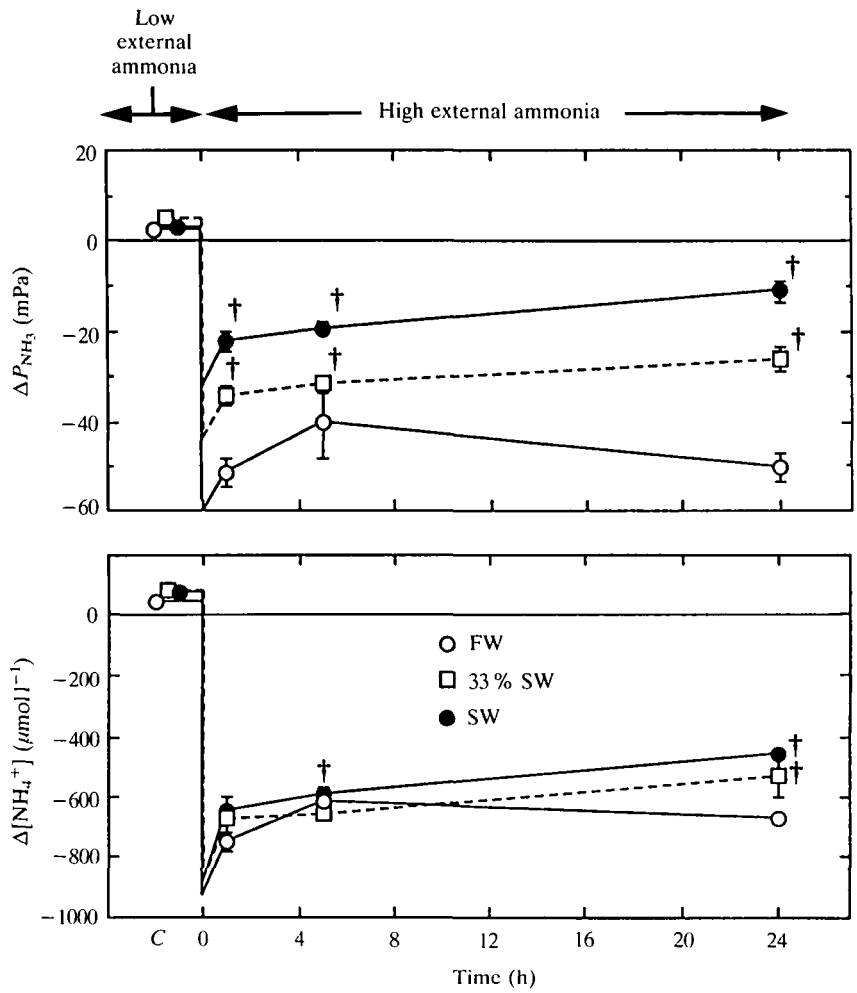

Fig. 2. Transbranchial P_{NH_3} and $[NH_4^+]$ gradients for FW, 33 %SW and SW trout during the control period (*C*) and during 24 h of exposure to high external ammonia concentration (mean±1 S.E.M.; for *N* values see Fig. 1). † denotes values significantly different when compared with the corresponding value from FW trout ($P<0.05$; Student's unpaired *t*-test).

trout had accumulated significantly more ammonia (34 % and 73 % more) than the FW trout by the end of the exposure ($P<0.05$).

In all cases ΔP_{NH_3} and ΔNH_4^+ remained negative throughout the high-ammonia treatment. However, FW trout maintained a more negative ΔP_{NH_3} than SW trout both as an absolute value (−50.5 mPa *vs* −11.2 mPa at 24 h; Fig. 2) and when compared with their respective ΔP_{NH_3} reversals at time zero (ΔP_{NH_3} values after 24 h were 87 % and 34 % of the original ΔP_{NH_3} in FW and SW trout, respectively). Although the original (time zero) reversal of ΔNH_4^+ was virtually

Table 1. *The calculated ambient* P_{NH_3} *during high-ammonia experiments at three different salinities*

	Fresh water	33 % sea water	Sea water
Temperature (°C)	15	15	12
pK'_{Amm}*	9.505	9.605	9.762
Water pH	7.85	7.87	7.93
$[T_{Amm}]$ (μmol l^{-1})	995	974	989
$[NH_3]$ (μmol l^{-1})	21.6	17.6	14.3
αNH_3 (μmol l^{-1} Pa^{-1})*	357.4	372.8	403.4
P_{NH_3} (mPa)	60.4	47.2	35.4

*From, or interpolated from, the data of Cameron and Heisler (1983).

