

AN ANALYSIS OF BRANCHIAL AMMONIA EXCRETION IN  
THE FRESHWATER RAINBOW TROUT:  
EFFECTS OF ENVIRONMENTAL pH CHANGE AND SODIUM  
UPTAKE BLOCKADE

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SUMMARY

Short-term treatments (3 h) designed to change the relative  $\text{NH}_3$  ( $\Delta P_{\text{NH}_3}$ ) and  $\text{NH}_4^+$  ( $\Delta \text{NH}_4^+$ ) gradients and sodium transport ( $J_{\text{Na}}$ ) across the gills were employed to analyse the normal mechanism(s) of branchial ammonia excretion ( $J_{\text{net}}^{\text{Amm}}$ ) in trout acclimated to fresh water of  $\text{pH} \approx 8.0$ . Control  $J_{\text{net}}^{\text{Amm}}$  occurred in the absence of, or against, an apparent  $\Delta P_{\text{NH}_3}$  gradient, while  $\Delta \text{NH}_4^+$  was positive. Severe acid exposure ( $\text{pH} = 4.06$ ) raised  $\Delta P_{\text{NH}_3}$  and  $\Delta \text{NH}_4^+$ , abolished  $J_{\text{in}}^{\text{Na}}$ , and reduced  $J_{\text{net}}^{\text{Amm}}$  by 28%, while moderate acidity ( $\text{pH} = 6.64$ ), which also elevated  $\Delta P_{\text{NH}_3}$ , had no significant influence on  $J_{\text{in}}^{\text{Na}}$  and  $J_{\text{net}}^{\text{Amm}}$ . Severe alkaline exposure ( $\text{pH} = 9.54$ ) raised  $\Delta \text{NH}_4^+$ , reduced  $\Delta P_{\text{NH}_3}$  to a very negative value, and decreased  $J_{\text{in}}^{\text{Na}}$  and  $J_{\text{net}}^{\text{Amm}}$  by equimolar amounts, representing 55% and 80% of control levels respectively. Moderate alkalinity ( $\text{pH} = 8.69$ ) had similar effects on  $\Delta P_{\text{NH}_3}$  and  $\Delta \text{NH}_4^+$ , but reduced  $J_{\text{in}}^{\text{Na}}$  and  $J_{\text{net}}^{\text{Amm}}$  by only ~25%. The sodium transport inhibitor amiloride ( $10^{-4} \text{ mol l}^{-1}$  in the external water,  $\text{pH} \approx 8.0$ ) had very similar effects to  $\text{pH} = 4.06$  on both  $J_{\text{in}}^{\text{Na}}$  and  $J_{\text{net}}^{\text{Amm}}$ , but did not alter  $\Delta P_{\text{NH}_3}$  or  $\Delta \text{NH}_4^+$ . The results discount the quantitative importance of  $\text{NH}_4^+$  diffusion and favour a flexible combination of  $\text{NH}_3$  diffusion and  $\text{Na}^+/\text{NH}_4^+$  exchange as the major mechanisms of  $J_{\text{net}}^{\text{Amm}}$ , with the latter dominating under the particular control conditions of the present study.

INTRODUCTION

Of the three major respiratory gases of aquatic animals, ammonia is probably the least understood. Metabolic processes are generally thought to produce ammonia in the  $\text{NH}_3$  form, but with a  $\text{pK}' \approx 9.5$ , the great majority exists in the  $\text{NH}_4^+$  form at physiological pH. Early studies by Smith (1929) established that the gills were the principal site of ammonia excretion. While some ammonia may arise *de novo* in the gill tissue (Goldstein & Forster, 1961), it is now clear that the major part of

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branchial ammonia excretion represents clearance from the blood (Pequin & Serfaty, 1963; Goldstein, Forster & Fanelli, 1964; Payan & Matty, 1975; Payan & Pic, 1977; Cameron & Heisler, 1983). However there is some question as to the actual mechanism(s) by which ammonia moves across the branchial epithelium (see Kormanik & Cameron, 1981a for review). It is well documented that ammonia can passively diffuse across the gills as  $\text{NH}_3$  (de Vooys, 1968; Kerstetter, Kirschner & Rafuse, 1970; Hillaby & Randall, 1979; Kormanik & Cameron, 1981b; Cameron & Kormanik, 1982; Cameron & Heisler, 1983; Høleton, Neumann & Heisler, 1983). There is also considerable evidence for a carrier-mediated  $\text{Na}^+/\text{NH}_4^+$  exchange (Maetz & García-Romeu, 1964; Payan & Matty, 1975; Payan, Matty & Maetz, 1975; Kerstetter & Keeler, 1976; Payan, 1978; Pressley, Graves & Krall, 1981) as well as some support for simple  $\text{NH}_4^+$  diffusion (Goldstein, Claiborne & Evans, 1982). It remains unclear whether one of these processes predominates, or whether a variable combination of two or more of these processes commonly occurs (e.g. Maetz, 1972, 1973; Maetz, Payan & De Renzis, 1976; Evans, 1977, 1982; Claiborne, Evans & Goldstein, 1982; Wood, Wheatley & Høbe, 1984).

Until recently, analysis of ammonia movements was limited by the lack of reliable physical constants ( $\text{pK}'$ , solubility) for ammonia in water and fish plasma at typical fish temperatures (cf. Kormanik & Cameron, 1981a). These parameters have now been measured for the rainbow trout (Cameron & Heisler, 1983). A further limitation in the past has been the absence of studies relating internal ammonia levels under resting conditions, as sampled by chronic cannulation, to external levels, ammonia excretion and unidirectional sodium fluxes at the gills. The present study employs this approach and the physical constants of Cameron & Heisler (1983) to reinvestigate the mechanism(s) of branchial ammonia efflux in the rainbow trout. Our aim has been to assess the mechanism(s) under normal laboratory conditions, rather than how they might change during long-term exposure to abnormal conditions. Thus we have examined the short-term effects, and their reversibility, of various treatments (environmental pH changes, amiloride) calculated to alter the relative  $\text{NH}_3$  and  $\text{NH}_4^+$  gradients and sodium transport across the gills.

#### MATERIALS AND METHODS

##### *Experimental animals*

Experiments were performed on 77 rainbow trout (*Salmo gairdneri*; mean weight, 430 g; range 180–786 g), obtained from Spring Valley Trout Farm, Petersburg, Ontario. The trout were initially held in flowing, dechlorinated Hamilton tapwater at seasonal temperatures and fed regularly with Silver Cup 3/16" Trout Chow. One week prior to experimentation, the fish were transferred in batches of 10–15 to a 350-l closed-circuit, temperature controlled ( $15 \pm 2^\circ\text{C}$ ) fibreglass tank containing the same tap water ( $[\text{Na}^+] \approx 0.6$ ,  $[\text{Cl}^-] \approx 0.8$ ,  $[\text{Ca}^{2+}] \approx 1.6$ ,  $[\text{Mg}^{2+}] \approx 0.3$ ,  $[\text{K}^+] = 0.05$  mequiv  $\text{l}^{-1}$ ; titration alkalinity  $\approx 2.0$  mequiv  $\text{l}^{-1}$ ; total hardness  $\approx 140$  mg  $\text{l}^{-1}$  as  $\text{CaCO}_3$ ; pH  $\approx 8.0$ ). The water was changed regularly to keep ammonia levels generally below  $200 \mu\text{mol l}^{-1}$ . During this acclimation period,

the fish were not fed in order to eliminate the influence of feeding history on ammonia excretion (Fromm, 1963).

Trout were fitted with chronic indwelling dorsal aortic cannulae (methods of Smith & Bell, 1964 or Soivio, Westman & Nyholm, 1972) and urinary bladder catheters (Wood & Randall, 1973) while under anaesthesia in a 1:10 000 dilution of MS-222 (Sigma). The fish were allowed to recover at least 48 h in individual flux boxes (see diagram and description in McDonald, 1983*b*). For measurements of branchial ammonia and sodium exchange, the flux boxes could be operated as closed, low-volume (3–6 l) recirculating systems at  $15 \pm 1^\circ\text{C}$ . As urine was collected externally, changes in water composition in the closed system were assumed to represent branchial fluxes, for skin and intestinal fluxes are considered negligible in fresh water (Fromm, 1968; Morii, Nishikata & Tamura, 1978; Cameron, 1978).

#### Experimental protocols

In each series, a 3-h control period (I) was followed by a 3-h experimental period and then a second control period (II; i.e. recovery) of equal length. At the start of each 3-h flux period, the box was flushed with fresh acclimation water and the volume reduced to a minimum (3–6 l depending on the fish and box size) so as to maximize the sensitivity with which changes in water ammonia and sodium concentration could be measured. This caused no apparent disturbance to the fish. At each time, new  $^{22}\text{Na}$  (New England Nuclear;  $1 \mu\text{Ci l}^{-1}$ ) was added to the water for measurement of unidirectional sodium fluxes at the gills. Water samples were drawn at the start of each 3-h period and every subsequent hour. Water pH was continually monitored and adjusted as necessary with  $1 \text{ mol l}^{-1} \text{ HCl}$  or  $1 \text{ mol l}^{-1} \text{ KOH}$ . Water samples were immediately frozen and later analysed for total ammonia ( $T_{\text{Amm}}$ ), total sodium and  $^{22}\text{Na}$  radioactivity. Arterial blood samples (300  $\mu\text{l}$ ) were drawn anaerobically from the dorsal aortic cannula and replaced by an equal volume of heparinized Cortland saline [Wolf, 1963; sodium heparin (Sigma); 100 i.u.  $\text{ml}^{-1}$ ]. Blood samples were taken at 0.5 h and 2.5 h of each 3-h period, and analysed for pH ( $\text{pH}_a$ ), total plasma  $\text{CO}_2$  content ( $C_T$ ) and total plasma ammonia content ( $T_{\text{Amm}}$ ).

