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AMMONIA, Na^+, K^+ AND Cl^- LEVELS IN RAINBOW TROUT YOLK-SAC FRY IN RESPONSE TO EXTERNAL AMMONIA

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Summary

Body ammonia content of rainbow trout alevins was about $0.6\,\mu mol\,g^{-1}$ but increased to $4\,\mu mol\,g^{-1}$ after 24h of exposure to an external ammonia concentration of $36.2\,\mu mol\,l^{-1}$ NH_3 ($15.8 mmol\,l^{-1}$ ammonia) at pH7 and 10°C. During ammonia loading, the mass of alevins remained unchanged, but body ion concentrations decreased by about 28% for Na^+ and Cl^- and by 35% for K^+. These effects were reduced at lower ammonia concentrations. Exposure for 24h to $36.2\,\mu mol\,l^{-1}$ NH_3 ($15.8 mmol\,l^{-1}$ ammonia) at pH7 and 10°C resulted in a build up of body ammonia that was almost complete within 10h, whereas Na^+ loss from the body was delayed and commenced after about 5h of exposure. After exposure, ammonia unloading from the body was complete in about 10h but there was a delay of about 5h before Na^+ uptake commenced. During ammonia exposure, alevins lost substantial amounts of K^+ ($14\,\mu mol\,g^{-1}$) that were not replaced for several days after exposure to ammonia. Ammonia exposure has major effects on ionic regulation in juvenile fish and possible regulatory processes are discussed.

Introduction

Ammonia is the major nitrogenous excretory product of teleost fish that is lost from the body across the gills to the aqueous environment. Ammonia is a weak base (pK value about 9.64 at 10°C) and at physiological pH values (pH7–8) less than 2.5% of the total ammonia exists as the unionised form, NH_3. Unionised ammonia accounts for the major part of metabolic ammonia excretion by diffusion down its concentration gradient (Cameron and Heisler, 1983; Wright and Wood, 1985). The ionised form, NH_4^+, may be excreted in exchange for Na^+ and possible mechanisms have been discussed on numerous occasions (e.g. Maetz, 1973; McDonald *et al.* 1989).

The chemistry of ammonia in fresh water has been extensively reviewed and toxicity to fish is usually expressed as NH_3 concentration (EIFAC, 1970; Alabaster and Lloyd, 1982; Erikson, 1985; WHO, 1986). Ammonia toxicity to rainbow trout is apparently not

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influenced by the nature of the ammonium salt (Thurston and Russo, 1983), although chloride is marginally more toxic than sulphate to channel catfish (Sheehan and Lewis, 1986). Ammonia has severe and often lethal effects on sensitive species such as salmonids at concentrations as low as 12 μmol l^{-1} NH_3 (EIFAC, 1970; Alabaster and Lloyd, 1982). Physiological effects include disturbances of ionic balance and acid–base balance (Maetz, 1973; Cameron and Heisler, 1983; Cameron, 1986; Twitchen and Eddy, 1993). However, in considering toxic effects, the temperature, pH and total ammonia content of the water should be known; e.g. an increase in pH by 1 unit will increase unionised ammonia by a factor of ten. The competitive inhibition of Na^+ influx by NH_4^+ at the gills may also be important (Maetz and Garcia-Romeu, 1964; Maetz, 1973; Twitchen and Eddy, 1993).

When considering the physiological effects of external ammonia, it is important to know its rate of entry into the body, how much can be accumulated and the rate of loss once the exposure has ended. There is very little information of this type apart from some values for changes in blood plasma concentrations (Cameron and Heisler, 1983; Cameron, 1986; Wilson and Taylor, 1992), and there are few data for tissue ammonia levels in fish (e.g. Randall and Wright, 1987; Dobson and Hochachka, 1987).

This paper describes experiments on the response of late yolk-sac fry to a pulse of ammonia, explores the rate of ammonia build up in the body, together with its effects on ionic balance, and examines post-exposure effects.