Values for water pH and $[T_{Amm}]$ are the averages of measured values over each 24 h experimental period (N=18, 24 and 15 for FW, 33 %SW and SW, respectively).

the same in all media, after 24 h $\Delta[NH_4^+]$ was significantly smaller in the 33 %SW and SW trout (Fig. 2).

Acid–base status during high-ammonia treatment

The pre-exposure pH and $[HCO_3^-]$ values of SW trout were lower than those of the FW and 33 %SW groups, but arterial P_{CO_2} was essentially the same at all three salinities (Fig. 3). Exposure to ammonia caused no significant blood pH changes in FW trout, and only a slight alkalosis after 1 h in the 33 %SW trout. In contrast, the SW trout experienced a gradual increase in blood pH as the exposure continued which became significant after 24 h (an increase of 0.1 pH units over the control value; Fig. 3). There was a small reduction in arterial P_{CO_2} in all groups during the exposure, which occurred more rapidly at the two lower salinities (significant after just 1 h). The trend for a reduction in P_{CO_2} continued for 24 h in FW and SW trout. The only significant changes in plasma $[HCO_3^-]$ were small decreases observed in the FW trout after 1 and 24 h.

Analysis of the non-respiratory component of the acid–base changes elicited during high-ammonia treatment (ΔH_m^+) revealed opposite responses in FW and SW trout (Fig. 4). After 24 h, FW trout had accumulated a significant acid load (ΔH_m^+=1.8±0.4 mequiv l^{-1}, N=6; P<0.05) whereas SW trout had developed a significant acid deficit (i.e. a base load; ΔH_m^+=−2.8±0.7 mequiv l^{-1}, N=5; P<0.05). No significant change was observed in the 33 % SW group.

Blood oxygen and plasma ions

Arterial P_{O_2} was unaffected by ammonia exposure apart from a slight increase in the 33 %SW trout after 5 h. There were no significant differences between the three groups, with average Pa_{O_2} values within the range 12.5–14.2 kPa.

Plasma ion concentrations also remained unchanged during ammonia exposure, regardless of salinity. FW and 33 %SW trout had plasma ion levels typical of