Six series of experiments were performed.

- (i) A control in which fish were subjected to unaltered acclimation water ( $\text{pH} = 8.07 \pm 0.02$ ,  $N = 8$ ) in the experimental period to check for any effects of the protocol itself.
- (ii) Exposure to severely acidic water ( $\text{pH} = 4.06 \pm 0.03$ ,  $N = 10$ ) obtained by titration with  $1 \text{ mol l}^{-1} \text{ HCl}$ ; the water was vigorously aerated for several hours before use to avoid any complicating effects of elevated  $\text{P}_{\text{CO}_2}$ .
- (iii) Exposure to moderately acidic water ( $\text{pH} = 6.64 \pm 0.04$ ,  $N = 17$ ) prepared in the same manner as the severely acidic.
- (iv) Exposure to severely alkaline water ( $\text{pH} = 9.54 \pm 0.02$ ,  $N = 9$ ) obtained by titration with  $1 \text{ mol l}^{-1} \text{ KOH}$ .
- (v) Exposure to moderately alkaline water ( $\text{pH} = 8.69 \pm 0.04$ ,  $N = 17$ ) prepared in the same manner as the severely alkaline.
- (vi) Exposure to the drug amiloride ( $\text{C}_6\text{H}_8\text{ClN}_7\text{O}$ ; Merck, Sharp & Dome

Laboratories), a specific sodium transport blocker (Benos, 1982) at  $10^{-4} \text{ mol l}^{-1}$  in water of unchanged pH ( $8.12 \pm 0.05$ ;  $N=9$ ).

A further seven trout were fitted with both dorsal aortic and ventral aortic cannulae, the latter by the method of Holeyton & Randall (1967a). These fish were employed to assess the relative concentrations of ammonia in pre- and post-gill blood plasma and were sampled only under control conditions.

#### *Analytical techniques*

Water pH was monitored with a Canlab polymer body, sealed reference, combination pH electrode (H5503-20) coupled to a Radiometer pH 71 MK2 acid-base analyser or Fisher 119 digital pH meter. Total water ammonia was measured by a micro-modification of the salicylate-hypochlorite assay (Verdouw, van Echedt & Dekkers, 1978). Total water sodium was determined by flame photometry (Eel Mark II), and  $^{22}\text{Na}$  radioactivity by counting 5 ml of water in 10 ml ACS fluor (Amersham) on a Beckman LS 250 liquid scintillation counter. Arterial  $\text{pH}_a$  and plasma  $C_T$  were measured immediately upon collection using Radiometer micro-electrodes and methodology outlined by Wood & Jackson (1980). Plasma was obtained by centrifugation at 9000 *g* for 2 min and frozen for later analysis of  $T_{\text{Amm}}$  by micro-modification of a commercial diagnostic kit (L-glutamic dehydrogenase/NAD method; Sigma, 1982). Different ammonia assays were used for plasma and water because the simpler salicylate-hypochlorite method occasionally gave spurious values for plasma. The two assays were cross-validated on the same water- and saline-based ammonia standards.

#### *Calculations*

Free ( $\text{NH}_3$ ) and ionized ( $\text{NH}_4^+$ ) ammonia concentrations in plasma and water were calculated from total ammonia concentrations ( $T_{\text{Amm}}$ ) and pH by the Henderson-Hasselbalch equation, using values of  $\text{pK}'$  appropriate for trout plasma and fresh water at experimental temperature from Cameron & Heisler (1983):

$$\text{NH}_4^+ = \frac{T_{\text{Amm}}}{1 + \text{antilog}(\text{pH} - \text{pK}')} \quad (1)$$

Therefore:

$$\text{NH}_3 = T_{\text{Amm}} - \text{NH}_4^+ \quad (2)$$

The partial pressure of free ammonia ( $P_{\text{NH}_3}$  in  $\mu\text{Torr}$ :  $1 \text{ Torr} \approx 133.322387 \text{ Pa}$ ) was then calculated using the appropriate solubility coefficient ( $\alpha\text{NH}_3$ ) from Cameron & Heisler (1983):

$$P_{\text{NH}_3} = \frac{\text{NH}_3}{\alpha\text{NH}_3} \quad (3)$$

An analogous series of equations based on the Henderson-Hasselbalch relationship was used to calculate plasma  $\text{HCO}_3^-$  and  $\text{PaCO}_2$  from  $\text{pH}_a$  and  $C_T$ , using appropriate values of  $\text{pK}_1'$  and  $\alpha\text{CO}_2$  (Severinghaus, 1965; Albers, 1970).

Since ammonia excreted by the gills is cleared from the blood (see Introduction), it is the mean blood plasma level  $[(\text{arterial } T_{\text{Amm}} + \text{venous } T_{\text{Amm}}) \div 2]$  which best defines blood to water gradients. However, the venous cannulation procedure is more difficult, more interventive and undoubtedly more stressful than the arterial. Therefore, we chose to predict the mean level from the arterial, rather than directly measuring it in all cases. Twelve simultaneous arterial and venous measurements under control conditions in the seven fish implanted with both cannulae yielded a mean venous  $T_{\text{Amm}}$  ( $241 \pm 31 \mu\text{mol l}^{-1}$ ) which was 1.66-fold mean arterial  $T_{\text{Amm}}$  ( $145 \pm 28 \mu\text{mol l}^{-1}$ ). As this was very similar to the ratio (1.81) reported by Cameron & Heisler (1983) for *Salmo gairdneri* and there is essentially no difference between  $\text{pH}_a$  and  $\text{pH}_v$ , mean blood plasma  $T_{\text{Amm}}$  was routinely calculated as  $1.33 \times$  arterial  $T_{\text{Amm}}$  for use in equations (1) and (2).

Diffusion gradients for free and ionized ammonia across the gill were estimated as:

$$\Delta P_{\text{NH}_3} = \text{mean plasma } P_{\text{NH}_3} - \text{water } P_{\text{NH}_3} \quad (4)$$

and

$$\Delta \text{NH}_4^+ = \text{mean plasma } \text{NH}_4^+ - \text{water } \text{NH}_4^+, \quad (5)$$

recognizing that the latter does not take into account the small (unmeasured) electrical gradient across the gill (McWilliams & Potts, 1978). In these calculations, for each 3-h flux period, the initial (0.5 h) and final (2.5 h) plasma measurements were related to the means of the 0 h and 1 h, and 2 h and 3 h water measurements, respectively.

Net branchial flux rates of total ammonia ( $J_{\text{net}}^{\text{Amm}}$ ) were calculated as:

$$J_{\text{net}}^{\text{Amm}} = \frac{(T_{\text{Amm},i} - T_{\text{Amm},f}) \times V}{t \times W}, \quad (6)$$

where  $i$  and  $f$  refer to initial and final concentrations in water in  $\mu\text{mol ml}^{-1}$ ,  $V$  is the volume of the system in ml (corrected for sampling deficits),  $t$  is the elapsed time in h, and  $W$  is the fish weight in kg. Several experiments were run with known  $T_{\text{Amm}}$  but no fish present to check for ammonia loss from the water to the atmosphere in alkaline exposures ( $\text{pH} = 9.54$ ); this proved to be negligible.

Net branchial sodium flux rates ( $J_{\text{net}}^{\text{Na}}$ ) were calculated by an equation analogous to (6). Unidirectional influx rate ( $J_{\text{in}}^{\text{Na}}$ ) was calculated as outlined by Maetz (1956):

$$J_{\text{in}}^{\text{Na}} = \frac{(R_i - R_f) \times V}{SA \times t \times W}, \quad (7)$$

where  $R_i$  and  $R_f$  are initial and final radioactivities of water in c.p.m.  $\text{ml}^{-1}$ ,  $SA$  is the mean specific activity of water (c.p.m.  $\mu\text{equiv}^{-1}$ ) over the flux period in question, and the other symbols are as in equation (6). In practice, reliable changes in  $R$  could only be detected over 3 h, so a single value for average  $J_{\text{in}}^{\text{Na}}$  was determined for each

3-h period. Internal SA never exceeded 10% of external SA, so correction for radioisotopic backflux in equation (7) was unnecessary (cf. Maetz, 1956). Unidirectional efflux rates ( $J_{out}^{Na}$ ) were calculated by the conservation equation:

$$J_{out}^{Na} = J_{net}^{Na} - J_{in}^{Na} \quad (8)$$

For all fluxes, losses by the animal have a negative sign, gains a positive sign.

Data have been expressed as mean  $\pm 1$  s.e.m. ( $N$ ) where  $N$  equals the number of animals sampled. Changes in selected parameters within flux periods are illustrated in the figures, while averaged values over each 3-h treatment period are summarized in the Tables. Student's two tailed  $t$ -test ( $P < 0.05$ ) has been used for comparisons within groups, with each animal serving as its own control.