Materials and methods

Rainbow trout [*Oncorhynchus mykiss* (Walbaum)] late alevins (triploids), similar to development stage 13–14 described for salmon alevins, *Salmo salar* L. (Pelluet, 1944), were obtained from a local hatchery (Cloan Hatcheries, Perthshire). Brown trout (*Salmo trutta* L.) late alevins at a similar developmental stage were obtained from Loch Leven Fisheries, Fife. Stocks were maintained in running Dundee aquarium water at 6.5–8°C. Towards the end of the experimental period, the fish were almost at the start-feed fry stage, but were not fed. The experimental temperature was 9–11°C. Mean values for water quality were (in mmol l^{-1}): Na^+, 0.19; K^+, 0.02; Ca^{2+}, 0.24; Mg^{2+}, 0.07; Cl^-, 0.3; free CO_2, 0.02. Alkalinity as $CaCO_3$ was 20.5mg l^{-1}; total hardness as $CaCO_3$, 31.2mg l^{-1} and non-bicarbonate hardness as $CaCO_3$, 10.6mg l^{-1}; pH was 8.2.

Between 12 and 18 rainbow trout alevins were placed in each of six darkened containers with 1l of constantly aerated aquarium water and were allowed to equilibrate at 10°C and pH7. After 24h, the water was replaced and ammonium chloride was added to give nominal concentrations of 0 (control), 7.2 (3.2), 14.4 (6.3), 21.7 (9.5), 28.9 (12.7) and 36.2 (15.8) μmol l^{-1} NH_3 (mmol l^{-1} ammonia), calculated according to the method of Cameron and Heisler (1983). The pH value was adjusted to and maintained at pH7 with dilute sulphuric acid. After 24h, eight alevins were removed, gently blotted to remove surface moisture, and the wet mass of each individual was determined. To avoid stress, alevins were weighed only at the end of each experiment (see below). Each alevin was individually homogenised in 1ml of ice-cold deionised water with a few strokes of a glass homogeniser, a procedure lasting for 10–20s. After centrifugation at 13000revsmin^{-1} for 2.5min, the supernatant was withdrawn, immediately frozen to −30°C and stored

until required for ammonia and Cl^- analysis. Since Na^+ may be released from glass homogenisers, this procedure was unsuitable for Na^+ analysis and an alternative method was selected. The remaining alevins (in most cases at least five, see Fig. 1) from each ammonia exposure were removed and surface water was blotted as before. They were individually wet weighed, each placed in a plastic tube, killed by compressing the head with forceps and dissolved in 0.5ml of concentrated nitric acid for subsequent Na^+ and K^+ analysis. This method is unsuitable for chloride determination, since HCl is lost during the acid digestion (Conway, 1957).

In a further experiment to examine loading and unloading of body ammonia, at least 300 rainbow trout alevins in 2.5l water were exposed to 36.2 $\mu mol\,l^{-1}$ NH_3, pH7, a level designed to provoke a maximal sublethal effect in 24h. Sixteen individuals were removed at 2, 4, 8 and 24h for analysis. Then fresh ammonia-free water, adjusted to pH7 as before, was substituted and further batches of 16 individuals were removed after 2, 4, 8, 24, 48, 72, 120 and 168h, together with water samples for ionic analysis. Water was changed every other day. A control experiment with 100 alevins was run concurrently. Alevins were individually weighed and processed for ammonia and ionic analysis, as described previously.

To investigate the effects of ammonia exposure on wet mass and percentage water content, 50 brown trout late alevins were placed in 1l of aquarium water containing 0 or 36.2 $\mu mol\,l^{-1}$ NH_3, pH7 and 10°C, as described above. Groups of eight alevins were removed at intervals, wet mass determined for each individual as described above, and then they were dried at 65°C for 48h before being reweighed (see Table 2). This involved wet weighing the alevin only once, since this procedure was very stressful. It was observed in a similar experiment that when individuals were wet weighed more than twice there was 100% mortality of both unexposed and exposed groups.