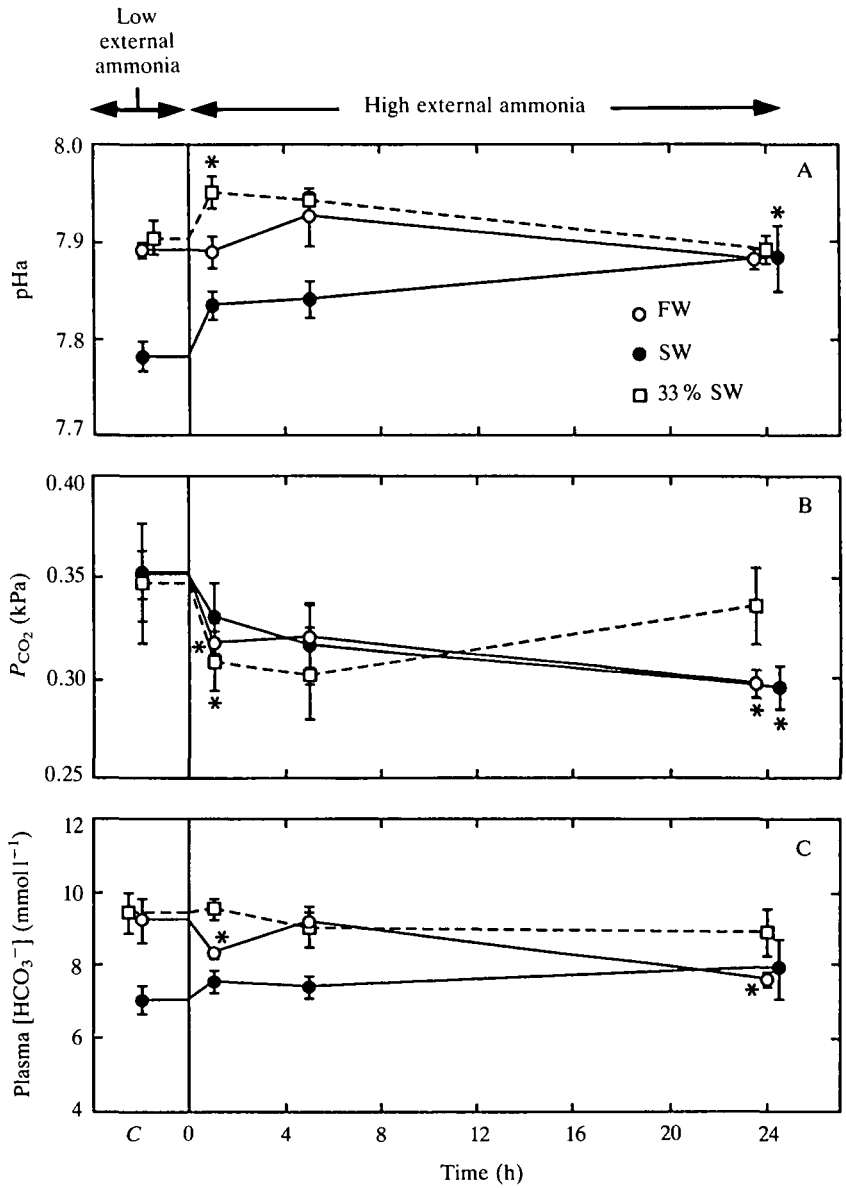

Fig. 3. Blood acid–base variables for FW, 33%SW and SW trout for the control period (*C*) and during 24 h of exposure to high external ammonia concentration; whole-blood pH (A), P_{CO_2} (B) and plasma [HCO_3^-] (C), (mean±1 S.E.M.; for *N* values see Fig. 1). * denotes a value significantly different from the control mean within the group ($P<0.05$).

previously published values for freshwater trout ($Na^+=148\pm3$, $Cl^-=126\pm2$, $K^+=2.6\pm0.1$, $Ca^{2+}=4.4\pm0.2$, $Mg^{2+}=1.8\pm0.1$ mequiv l^{-1}). However, SW trout had significantly higher plasma levels of all the measured ions except magnesium

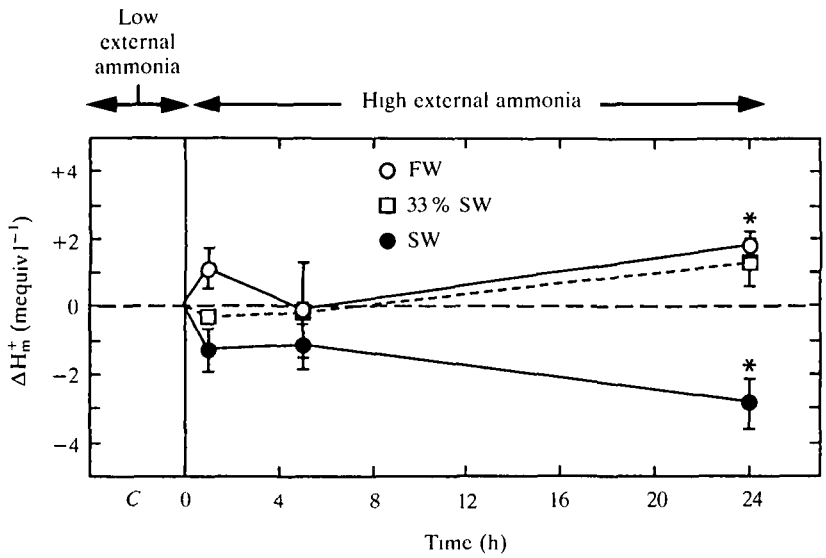

Fig. 4. The non-respiratory acid load for FW, 33%SW and SW trout during the control period (*C*) and during 24 h of exposure to high external ammonia concentration (mean±1 S.E.M.; for *N* values see Fig. 1). * denotes a value significantly different from the control mean within the group ($P<0.05$).

(Na^+=159±4, Cl^-=152±2, K^+=3.1±0.3, Ca^{2+}=5.8±0.5, Mg^{2+}=1.9±0.2 mequiv l^{-1}).

Discussion

The changes in plasma ammonia levels are consistent with those of previous reversed-gradient studies using freshwater rainbow trout (Cameron and Heisler, 1983), freshwater channel catfish *Ictalurus punctatus* (Cameron, 1986) and the marine long-horned sculpin *Myoxocephalus octodecimspinosus* (Claiborne and Evans, 1988). In all cases, plasma T_{Amm} increased rapidly, but approached a new steady state within 3–5 h, at a point where both $\Delta[NH_4^+]$ and ΔP_{NH_3} were either negative or negligible. Previous reversed-gradient studies have employed exposure times of 6 h or less. It is clear from the present investigation that trout can maintain reversed ammonia gradients for much longer periods (we have continued exposures for up to 48 h) and, indeed, some of the relevant acid–base changes only become apparent after at least 5 h of exposure, subsequent to the stabilisation of plasma $[T_{Amm}]$.