## RESULTS

### *Control series and resting values*

$J_{net}^{Amm}$ , measured on an hourly basis, declined during each 3-h flux period, and then recovered after the flush (Fig. 1D), a pattern also seen in the control periods and the experimental periods of all the other experimental series (Figs 2D-6D). A similar pattern was seen by Kormanik & Cameron (1981b) in their work on blue crabs. In the present study, the fish were preadapted to the experimental chambers for at least 48 h, so it is unlikely to be a stress effect. Rather, this decline in  $J_{net}^{Amm}$  appeared to be correlated with the rise in external  $T_{Amm}$  in the closed system over time. Plasma  $T_{Amm}$ , while variable, did not increase significantly over the same period (Fig. 1A). Consequently both  $\Delta P_{NH_3}$  and  $\Delta NH_4^+$  progressively fell (Fig. 1B,C); again the same patterns were seen in the control periods of all other protocols (Figs 2-6). There was no significant variation in plasma acid-base status (i.e.  $pH_a$ ,  $HCO_3^-$ ,  $P_{aCO_2}$ ; data not shown) within the 3-h periods.

Averaged data for each 3-h period are summarized in Table 1. All measured parameters were unchanged in the experimental period relative to control I. However, in control II, both plasma and water  $P_{NH_3}$ ,  $NH_4^+$ , and thus  $T_{Amm}$ , were significantly lower relative to control I. This lowering of average ammonia levels in the system was due both to a small reduction in  $J_{net}^{Amm}$  in control II, at least relative to the experimental period, and to slightly lower starting ammonia levels (cf. Fig. 1A). This probably resulted from the longer flush performed prior to control II, which was performed to duplicate the experimental protocols where thorough flushes were employed to ensure washout of the experimental water.

Fig. 1. Changes over time in selected parameters related to branchial ammonia excretion in rainbow trout subjected to a control protocol identical to that employed in the various experimental series. During the 3-h experimental period, the flux box was simply flushed with fresh acclimation water at control pH. Means  $\pm 1$  s.e.m. (A) Total ammonia levels ( $T_{Amm}$ ) in plasma (●) and water (○),  $N = 5$ . (B) The calculated partial pressure gradient for  $NH_3$  ( $\Delta P_{NH_3}$ ; inside minus outside) across the gills,  $N = 5$ . (C) The calculated concentration gradient for  $NH_4^+$  ( $\Delta NH_4^+$ ; inside minus outside) across the gills,  $N = 5$ . (D) The net rate of ammonia excretion at the gills ( $J_{net}^{Amm}$ ) during each hour,  $N = 8$ . (E) The mean unidirectional ( $J_{in}^{Na}$ ,  $J_{out}^{Na}$ ) and net ( $J_{net}^{Na}$ ; cross-hatched) flux rates of sodium across the gills during each 3-h period,  $N = 8$ .

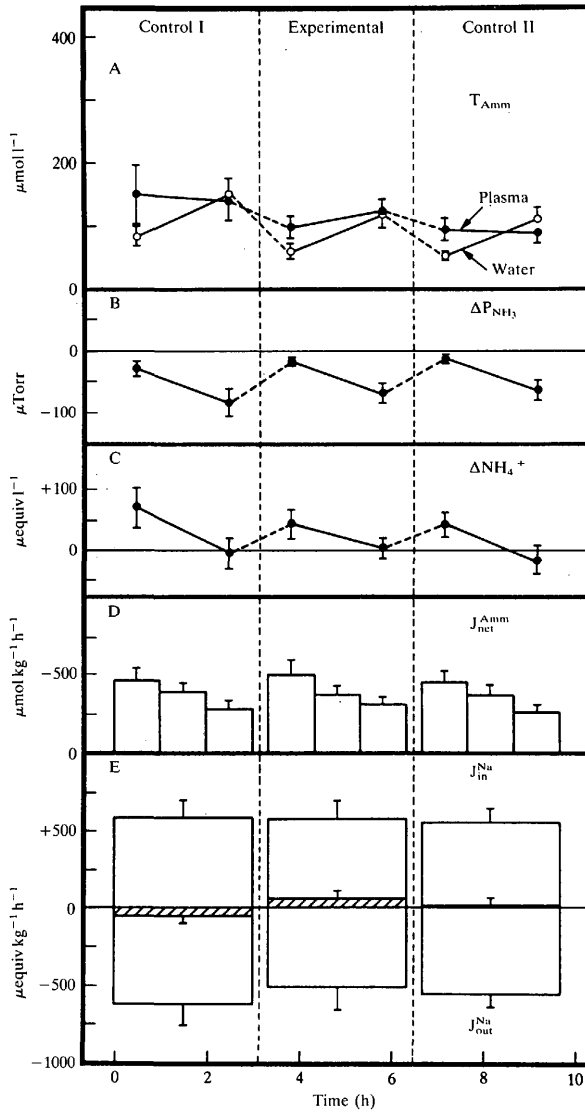


Fig. 1

Table 1. Mean values of parameters related to branchial ammonia excretion during each 3-h period in rainbow trout subjected to the control protocol

	N	Control I	Experimental	Control II
Water pH	8	8.10 ± 0.04	8.11 ± 0.04	8.09 ± 0.04
asma P <sub>H<sub>2</sub>O</sub> (Torr)	7	7.79 ± 0.02	7.80 ± 0.02	7.81 ± 0.02
asma P <sub>HCO<sub>3</sub><sup>-</sup></sub> (nequiv l <sup>-1</sup> )	7	2.94 ± 0.27	2.75 ± 0.20	2.54 ± 0.24
asma P <sub>NH<sub>3</sub></sub> (μTorr)	7	6.41 ± 0.42	6.26 ± 0.26	5.76 ± 0.44
ater P <sub>NH<sub>3</sub></sub> (μTorr)	5	42 ± 11	33 ± 5	27 ± 5*
P <sub>NH<sub>3</sub></sub> (μTorr)	5	99 ± 17	77 ± 10	65 ± 7*
asma NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	5	57 ± 12	44 ± 8	38 ± 10
ater NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	5	145 ± 38	108 ± 18	89 ± 20*
NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	5	113 ± 17	86 ± 12	78 ± 8*
NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	5	+32 ± 34	+22 ± 17	+11 ± 25
† (μmol kg <sup>-1</sup> h <sup>-1</sup> )	8	-387 ± 57	-394 ± 61	-358 ± 51†
‡ (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	8	+579 ± 121	+573 ± 137	+550 ± 88
§ (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	8	-625 ± 124	-513 ± 145	-549 ± 117
¶ (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	8	-46 ± 33	+60 ± 54	+1 ± 57

± 1 s.e.m. \* P < 0.05 relative to control I. † P < 0.05 relative to experimental.

Table 2. Mean values of parameters related to branchial ammonia excretion during each 3-h period in rainbow trout subjected to severe acid exposure during the experimental period

	N	Control I	Severe acid	Control II
Water pH	10	7.96 ± 0.06	4.06 ± 0.03*	7.85 ± 0.03†
asma P <sub>H<sub>2</sub>O</sub> (Torr)	7	7.78 ± 0.02	7.72 ± 0.03*	7.73 ± 0.02
asma P <sub>HCO<sub>3</sub><sup>-</sup></sub> (nequiv l <sup>-1</sup> )	7	2.64 ± 0.18	2.99 ± 0.21	3.10 ± 0.20
asma P <sub>HCO<sub>3</sub><sup>-</sup></sub> (nequiv l <sup>-1</sup> )	7	5.70 ± 0.26	5.34 ± 0.24	5.81 ± 0.53
asma P <sub>NH<sub>3</sub></sub> (μTorr)	7	73 ± 21	70 ± 14	52 ± 11†
ater P <sub>NH<sub>3</sub></sub> (μTorr)	7	91 ± 17	0 ± 0*	48 ± 8*†
P <sub>NH<sub>3</sub></sub> (μTorr)	7	-18 ± 15	+70 ± 14	+4 ± 11†
asma NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	7	220 ± 51	281 ± 62	194 ± 40†
ater NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	7	123 ± 22	84 ± 19*	95 ± 22
NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	7	+97 ± 41	+197 ± 44*	+99 ± 32†
NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	10	-379 ± 53	-273 ± 38*	-354 ± 53†
† (μmol kg <sup>-1</sup> h <sup>-1</sup> )	7	+433 ± 68	-55 ± 13*	+265 ± 44*†
‡ (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	7	-363 ± 73	-396 ± 93	-310 ± 107
§ (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	7	+70 ± 53	-451 ± 90*	-45 ± 82†

± 1 s.e.m. \* P < 0.05 relative to control I. † P < 0.05 relative to severe acid.



Resting levels of plasma  $T_{\text{Amm}}$ ,  $P_{\text{NH}_3}$ ,  $\text{NH}_4^+$ , acid-base status,  $\Delta\text{NH}_4^+$  across the gills and  $J_{\text{net}}^{\text{Amm}}$  were very similar to those found by Cameron & Heisler (1983), who employed comparable methodology, though  $\Delta P_{\text{NH}_3}$  across the gills was very different. This difference was probably due to the very different control water pH levels employed in the two studies (see Discussion). The present fish started each control period with  $\Delta P_{\text{NH}_3}$  close to zero, which became significantly negative by 3 h as water  $P_{\text{NH}_3}$  increased (Figs 1B–6B). Thus our fish excreted ammonia in the apparent absence of or even against a  $P_{\text{NH}_3}$  gradient, but in the direction of the  $\text{NH}_4^+$  gradient (Tables 1–6).