Ammonia analysis of homogenates was by the Boehringer Mannheim UV method, where ammonia content of the sample is equivalent to the amount of NADH oxidised by glutamate dehydrogenase. Known amounts of ammonia were added to some of the samples, resulting in recoveries in excess of 75%. The presence of glutamate dehydrogenase (or other ammonia-metabolising enzymes) in tissue samples may result in an underestimation of tissue ammonia content, although this would be minimal since tissue samples were immediately frozen following preparation. Reanalysis of samples which had been frozen for several weeks showed no loss of ammonia. This method of tissue preparation was preferred since it offered few opportunities for ammonia release from proteins, a possible cause of overestimation of tissue ammonia. As all rainbow trout alevins were processed in the same way, differences between control and ammonia-exposed alevins are directly comparable. Ammonia content of the water was determined by the indophenol method (Solorzano, 1969). Na^+ and K^+ concentrations were determined using an EEL 100 flame photometer, and chloride levels in the homogenate were determined with a Jenway chloride meter.

Definitions

Ammonia refers to total ammonia, NH_3 refers to unionised ammonia and NH_4^+ to ionised ammonia. In the Discussion, terms referring to ion fluxes are: unidirectional

influx, influx; unidirectional efflux, efflux. The difference between unidirectional fluxes represents the net flux: net gain, uptake; net loss, loss.

Results

The body ammonia concentration of rainbow trout alevins increased from about 0.6 μmol g^{-1} for unexposed fish to nearly 4 μmol g^{-1} after 24h of exposure to 36.2 μmol l^{-1} NH_3 (15.8mmol l^{-1} ammonia) (Fig. 1D). As well as gaining ammonia, the fish also lost body ions, about 10 μmol g^{-1} alevin for Na^+ and Cl^-, and over double that value for K^+ at 36.2 μmol l^{-1} NH_3. Lower ammonia exposure levels produced lesser

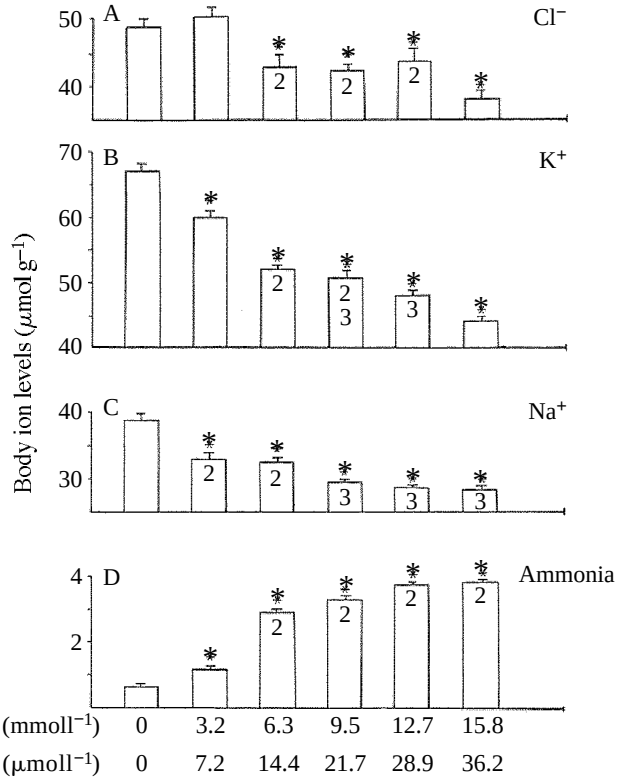

Fig. 1. Body Cl^- (A), K^+ (B), Na^+ (C) and total ammonia (D) levels in rainbow trout late alevins after exposure for 24h to 0, 7.2 (3.2), 14.4 (6.3), 21.7 (9.5), 28.9 (12.7) and 36.2 (15.8) μmol l^{-1} NH_3 (mmol l^{-1} ammonia), pH7, 10°C. Values are mean ± standard error. Values marked with an asterisk are significantly different from the control values for each ion. All other values are significantly different from each other, except those marked 2 and 3. Those values marked 2 within the columns are insignificantly different from each other, as are those marked 3. For Cl^{-1} and ammonia, N=8; for Na^+ and K^+, N is at least 6, apart from 28.9 and 36.2 μmol l^{-1} NH_3, where N=4 and 5, respectively. Significant differences are at P<0.05, analysis of variance and Student's t-test.

ammonia burdens and lower ionic losses (Fig. 1). There was no significant difference in mass of rainbow trout alevins following ammonia exposure, suggesting that changes in body ion content were not due to tissue dilution and mass increase (Table 1).