It is unlikely that any change in the form of the total excreted waste nitrogen occurred (e.g. a switch from mainly ammonia to urea excretion), since we have found that during similar ammonia exposure regimes changes in urea excretion play no significant role in the restoration of nitrogenous waste excretion in

rainbow trout (R. W. Wilson and R. S. Munger, unpublished results). If we also assume that no change in the tissue ammonia *production* rate occurred, then the maintenance of negative P_{NH_3} and $[NH_4^+]$ gradients requires that an active NH_4^+ extrusion mechanism was operating, which was sufficient to counterbalance the influx of ammonia in addition to excreting the endogenously produced ammonia.

NH_4^+/Na^+ or NH_4^+/H^+ exchange

A likely mechanism for the active extrusion of NH_4^+ is *via* branchial Na^+/NH_4^+ exchange. Many authors have provided evidence that supports the existence of a carrier-mediated Na^+/NH_4^+ exchange across the fish gill epithelium (Maetz and Garcia-Romeu, 1964; Maetz, 1973; Payan and Matty, 1975; Evans, 1977; Payan, 1978; Wright and Wood, 1985; Balm *et al.* 1988; McDonald and Prior, 1989). When transbranchial ammonia gradients were reversed in the freshwater trout, Cameron and Heisler (1983) suggested that Na^+/NH_4^+ exchange could be operating to counterbalance the diffusive uptake of NH_3. However, Cameron (1986) more recently found that exchange of internal NH_4^+ for external H^+ was the only plausible mechanism that could be fitted to his data from the channel catfish to explain the maintenance of reversed NH_3 and NH_4^+ gradients (e.g. the changes in titratable acidity, net apparent H^+ efflux and lack of change in Na^+ uptake).

The two suggested mechanisms for active excretion of ammonium ions (NH_4^+/Na^+ and NH_4^+/H^+ exchange) should have opposite acid–base consequences if operating to counteract the inward diffusion of ammonia. If the majority of ammonia enters the fish as NH_3, then an internal alkalosis might be expected, since at the pH of the fish plasma at 12–15°C (approximately 7.9) any inwardly diffusing molecular NH_3 would associate with protons to form NH_4^+. Any ammonia entering the fish as NH_4^+ would have no acid–base effect. However, transbranchial ammonia movements will only cause changes in blood pH if the respiratory component of blood acid–base status (P_{CO_2}) remains constant. For this reason one must use the non-respiratory component (ΔH_m^+) of the blood acid–base status when discussing the acid–base consequences of branchial ammonia movements. The schematic diagram in Fig. 5A shows that operation of NH_4^+/Na^+ exchange would complete an H^+ 'shuttle', exporting protons 'trapped' in NH_4^+ out of the blood and would therefore be expected to cause an acid deficit or 'base load' ($-\Delta H_m^+$). In contrast, operation of NH_4^+/H^+ exchange (Fig. 5B) would counteract the alkalising effect of passive NH_3 influx, by taking up H^+ from the ambient water in exchange for each NH_4^+ exported. In this respect, NH_4^+/H^+ exchange would be both electrically and acid–base neutral. However, if any ammonia were to diffuse into the blood as NH_4^+ then a small acid load would be expected. In our FW trout, blood pH remained constant but a significant non-respiratory acid load ($\Delta H_m^+ = +1.8 \pm 0.4$ mequiv l^{-1}) had accumulated after 24 h. Although the gills of freshwater fish are considered to be relatively impermeable to NH_4^+, the large diffusion gradients during the present experimental regime would probably result in some inward diffusion of NH_4^+ (McDonald and Prior, 1989). If even a small proportion of the ammonia influx in these