$J_{\text{in}}^{\text{Na}}$  and  $J_{\text{out}}^{\text{Na}}$  were rather variable both within and between different experimental groups ( $400\text{--}700 \mu\text{equiv kg}^{-1} \text{h}^{-1}$  in control I; Tables 1–6), but a  $J_{\text{net}}^{\text{Na}}$  close to zero balance was usual (Figs 1E–6E). These unidirectional fluxes were approximately twice as large as those measured by Wood *et al.* (1984) in trout in water of the same ionic composition and temperature.  $J_{\text{net}}^{\text{Amm}}$  in the present study was also twice as high, perhaps because Wood *et al.* (1984) allowed external  $T_{\text{Amm}}$  to rise to a level 3–5 times greater. Elevated external  $T_{\text{Amm}}$  may well interfere with both  $\text{Na}^+/\text{NH}_4^+$  exchange and diffusive ammonia efflux.

#### Severe acid exposure

The aim here was to apply a condition [water  $\text{pH} = 4.06 \pm 0.03$  (10)] which would enhance  $\Delta P_{\text{NH}_3}$  while simultaneously blocking  $J_{\text{in}}^{\text{Na}}$  (cf. McDonald, 1983b). If branchial  $\text{Na}^+/\text{NH}_4^+$  exchange is unimportant and  $\text{NH}_3$  diffusion predominates, the predicted result would be an increase in  $J_{\text{net}}^{\text{Amm}}$ . Since at this pH essentially all ammonia is protonated, the water becomes an immense sink for  $\text{NH}_3$ .

$J_{\text{net}}^{\text{Amm}}$ , rather than increasing, declined significantly by 28% relative to control I and then recovered in control II (Fig. 2B, Table 2). This occurred despite a reversal of  $\Delta P_{\text{NH}_3}$  to a positive value as water  $P_{\text{NH}_3}$  became zero (Fig. 2B, Table 2).  $\Delta\text{NH}_4^+$  also increased (Fig. 2C) because the reduction in  $J_{\text{net}}^{\text{Amm}}$  both raised plasma  $T_{\text{Amm}}$  (and thus  $\text{NH}_4^+$ ) and lowered water  $T_{\text{Amm}}$  (Fig. 2A, Table 2). This rise in internal ammonia shows that the reduction in  $J_{\text{net}}^{\text{Amm}}$  could not be attributed to a reduction in endogenous ammonia production. As anticipated,  $J_{\text{in}}^{\text{Na}}$  was abolished so that  $J_{\text{net}}^{\text{Na}}$  became highly negative and equal to  $J_{\text{out}}^{\text{Na}}$  (Fig. 2E, Table 2), an effect which was only partially reversed in control II. A slight but significant blood acidosis occurred during severe acid exposure attributable to both respiratory ( $\text{PaCO}_2$  elevation) and metabolic ( $\text{HCO}_3^-$  reduction) components, neither of which was significant by itself (Table 2). The magnitude of this acidosis after 3 h at  $\text{pH} = 4.06$  was approximately equal to that which would be predicted from an earlier study under comparable conditions (McDonald & Wood, 1981).

These results indicate a significant contribution of  $\text{Na}^+/\text{NH}_4^+$  exchange to resting  $J_{\text{net}}^{\text{Amm}}$ . However 72% of control  $J_{\text{net}}^{\text{Amm}}$  continued in the complete absence of  $J_{\text{in}}^{\text{Na}}$ , suggesting that diffusive processes, probably enhanced by the elevated gradients, were important in sustaining  $J_{\text{net}}^{\text{Amm}}$  under conditions where  $\text{Na}^+/\text{NH}_4^+$  exchange was blocked.

*Moderate acid exposure*

The goal of this treatment was again to elevate  $\Delta P_{\text{NH}_3}$  by reducing water  $P_{\text{NH}_3}$  close to zero, but without inducing a blockage of  $J_{\text{in}}^{\text{Na}}$ . A water pH of  $6.64 \pm 0.04$  (17) was calculated to represent the minimum acidity level to ensure the former objective.

$J_{\text{net}}^{\text{Amm}}$  increased only slightly (+7%) and non-significantly relative to control I during moderate acid exposure (Fig. 3D, Table 3). While this  $J_{\text{net}}^{\text{Amm}}$  was significantly higher (+23%) than in control II, the same trend was also seen in the control series (Table 1). As planned,  $\Delta P_{\text{NH}_3}$  was reversed to a positive value (Fig. 3B, Table 3), though not as high as in the severe acid series (Table 2) because internal ammonia levels fell significantly (Table 3), an effect which was not corrected during control II. It is not clear whether this was the result of the marginally elevated  $J_{\text{net}}^{\text{Amm}}$ , or reflected a true decrease in the endogenous ammonia production, though the severe acid results would argue against this. In any event, one important consequence was a significant reduction in  $\Delta \text{NH}_4^+$  during the experimental period (Fig. 3C, Table 3). The goal of preserving sodium exchanges at control levels was largely achieved, with only a non-significant 23% decrease in  $J_{\text{in}}^{\text{Na}}$  and continued positive  $J_{\text{net}}^{\text{Na}}$  occurring at pH = 6.64 (Fig. 3E, Table 3). There were no further changes in these parameters in control II. As with severe acid exposure, a slight blood acidosis of compound respiratory and metabolic origin again occurred (Table 3).

Under these conditions, a significant elevation of  $\Delta P_{\text{NH}_3}$  had only a very small stimulating effect on  $J_{\text{net}}^{\text{Amm}}$ , and  $\Delta \text{NH}_4^+$  again changed in the opposite direction from  $J_{\text{net}}^{\text{Amm}}$ .  $J_{\text{net}}^{\text{Amm}}$  was therefore only slightly altered by a treatment whereas  $J_{\text{in}}^{\text{Na}}$  was largely unaffected.

*Severe alkaline exposure*

Exposure to alkaline water was selected as a treatment to separate the relative importance of  $\text{NH}_3$  and  $\text{NH}_4^+$  diffusion across the gills, for elevated water pH should increase external  $P_{\text{NH}_3}$  and decrease external  $\text{NH}_4^+$ . An alkaline pH [ $9.54 \pm 0.04$  (9)] close to the  $\text{pK}'$  was initially tested to ensure pronounced changes in  $\Delta P_{\text{NH}_3}$  and  $\Delta \text{NH}_4^+$ . As alkaline effects on sodium transport in trout have not been studied previously, it was not known what changes, if any, might occur in  $J_{\text{in}}^{\text{Na}}$ .

$J_{\text{net}}^{\text{Amm}}$  declined dramatically by 80% during severe alkaline exposure and then recovered to a level significantly above control I (+22%) during control II (Fig. 4D, Table 4). Plasma  $T_{\text{Amm}}$  increased 2.5-fold during the experimental period and then dropped during recovery (Fig. 4A), showing that the reduction in  $J_{\text{net}}^{\text{Amm}}$  was not due to a blockage of endogenous ammonia production. At the same time,  $\Delta \text{NH}_4^+$  increased over 4-fold to a highly positive value, while  $\Delta P_{\text{NH}_3}$  fell from its initial level close to zero to a very negative value (Fig. 4B,C, Table 4). These alterations in gradients, which were the intention of the experiment, were

Fig. 2. The influence of severe acid exposure (pH = 4.06) during the experimental period on selected parameters related to branchial ammonia excretion in rainbow trout.  $N = 7$  in (A)–(C), 10 in (D) and 7 in (E). Other details as in legend of Fig. 1.

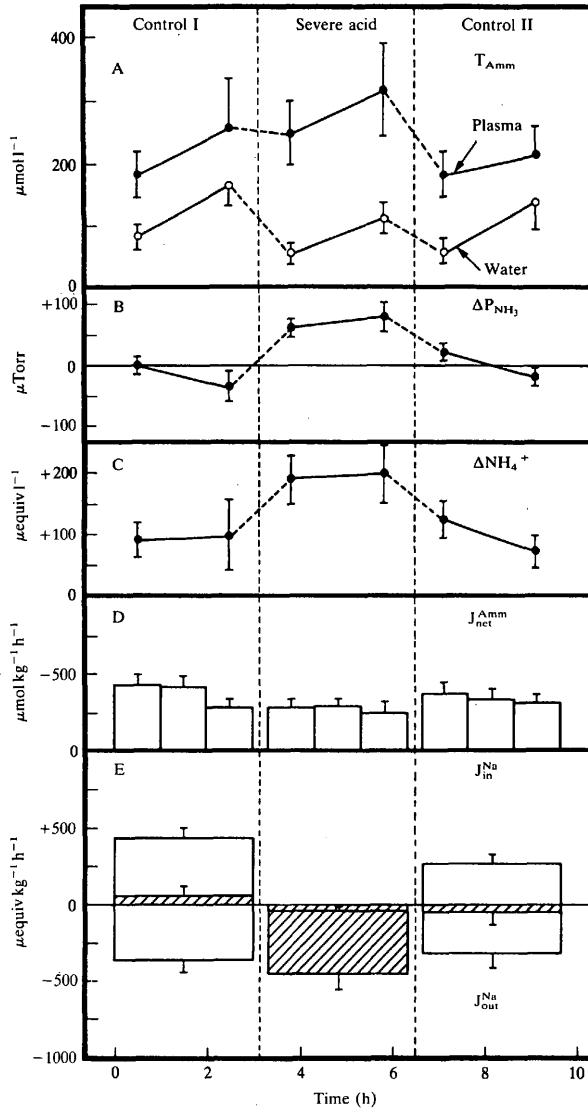


Fig. 2

reversed during control II. High water pH also markedly affected  $J_{in}^{Na}$ , which fell by 55 % during the experimental period, and returned to normal in control II (Fig. 4E, Table 4).  $J_{net}^{Na}$  mirrored these changes. A pronounced blood alkalosis (+0.3 pH units) of wholly respiratory origin occurred during severe alkaline exposure and partially persisted during recovery (Table 4).  $P_{aCO_2}$  was approximately halved while  $[HCO_3^-]$  remained unchanged because water at pH = 9.54 essentially acts as a vacuum for  $CO_2$ .