There was no significant difference in wet mass or percentage water content between unexposed and exposed late brown trout alevins (Table 2), confirming that mass increase through increased water content during ammonia exposure of rainbow trout alevins is extremely unlikely (Tables 1 and 3). Subsequent calculations are therefore based on this assumption.

Upon exposure to 36.2 μmol l^{-1} NH_3, rainbow trout alevins showed a rapid build up of body ammonia that was almost complete by 10h and reached a value of about 6 μmol g^{-1} alevin by 24h (Fig. 2D). Subsequent exposure to ammonia-free water resulted in a decrease in body ammonia, that was again almost complete in 10h, to a value of 2–3 μmol g^{-1}, which is higher than the initial level of around 1 μmol g^{-1} but comparable to that of the control group at this time (Fig. 2). Ammonia exposure did not result in any significant mass differences between exposed and corresponding unexposed alevins (Table 3).

Table 1. *Wet mass (mg) of late rainbow trout alevins exposed to various concentrations of external ammonia at pH7, 10°C for 24 h*

Total ammonia ($mmol\,l^{-1}$)	NH_3 ($\mu mol\,l^{-1}$)	*N*	Mean wet mass (mg)	S.E.M.
0	0	18	93.40	3.55
3.2	7.2	14	88.43	4.07
6.3	14.4	14	93.29	5.64
9.5	21.7	14	92.00	4.15
12.7	28.9	12	96.67	3.50
15.8	36.2	13	91.92	5.84

There were no significant differences between mean masses for control and ammonia-exposed alevins by analysis of variance.

N, number of alevins.

Table 2. *Wet mass (mg) and percentage water content of Loch Leven brown trout late alevins exposed to 36.2 μmol l^{-1} NH_3 (15.8mmol l^{-1} ammonia), pH7, 10°C for up to 24 h*

	Control				Exposed			
Time (h)	Wet mass (mg)	S.E.M.	Water content (%)	S.E.M.	Wet mass (mg)	S.E.M.	Water content (%)	S.E.M.
2.5	105.75	4.33	70.13	0.743	96.88	4.24	69.10	1.16
5.5	102.75	6.39	70.12	1.14	98.13	6.75	69.72	0.89
24	108.25	2.53	71.57	0.617	99.63	7.96	70.76	0.781

The control group was an unexposed group run concurrently.

Mean values and standard errors for *N*=8 are indicated.

There was no significant difference in wet mass or percentage water content within or between the unexposed and exposed groups (analysis of variance).

Table 3. *Wet mass (mg) of late rainbow trout alevins exposed to 36.2 μmol l^{-1} NH_3 (15.8mmol l^{-1} ammonia), pH7, 10°C for 24h (Ammonia exposure), followed by return to ammonia-free water for up to 168h (Post exposure)*

Time (h)	Control wet mass (mg)	*N*	S.E.M.	Exposed wet mass (mg)	*N*	S.E.M.
Ammonia exposure						
0	102.62	16	3.99	–	–	–
2				90.12	16	4.33
4				99.62	16	4.72
8				94.75	16	3.50
24	93.4	10	3.55	106.37	16	3.28
Post exposure						
26				112.06	16	3.39
28				101.56	16	4.47
32				94.12	16	4.82
48	100.94	16	4.88	94.19	16	3.31
72				95.81	16	4.87
96				94.62	16	3.81
144				96.94	16	2.64
192	115.31	16	3.11	105.19	16	4.49

An unexposed group was run concurrently (Control).

Control and exposed/post-exposed masses were insignificantly different at corresponding times (analysis of variance).