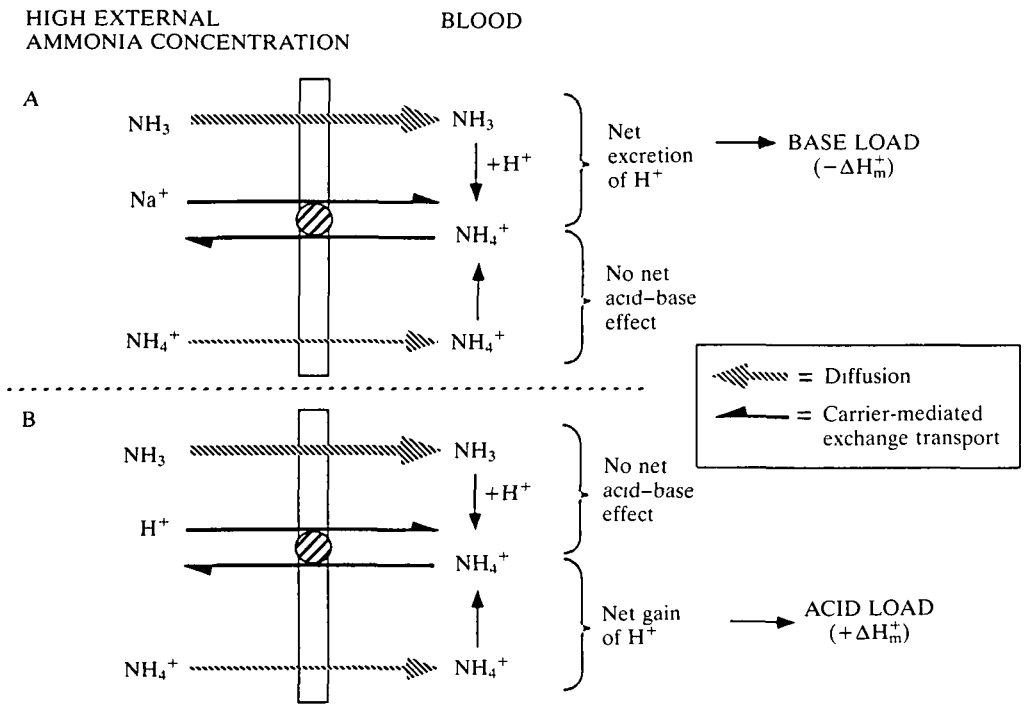

Fig. 5. A schematic representation of the predicted acid–base changes when either branchial NH_4^+/Na^+ (A) or NH_4^+/H^+ exchange (B) is employed to counteract diffusion of ammonia into the blood during exposure to a high external ammonia concentration. The vertical open bar represents the gill epithelium separating the blood from the external medium.

freshwater fish was the result of NH_4^+ diffusion, then any subsequent NH_4^+/H^+ exchange would cause an acid load resulting from the import of external H^+ in exchange for the inwardly diffusing NH_4^+ (see Fig. 5B). It would therefore appear that the NH_4^+/H^+ exchange proposed by Cameron (1986) to explain how freshwater channel catfish maintain negative transbranchial ammonia gradients will also explain the acid–base response in FW trout under similar conditions. In contrast, the SW trout developed an acid deficit or 'base load' ($\Delta H_m^+ = -2.8 \pm 0.7$ mequiv l^{-1}). Although the inward diffusion of NH_4^+ probably does contribute to the plasma hyperammoniaemia in SW trout (see below), any subsequent exchange of NH_4^+ with external Na^+ would be acid–base neutral. The acid deficit in the SW trout can be explained by the inward diffusion of NH_3 followed by extrusion of the subsequently formed NH_4^+ *via* NH_4^+/Na^+ exchange (see Fig. 5A).

On the basis of the differential acid–base changes observed in FW and SW trout, we suggest that NH_4^+/Na^+ exchange predominates in SW-adapted trout but that NH_4^+/H^+ is the primary exchange utilised by FW-adapted trout. Given

the very high concentration of Na^+ in sea water (approximately 460 mequiv l^{-1}), the former seems likely purely from the aspect of counterion availability. In terms of acid–base balance, operation of NH_4^+/H^+ exchange under these conditions in fresh water is actually more appropriate than NH_4^+/Na^+ exchange if one assumes that the majority of the ammonia influx is *via* NH_3 diffusion, as removal of NH_4^+ by this exchange is acid–base neutral and would not involve an increased rate of Na^+ uptake that might cause a Na^+ load (plasma $[Na^+]$ did not change). In seawater teleosts the unidirectional ion fluxes are an order of magnitude greater than in freshwater fish (Evans, 1984). Increasing the unidirectional Na^+ uptake (through NH_4^+/Na^+ exchange) for the purpose of active NH_4^+ excretion would probably have little effect on *net* sodium uptake in SW trout. This is presumably why plasma $[Na^+]$ also remained unchanged in the SW trout exposed to a high ammonia concentration.