Of all the experimental treatments, this caused the largest drop in  $J_{net}^{Amm}$ . In this situation, where  $\Delta NH_4^+$  increased over 4-fold and where  $\Delta P_{NH_3}$  was rendered highly negative and therefore the net outward diffusion of  $NH_3$  clearly prevented, the decreases ( $\sim -270 \mu\text{equiv kg}^{-1} \text{h}^{-1}$ ) in  $J_{net}^{Amm}$  and  $J_{in}^{Na}$  were equal in size (Table 4). These data were therefore consistent with the results of the two acid series.

#### *Moderate alkaline exposure*

A much more moderate alkaline pH was tested in the hope that a very negative  $\Delta P_{NH_3}$  could still be attained without affecting  $J_{in}^{Na}$ . A pH of  $8.69 \pm 0.04$  (17) was selected as the minimum level of alkalinity to ensure the former objective.

As intended, moderate alkaline exposure changed  $\Delta P_{NH_3}$  to a very negative value (Fig. 5B, Table 5) almost equal to and less variable than that during severe alkaline exposure (Fig. 4B, Table 4). However in this case, the drop in  $J_{net}^{Amm}$ , while significant, was only 23 % (Fig. 5D, Table 5). Even at this much more moderate alkaline pH,  $J_{in}^{Na}$  declined significantly by 28 % (Fig. 5E, Table 5). The absolute size of this decrease was adequate to explain the observed fall in  $J_{net}^{Amm}$ . As with severe alkaline exposure, plasma  $T_{Amm}$  and  $\Delta NH_4^+$  both rose during the experimental period as ammonia accumulated internally (Fig. 5A,C), and a respiratory alkalosis again developed (Table 5). Most changes were reversed during control II.

Thus in water of pH = 8.69, 77 % of control I  $J_{net}^{Amm}$  persisted in the face of a very negative  $\Delta P_{NH_3}$ . This small reduction in  $J_{net}^{Amm}$  was accompanied by a comparable fall in  $J_{in}^{Na}$ .

#### *Amiloride exposure*

The results of the previous four experiments suggested that a large decrease in  $J_{net}^{Amm}$  would be the predicted result of a treatment which blocked  $J_{in}^{Na}$  without altering  $\Delta P_{NH_3}$  or  $\Delta NH_4^+$ . The drug amiloride, a specific sodium transport inhibitor (Benos, 1982), was selected for this purpose.

As intended, amiloride at  $10^{-4} \text{ mol l}^{-1}$  in the external water (pH =  $8.12 \pm 0.05$ ) had no effect on  $\Delta P_{NH_3}$  or  $\Delta NH_4^+$  (Fig. 6B,C, Table 6), but essentially obliterated  $J_{in}^{Na}$ , with only 6 % of the control I influx persisting (Fig. 6E, Table 6).  $J_{net}^{Na}$  therefore became very negative. These effects were largely reversed during control II. However, contrary to prediction, the fall in  $J_{net}^{Amm}$ , while significant, was only

Fig. 3. The influence of moderate acid exposure (pH = 6.64) during the experimental period on selected parameters related to branchial ammonia excretion in rainbow trout.  $N = 15$  in (A)–(C), 17 in (D) and 9 in (E). Other details as in legend of Fig. 1.

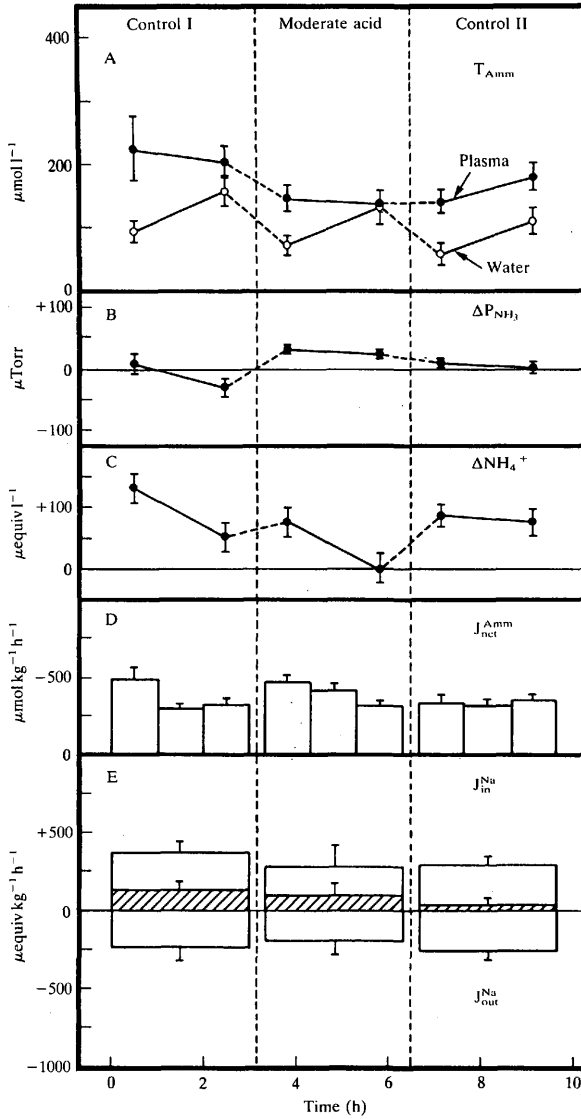


Fig. 3

ble 3. Mean values of parameters related to branchial ammonia excretion during each 3-h period in rainbow trout subjected to moderate acid exposure during the experimental period

	N	Control I	Moderate acid	Control II
Water pH	12	7.85 ± 0.08	6.64 ± 0.04*	7.61 ± 0.06†
asma P <sub>a</sub> CO <sub>2</sub> (Torr)	15	7.80 ± 0.01	7.73 ± 0.02*	7.75 ± 0.02
asma HCO <sub>3</sub> <sup>-</sup> (mequiv l <sup>-1</sup> )	15	2.65 ± 0.12	2.97 ± 0.24	2.64 ± 0.19
asma P <sub>a</sub> NH <sub>3</sub> (μTorr)	15	6.12 ± 0.32	5.42 ± 0.32	5.28 ± 0.38
ater P <sub>a</sub> NH <sub>3</sub> (μTorr)	15	56 ± 6	32 ± 5*	35 ± 3*
P <sub>a</sub> NH <sub>3</sub> (μTorr)	15	62 ± 10	3 ± 0*	32 ± 6*†
asma NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	15	-6 ± 14	+29 ± 5*	+3 ± 9†
ater NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	15	1.98 ± 33	129 ± 23*	137 ± 23*
asma NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	15	1.21 ± 18	102 ± 14*	78 ± 12*†
ater NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	15	+77 ± 32	+27 ± 31*	+59 ± 24*
asma (μmol kg <sup>-1</sup> h <sup>-1</sup> )	17	-375 ± 38	-403 ± 30	-327 ± 28†
ater (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	9	+370 ± 86	+285 ± 143	+297 ± 43
asma (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	9	-238 ± 86	-188 ± 84	-261 ± 69
ater (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	9	+132 ± 55	+97 ± 88	+36 ± 40

± 1 s.e.m. \* P < 0.05 relative to control I. † P < 0.05 relative to moderate acid.

ble 4. Mean values of parameters related to branchial ammonia excretion during each 3-h period in rainbow trout subjected to severe alkaline exposure during the experimental period

	N	Control I	Severe alkaline	Control II
Water pH	9	7.97 ± 0.06	9.54 ± 0.04*	7.99 ± 0.08†
asma P <sub>a</sub> CO <sub>2</sub> (Torr)	5	7.78 ± 0.02	8.08 ± 0.07*	7.89 ± 0.02*†
asma HCO <sub>3</sub> <sup>-</sup> (mequiv l <sup>-1</sup> )	5	2.95 ± 0.50	1.61 ± 0.12*	2.12 ± 0.47†
asma P <sub>a</sub> NH <sub>3</sub> (μTorr)	5	6.60 ± 0.83	6.91 ± 0.86	6.76 ± 0.69
ater P <sub>a</sub> NH <sub>3</sub> (μTorr)	5	59 ± 10	285 ± 65*	83 ± 13*†
P <sub>a</sub> NH <sub>3</sub> (μTorr)	5	81 ± 20	424 ± 89*	95 ± 22†
asma NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	5	-22 ± 17	-139 ± 83	-12 ± 26
ater NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	5	186 ± 22	408 ± 51*	215 ± 21†
asma NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	5	103 ± 18	16 ± 3*	97 ± 20†
ater NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	5	83 ± 22	392 ± 49*	+118 ± 34†
asma (μmol kg <sup>-1</sup> h <sup>-1</sup> )	9	-345 ± 91	-68 ± 13*	-420 ± 45*†
ater (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	7	+496 ± 56	+226 ± 60*	+465 ± 86†
asma (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	7	-530 ± 66	-745 ± 193	-381 ± 92
ater (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	7	-34 ± 33	-519 ± 194*	+84 ± 66

± 1 s.e.m. \* P < 0.05 relative to control I. † P < 0.05 relative to severe alkaline.

23% relative to control I (Fig. 6D, Table 6). This inhibition disappeared during control II. Blood acid-base status was not significantly affected by amiloride (Table 6). The effects of amiloride on  $J_{in}^{Na}$  and  $J_{net}^{Amm}$  were therefore very similar to those of severe acid exposure (cf. Fig. 2, Table 2). This occurred despite the fact that elevated  $\Delta P_{NH_3}$  (and  $\Delta NH_4^+$ ) gradients, interpreted as sustaining  $J_{net}^{Amm}$  when  $Na^+/NH_4^+$  exchange was blocked at pH = 4.06, were not seen with amiloride.

#### DISCUSSION

##### *Ammonia gradients*

There are numerous sources of uncertainty in the estimation of ammonia gradients across the gills. As Cameron & Heisler (1983) point out, relatively small errors in physical constants and pH may have large effects on calculated  $\Delta P_{NH_3}$ . Employing Cameron & Heisler's physical constants, we have obtained internal  $P_{NH_3}$  and  $NH_4^+$  levels similar to their values, which at least suggests consistency between the two studies, and that our procedure of predicting mean plasma  $T_{Amm}$  levels from arterial  $T_{Amm}$  was not a major source of error. The pH values are a more serious concern. While blood plasma pH is a routine and precise measurement ( $\pm 0.01$  units), the value obtained represents an equilibrium condition in the electrode which may or may not be the same as that in gill blood plasma depending on the extent of carbonic anhydrase catalysis. One may even question whether blood plasma pH is the correct internal value to use. The relevant gradient could in fact be from the cytoplasm of branchial epithelial cells to the water, rather than from blood to water, especially if ammonia is transported rather than diffuses across the baso-lateral boundary (cf. Claiborne *et al.* 1982). The intracellular pH of the gill epithelium is unknown, but  $pH_i$  in other trout tissues is 0.4–0.5 pH units below  $pH_a$  (Höbe, Wood & Wheatly, 1984b; C. L. Milligan & C. M. Wood, unpublished data). Furthermore, while branchial ammonia production appears to be of little importance (see Introduction), it must be noted that the enzymes involved in ammoniogenesis have been located in the gills (Goldstein & Forster, 1961; Makarawicz & Zydowo, 1962). It remains conceivable that under appropriate conditions, ammonia production within the epithelial cells could be turned on.

The measurement of water pH ( $\pm 0.02$  units) is somewhat less precise than that of plasma because of the lower ionic strength. However, a much more serious concern is that like other workers (e.g. Maetz, 1972, 1973; Cameron & Heisler, 1983), we have employed equilibrium pH measurements in the external bath where water  $P_{CO_2}$  is kept relatively constant. Lloyd & Herbert (1960) theorized that  $P_{CO_2}$  was higher and pH lower in lamellar water, assuming that the  $CO_2$  hydration reaction was rapid. However in the absence of external carbonic anhydrase, the  $CO_2/HCO_3^-$  system is undoubtedly not at equilibrium in lamellar water. Holeton & Randall (1967b) reported that mixed expired water pH, at equilibrium, was  $\sim 0.2$  pH units below inspired in rainbow trout examined in very soft water. Our water is more alkaline and better buffered, so a smaller difference is anticipated. However actual lamellar water pH has yet to be measured and is difficult to predict accurately, for it will depend on the extent of this disequilibrium, the size of the unstirred layer and the magnitude of the  $CO_2$  flux relative to the ventilatory flow, as

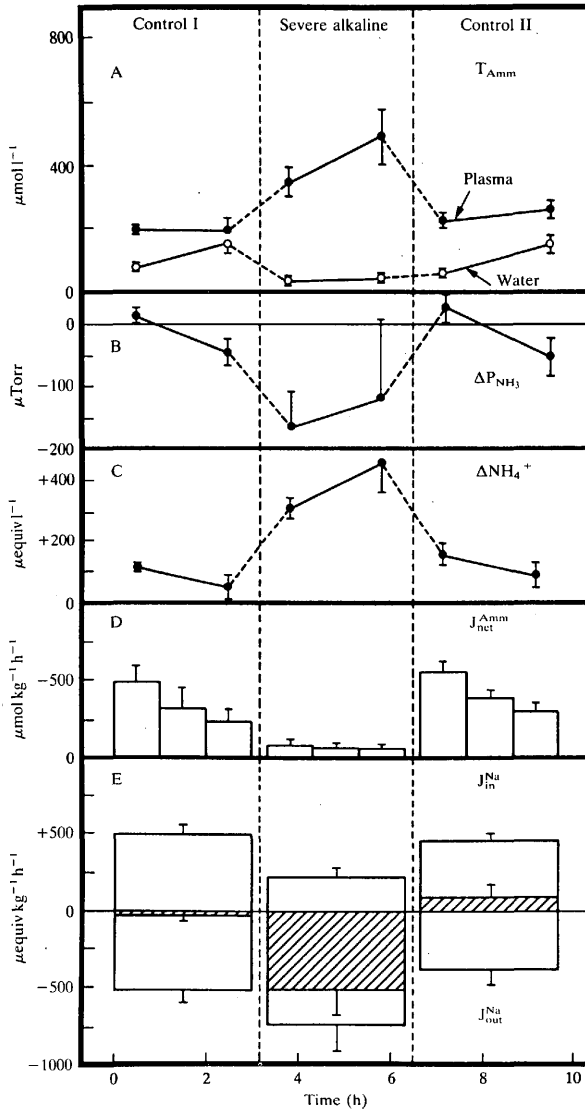


Fig. 4



well as the fluxes of other acidifying ( $H^+$ ) or alkalinizing species (e.g.  $NH_3$ ,  $HCO_3^-$ ,  $OH^-$ ). In view of these difficulties, we have much more confidence in relative changes in ammonia gradients caused by experimental treatments than in their absolute values.

#### Experimental responses

The immediate and complete blockage of  $J_{in}^{Na}$  during severe acid exposure has been observed and analysed repeatedly (see Wood & McDonald, 1982; McDonald, 1983a for reviews). However, the simultaneous reduction in  $J_{net}^{Amm}$ , in the face of greatly elevated  $\Delta P_{NH_3}$  during the initial exposure period has received little attention, though it has been noted in three other recent studies (Ultsch, Ott & Heisler, 1981; McDonald, Walker & Wilkes, 1983; Höbe *et al.* 1984a,b). Instead, most authors have concentrated on the fact that  $J_{net}^{Amm}$  increases well above the control level during longer term acid exposure despite minimal recovery of  $J_{in}^{Na}$  (e.g. Ultsch *et al.* 1981; McDonald & Wood, 1981; Booth, Jansz & Holeton, 1982; McDonald, 1983a,b; McDonald *et al.* 1983). Plasma  $T_{Amm}$  also rises during longer term exposure (McDonald, 1983b; Höbe *et al.* 1984a) so elevated ammoniagenesis is probably involved. Moderate acid exposure, which also elevated  $\Delta P_{NH_3}$ , did not significantly alter  $J_{in}^{Na}$  and only minimally elevated  $J_{net}^{Amm}$ . These observations are all consistent with the interpretation that a large  $Na^+/NH_4^+$  exchange under the control conditions of the present laboratory experiments is blocked by severe acid exposure; the continuation of ammonia efflux is then dependent upon increased  $NH_3$  diffusion due to the elevated  $\Delta P_{NH_3}$ .

Trout branchial function appears very sensitive to alkaline conditions, for raising the water pH to only 8.69 depressed both  $J_{net}^{Amm}$  and  $J_{in}^{Na}$  by ~25%, and 9.54 reduced these parameters by 80% and 55% respectively, the latter representing an equimolar reduction. We are aware of no previous reports of alkaline effects on ammonia excretion, and only one on sodium exchange. In contrast to the present data, Maetz & De Renzis (1978) found a 10-fold stimulation of  $J_{in}^{Na}$  in *Tilapia mossambica* raised from water pH = 7.8 to 9.7, though their tests were conducted in 10% sea water. The inhibitory effect of low pH on  $J_{in}^{Na}$  has been variously explained by  $H^+$  competition for the carrier,  $H^+$  titration of negative charges on channels leading to the carrier, or a direct effect on the carrier itself (cf. McDonald, 1983a). Only the latter would seem a reasonable mechanism for high pH inhibition of  $J_{in}^{Na}$ , though the effect was reversible in the present study. The persistence of over 75% of control  $J_{net}^{Amm}$  in the face of a very negative  $\Delta P_{NH_3}$  (at pH = 8.69) and the large equimolar reduction in both  $J_{in}^{Na}$  and  $J_{net}^{Amm}$  (at pH = 9.54) were again consistent with a dominant role for  $Na^+/NH_4^+$  exchange under the present control conditions.

The observed 94% reduction in  $J_{in}^{Na}$  caused by amiloride agrees with previous studies showing a 70–92% reduction in various trout preparations (Kirschner, Greenwald & Kerstetter, 1973; Kerstetter & Keeler, 1976; Payan, 1978; Perry,

Fig. 4. The influence of severe alkaline exposure (pH = 9.54) during the experimental period on selected parameters related to branchial ammonia excretion in rainbow trout.  $N = 5$  in (A)–(C), 9 in (D) and 7 in (E). Other details as in legend of Fig. 1.