NH_4^+ permeability in freshwater and seawater trout

The initially rapid increase in plasma $[T_{Amm}]$ is likely to be at least partly due to inward diffusion of NH_3 down its partial pressure gradient since the calculated gill diffusion coefficient for NH_3 is about the same as that for CO_2 in rainbow trout (Cameron and Heisler, 1983; Avella and Bornancin, 1989). However, the hyperammoniaemia developed more rapidly and was far more pronounced (73 % higher) in the SW trout where the inward P_{NH_3} gradient was 40 % smaller at the start (−35.4 mPa in seawater *vs.* −60.4 mPa in freshwater fish) and 78 % smaller after 24 h (−11.2 mPa in seawater *vs* a freshwater value of −50.5 mPa at 24 h). Assuming that the gill permeability to gaseous NH_3 does not change with salinity, and that ammonia production/excretion rates are the same in freshwater and seawater fish (the transbranchial gradients supporting excretion were not significantly different), then there are two possible explanations for this: (i) SW trout may have a greater permeability to NH_4^+ than their FW-acclimated counterparts, and/or (ii) SW trout have a reduced capacity to excrete NH_4^+ against a negative gradient. The latter seems extremely unlikely given that SW trout are known to have a fivefold higher capacity than FW trout for the excretion of acidic equivalents (H^+ and NH_4^+) following exhaustive exercise (Tang *et al.* 1989), which is correlated with the availability of Na^+ as an external counterion (McDonald *et al.* 1989). In the present study the level of Na^+ available for active NH_4^+ excretion is about 660 times greater in sea water. The most plausible explanation for the more rapid and greater accumulation of ammonia in SW trout (which were subjected to a much smaller reversal of ΔP_{NH_3}, but a near identical reversal of $\Delta[NH_4^+]$ compared to the FW trout) would appear to be that an increased permeability to NH_4^+ accompanies the adaptation from fresh water to sea water. This is in keeping with the notion that marine teleosts have higher ionic permeabilities than freshwater fish (Girard and Payan, 1980; Evans, 1979, 1984). Branchial permeability to NH_4^+ has previously been shown to be important in two marine teleost species, the long-horned sculpin *Myoxocephalus octodecimspinosus*

and the Gulf toadfish *Opsanus beta* (Goldstein *et al.* 1982; Claiborne and Evans, 1988; Evans *et al.* 1989). Evans *et al.* (1989) reported some preliminary results from experiments using perfused heads of toadfish acclimated to a reduced salinity (5% sea water) that showed the same correlation between salinity and NH_4^+ permeability. However, this is the first time that a complete comparison has been reported for animals of the same species acclimated to both fresh water and full-strength sea water.

It is of course the *electro*chemical gradient that provides the driving force behind the passive diffusion of ions across the gill. Analysis of branchial NH_4^+ diffusion should take into account the gill transepithelial potential (TEP). The TEP of most euryhaline fish in sea water is positive (inside relative to outside) and generally in the range of +10 to +35 mV (Potts, 1984), which will promote the outward diffusion of NH_4^+ under normal conditions (i.e. a low external ammonia concentration). Although NH_4^+ diffusion is not considered important in freshwater fish, the greater permeability coupled with a positive TEP in seawater teleosts suggests that NH_4^+ diffusion may have a substantial role in branchial ammonia transfer under normal conditions. Evans *et al.* (1989) determined that passive NH_4^+ diffusion contributed at least 21% to the overall rate of ammonia excretion in the perfused head of marine *Opsanus beta*. Future research should seek to clarify and quantify the role of this pathway in seawater fish by simultaneous measurements of gill NH_4^+ and NH_3 gradients, ammonia fluxes and TEP.

Toxicological implications

Acute ammonia toxicity occurs when the internal ammonia concentration reaches a critical level causing impairment of cerebral energy metabolism and nerve function (see Smart, 1978; Randall and Wright, 1987). This is obviously dependent on the rate of influx of ammonia during ammonia exposure which, in turn, will be determined by the permeability to NH_3 and NH_4^+. Ammonia toxicity to fish is usually described in terms of the ambient un-ionised ammonia concentration, since this is the variable best correlated with toxicity (Alabaster and Lloyd, 1980; Thurston and Russo, 1983; Thurston *et al.* 1984). This is not surprising considering that the above criterion is based almost exclusively upon data obtained from freshwater fish, which are considered to be relatively impermeable to NH_4^+. Since seawater-adapted teleosts appear to have enhanced NH_4^+ permeability, the use of un-ionised ammonia concentrations to describe toxicity may prove misleading when considering tests on marine species.

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