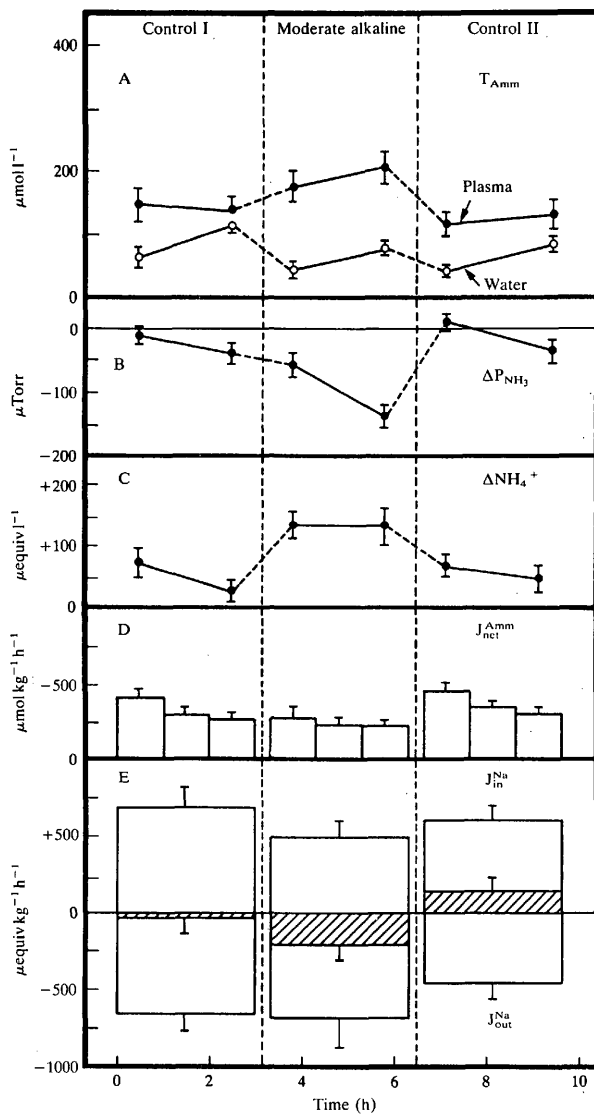


Fig. 5

Haswell, Randall & Farrell, 1981; Perry & Randall, 1981). Amiloride also reduced  $J_{\text{net}}^{\text{Amm}}$  by 23%, a figure consistent with 30% reductions reported in perfused and/or irrigated trout gill preparations (Kirschner *et al.* 1973; Payan, 1978). A much larger inhibition of  $J_{\text{net}}^{\text{Amm}}$  would be expected if  $\text{Na}^+/\text{NH}_4^+$  were the dominant process, for no elevation of diffusion gradients occurred to sustain excretion as during severe acid exposure. Recently Perry & Randall (1981) have shown that  $10^{-4} \text{ mol l}^{-1}$  amiloride also inhibits  $J_{\text{in}}^{\text{Cl}}$  by 55% in intact trout, an effect they attributed to a predicted acidosis within the branchial epithelial cells. As this could alter the effective driving gradients for  $\text{NH}_3$  and  $\text{NH}_4^+$  movements at both the baso-lateral and apical surfaces (see above), the interpretation of this experiment is unclear.

#### The mechanism(s) of ammonia excretion

The results of all five experimental series were consistent with the conclusion that  $\text{Na}^+/\text{NH}_4^+$  exchange plays a significant role in  $J_{\text{net}}^{\text{Amm}}$  in trout under the present control conditions, and all but the amiloride experiment suggest it may be the dominant mechanism.  $\text{NH}_3$  diffusion also occurs, and this becomes more important in treatments where  $\Delta P_{\text{NH}_3}$  is elevated and/or  $J_{\text{in}}^{\text{Na}}$  is inhibited. While  $\text{NH}_4^+$  diffusion cannot be ruled out, it is probably of minor quantitative importance; for  $J_{\text{net}}^{\text{Amm}}$  always changed in the opposite direction from  $\Delta \text{NH}_4^+$ . It must be noted that we did not take the transepithelial potential (TEP) into account. However, this was probably unimportant to our conclusion, for McWilliams & Potts (1978) showed TEP in the brown trout, *Salmo trutta*, to be stable down to  $\text{pH} = 6.0$ , and then to become progressively more positive at lower pH values. Our calculated elevation in  $\Delta \text{NH}_4^+$  at  $\text{pH} = 4.06$  was therefore probably an underestimate. The effect of alkaline pH on TEP has not been studied in trout, but is reported to elevate the TEP in goldfish *Carassius auratus* (Eddy, 1975). Our calculated elevation in  $\Delta \text{NH}_4^+$  at  $\text{pH} = 8.69$  and  $9.54$  would again be an underestimate if this were also true in trout. In any event, when one compares the relative gradients available to drive the passive effluxes of  $\text{Na}^+$  and  $\text{NH}_4^+$  across the gills, only a very small  $\text{NH}_4^+$  diffusion is theoretically likely. This argument has been developed in detail by Kormanik & Cameron (1981a).

We have attempted to relate the influx of  $\text{Na}^+$  ( $J_{\text{in}}^{\text{Na}}$ ) to  $\text{NH}_4^+$  excretion ( $J_{\text{net}}^{\text{NH}_4}$ ) in all series by assuming that at  $\text{pH} = 4.06$ , net ammonia excretion ( $J_{\text{net}}^{\text{Amm}}$ ) was entirely by  $\text{NH}_3$  diffusion (i.e.  $J_{\text{net}}^{\text{NH}_4} = 0$ ) since  $J_{\text{in}}^{\text{Na}}$  was obliterated and  $\text{NH}_4^+$  diffusion appears insignificant. The diffusivity of the gills to  $\text{NH}_3$  ( $D_{\text{NH}_3} = J_{\text{net}}^{\text{Amm}}/\Delta P_{\text{NH}_3}$ ) under these conditions was  $4 \mu\text{mol kg}^{-1} \text{ h}^{-1} \mu\text{Torr}^{-1}$ , which is about 65% of the value estimated by Cameron & Heisler (1983) for the same species. This  $D_{\text{NH}_3}$  value was then applied to the other series and the  $\text{NH}_3$  contribution ( $J_{\text{net}}^{\text{NH}_3}$ ) to  $J_{\text{net}}^{\text{Amm}}$  determined from the prevailing  $\Delta P_{\text{NH}_3}$ .  $J_{\text{net}}^{\text{NH}_4}$  was then calculated as the difference between  $J_{\text{net}}^{\text{Amm}}$  and  $J_{\text{net}}^{\text{NH}_3}$ , signs considered. The analysis therefore relies on the absolute  $\Delta P_{\text{NH}_3}$  values (see above), assumes that

Fig. 5. The influence of moderate alkaline exposure ( $\text{pH} = 8.69$ ) during the experimental period on selected parameters related to branchial ammonia excretion in rainbow trout.  $N = 13$  in (A)–(D) and 7 in (E). Other details are in legend of Fig. 1.

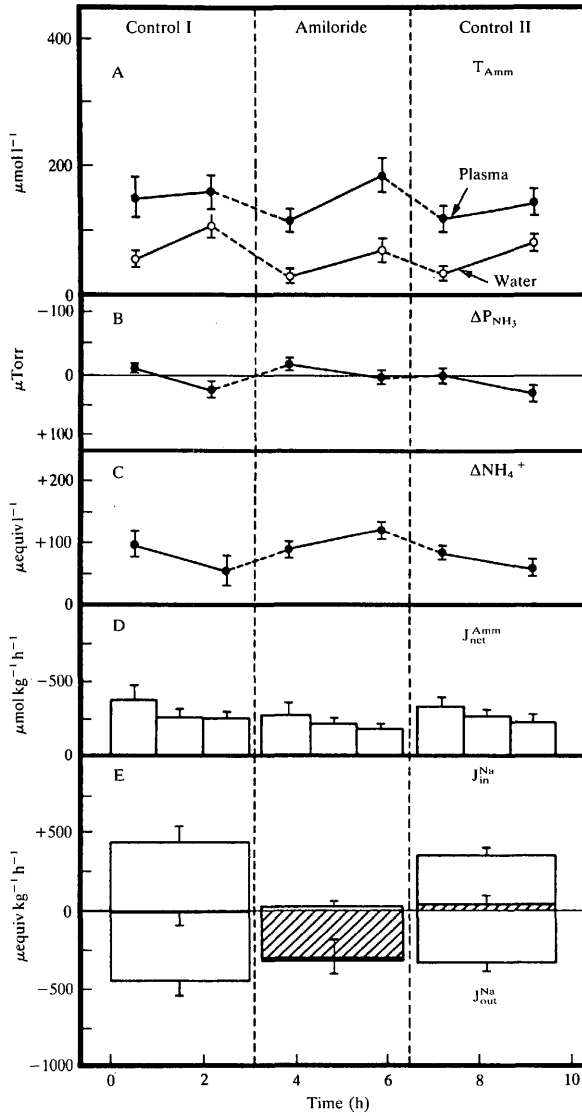


Fig. 6

NH<sub>3</sub> excretion is diffusion limited (i.e. any ventilatory or cardiovascular effects of the various treatments were unimportant), and assumes that  $D_{\text{NH}_3}$  is the same under all treatments and in both directions of NH<sub>3</sub> flux: all of which are possible sources of error.

Fig. 7 illustrates the relationship between  $J_{\text{net}}^{\text{NH}_4}$  and  $J_{\text{in}}^{\text{Na}}$  based on this analysis. The two parameters appear to be more or less linearly related and increase with pH from 4.1 to 8.7. If the analysis is valid, Na<sup>+</sup>/NH<sub>4</sub><sup>+</sup> exchange clearly dominates over diffusive processes in the control pH range. At pH = 9.5, where  $J_{\text{net}}^{\text{NH}_4}$  appears greatly to exceed  $J_{\text{in}}^{\text{Na}}$ , any overestimation in the very negative (and highly variable)  $\Delta P_{\text{NH}_3}$  measurement will greatly exaggerate the calculated  $J_{\text{net}}^{\text{NH}_4}$ . Under these conditions, there may well have been a significant branchial H<sup>+</sup> efflux or HCO<sub>3</sub><sup>-</sup> uptake, lowering the pH of lamellar water and decreasing the  $\Delta P_{\text{NH}_3}$  below the measured value.

These conclusions about the dominance of Na<sup>+</sup>/NH<sub>4</sub><sup>+</sup> exchange relative to NH<sub>3</sub> diffusion under our control conditions agree completely with the work of Maetz (1972, 1973) on goldfish where control pH (~7.9) was similar to the present. Maetz later questioned the absolute values of the ammonia gradients calculated in his experiments, but not the basic conclusions (Maetz *et al.* 1976). In contrast, Cameron & Heisler's (1983) investigation of rainbow trout, while not ruling out Na<sup>+</sup>/NH<sub>4</sub><sup>+</sup> exchange, concluded that NH<sub>3</sub> diffusion was sufficient to explain all resting  $J_{\text{net}}^{\text{Amm}}$ , assuming that NH<sub>3</sub> and CO<sub>2</sub> have similar permeabilities in gill tissue. This difference is probably related to the lower control pH = 7.0 used in their study. Thus comparable internal and external  $T_{\text{Amm}}$  in the two studies gave rise to a reasonable  $\Delta P_{\text{NH}_3}$  (+55  $\mu\text{Torr}$ ) to drive resting  $J_{\text{net}}^{\text{Amm}}$  by passive NH<sub>3</sub> diffusion at pH = 7.0 (Cameron & Heisler, 1983), but negligible or negative  $\Delta P_{\text{NH}_3}$  at pH = 8.0 (present study) due to the approximately 10-fold higher water  $P_{\text{NH}_3}$ . In any event, absolute values of  $\Delta P_{\text{NH}_3}$  should be viewed with caution and relative changes may well be more informative (see above): Indeed, Cameron & Heisler (1983) postulated a high rate of Na<sup>+</sup>/NH<sub>4</sub><sup>+</sup> exchange to explain the response to a treatment (pH = 8.0, elevated external  $T_{\text{Amm}}$ ) which changed  $\Delta P_{\text{NH}_3}$  to a very negative value. Hence there appears to be no conflict in observation between our study and that of Cameron & Heisler (1983), but subtle differences in interpretation.

We favour a flexible model in which  $J_{\text{net}}^{\text{Amm}}$  is achieved by a variable combination of Na<sup>+</sup>/NH<sub>4</sub><sup>+</sup> exchange and NH<sub>3</sub> diffusion. The exact proportions occurring through each pathway will depend upon the relative  $\Delta P_{\text{NH}_3}$ , the extent of  $J_{\text{in}}^{\text{Na}}$ , and quite possibly the acid-base status of the animal. Ammonia excretion *via* Na<sup>+</sup>/NH<sub>4</sub><sup>+</sup> exchange represents acidic equivalent excretion, while NH<sub>3</sub> efflux is neutral in net acid-base terms, though inequalities between NH<sub>3</sub> production and excretion rates will affect internal acid-base status. Branchial Na<sup>+</sup>/H<sup>+</sup> exchange may also occur (e.g. Kerstetter *et al.* 1970; Kirschner *et al.* 1973), and the relative  $\Delta\text{H}^+$  and  $\Delta\text{NH}_4^+$  gradients will determine which mechanism is an energetically

Fig. 6. The influence of exposure to  $10^{-4} \text{ mol l}^{-1}$  amiloride in water at control pH during the experimental period on selected parameters related to branchial ammonia excretion in rainbow trout.  $N = 8$  in (A)–(C), 9 in (D) and 5 in (E). Other details as in legend of Fig. 1.

Table 5. Mean values of parameters related to branchial ammonia excretion during each 3-h period in rainbow trout subjected to moderate alkaline exposure during the experimental period

	N	Control I	Moderate alkaline	Control II
Water pH	17	7.98 ± 0.06	8.69 ± 0.04*	8.11 ± 0.06†
Plasma pH <sub>a</sub>	13	7.79 ± 0.02	7.87 ± 0.02*	7.83 ± 0.02
Plasma P <sub>a</sub> CO <sub>2</sub> (Torr)	13	3.13 ± 0.22	2.61 ± 0.14*	2.67 ± 0.16
Plasma HCO <sub>3</sub> <sup>-</sup> (mequiv l <sup>-1</sup> )	13	6.91 ± 0.44	7.09 ± 0.29	6.44 ± 0.47†
Plasma P <sub>a</sub> NH <sub>3</sub> (μTorr)	13	39 ± 9*	60 ± 9*	35 ± 6†
Water P <sub>a</sub> NH <sub>3</sub> (μTorr)	13	58 ± 6	160 ± 16*	52 ± 7†
P <sub>a</sub> NH <sub>3</sub> (μTorr)	13	-19 ± 12	-100 ± 18*	-17 ± 11†
Plasma NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	13	144 ± 21	187 ± 26*	120 ± 22†
Water NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	13	90 ± 8	53 ± 5*	60 ± 6*
NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	13	+54 ± 17	+134 ± 24*	+60 ± 20†
V <sub>am</sub> (μmol kg <sup>-1</sup> h <sup>-1</sup> )	13	-342 ± 34	-262 ± 28*	-380 ± 37†
V <sub>am</sub> (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	7	+688 ± 147	+492 ± 123*	+600 ± 105
V <sub>am</sub> (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	7	-658 ± 121	-698 ± 171	-458 ± 91
V <sub>am</sub> (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	7	+30 ± 133	-206 ± 105	+142 ± 78†

‡ ± 1 s.e.m. \* P < 0.05 relative to control I. † P < 0.05 relative to moderate alkaline.

Table 6. Mean values of parameters related to branchial ammonia excretion during each 3-h period in rainbow trout subjected to 10<sup>-4</sup> mol l<sup>-1</sup> amiloride in water at control pH during the experimental period

	N	Control I	Amiloride	Control II
Water pH	9	7.97 ± 0.10	8.12 ± 0.05	8.14 ± 0.05
Plasma pH <sub>a</sub>	8	7.84 ± 0.06	7.81 ± 0.04	7.79 ± 0.03
Plasma P <sub>a</sub> CO <sub>2</sub> (Torr)	8	2.88 ± 0.29	2.45 ± 0.19	2.56 ± 0.34
Plasma HCO <sub>3</sub> <sup>-</sup> (mequiv l <sup>-1</sup> )	8	7.05 ± 0.54	6.02 ± 0.43	5.54 ± 0.48
Plasma P <sub>a</sub> NH <sub>3</sub> (μTorr)	8	51 ± 18	42 ± 9	33 ± 6
Water P <sub>a</sub> NH <sub>3</sub> (μTorr)	8	61 ± 24	39 ± 16	49 ± 16
Water P <sub>a</sub> NH <sub>3</sub> (μTorr)	8	-10 ± 12	+3 ± 16	-16 ± 12†
Plasma NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	8	128 ± 27	127 ± 15	106 ± 15
Water NH <sub>4</sub> <sup>+</sup> (μequiv l <sup>-1</sup> )	8	75 ± 19	44 ± 13*	53 ± 11*†
V <sub>am</sub> (μequiv l <sup>-1</sup> )	8	+53 ± 29	+83 ± 20	+53 ± 22
V <sub>am</sub> (μequiv l <sup>-1</sup> )	8	-306 ± 61	-237 ± 45*	-290 ± 40†
V <sub>am</sub> (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	5	+444 ± 101	+28 ± 49*	+363 ± 40†
V <sub>am</sub> (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	5	-447 ± 105	-319 ± 65	-323 ± 54
V <sub>am</sub> (μequiv kg <sup>-1</sup> h <sup>-1</sup> )	5	-3 ± 91	-292 ± 125	+40 ± 62†

‡ ± 1 s.e.m. \* P < 0.05 relative to control I. † P < 0.05 relative to amiloride.

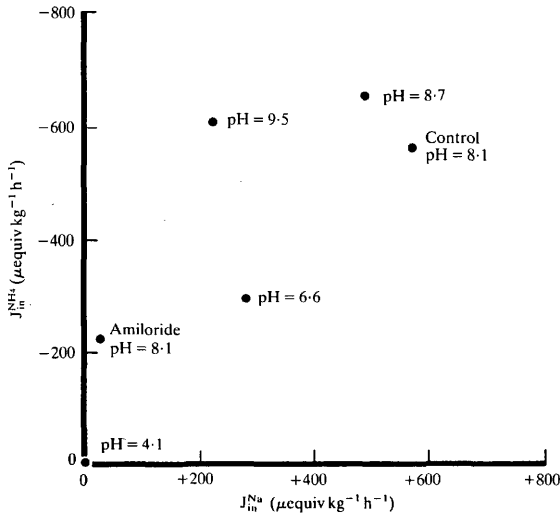


Fig. 7. The relationship between estimated  $J_{net}^{NH_4}$  and measured  $J_{in}^{Na}$  in rainbow trout in each experimental series. See text for details of the  $J_{net}^{NH_4}$  calculation.

more favourable means of acidic equivalent excretion in any given situation.  $Na^+/H^+$  exchange plus  $NH_3$  diffusion is equivalent to  $Na^+/NH_4^+$  exchange in terms of both mass and acid-base balance.  $Na^+/Na^+$  exchange diffusion also undoubtedly occurs in the gills (cf. Maetz *et al.* 1976; Wood *et al.* 1984). The factors controlling the relative rates of these various processes is a challenging field for future investigation.

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