Mean values, standard error (S.E.M.) and number of alevins (*N*) are indicated.

During ammonia exposure, 4–5h elapsed before body Na^+ levels began to fall, and by 24h some 4–5 μmol g^{-1} had been lost. Following return to ammonia-free water, there was again a delay of some 4–5h before body Na^+ values began to recover, reaching normal levels 8h after exposure (Fig. 2). Body Cl^- levels fell by 8 μmol g^{-1} after 24h of ammonia exposure, but did not show the delay noted for Na^+. Cl^- recovery was complete within 10h but was subsequently unstable compared with the control. Body K^+ values fell by about 14 μmol g^{-1} after 24h of ammonia exposure, but recovery to normal values of about 70 μmol g^{-1} was slow and incomplete even after a week in ammonia-free water. Over the 8-day experiment, Na^+, K^+ and ammonia values for unexposed rainbow trout alevins showed a steady increase, while Cl^- values decreased (Fig. 2).

Net fluxes of Na^+, K^+, ammonia and Cl^- were calculated for each group of rainbow trout alevins from the change in body ionic content during the ammonia exposure (Tables 4 and 5) and the results parallel the trends shown in Figs 1 and 2.

Following exposure of rainbow trout alevins to ammonia for 24h, the water Na^+ and K^+ values increased (Table 6), reflecting net Na^+ and K^+ loss from the alevins (Fig. 2 and Table 5). The net fluxes calculated from changes in water content (assuming 300 alevins of average mass 100mg) were about 1.11 μmol g^{-1} h^{-1} for K^+ and 0.49 μmol g^{-1} h^{-1} for Na^+, which are greater than those estimated from changes in body ionic content (Table 5). This suggests that net ion flux rates based on body ionic content changes (Table 5) may

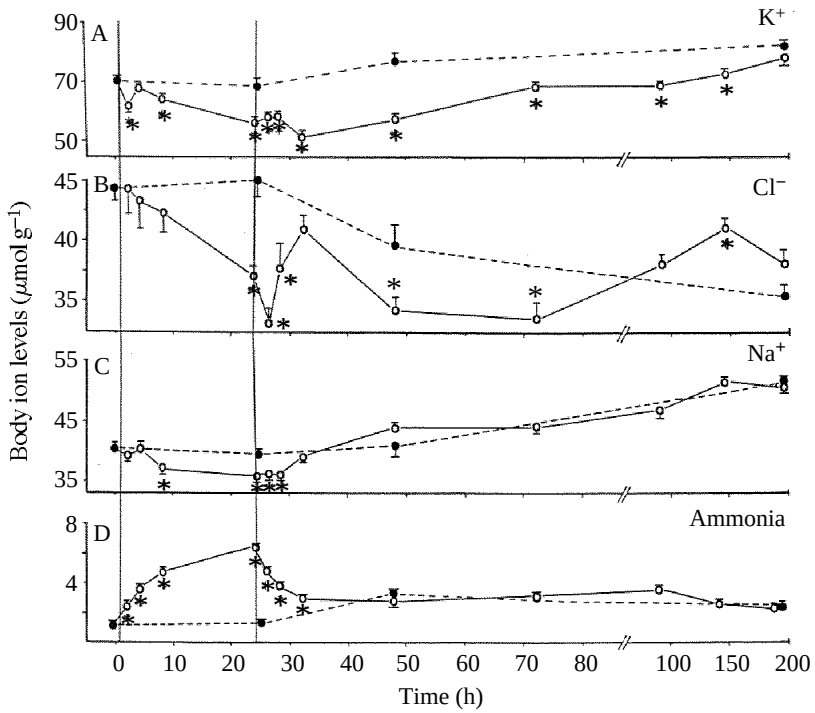

Fig. 2. Body K^+ (A), Cl^- (B), Na^+ (C) and total ammonia (D) levels in rainbow trout late alevins exposed to 36.2 μmol l^{-1} NH_3, pH7, 10°C for 24h, followed by return to ammonia-free water for up to 168h (open symbols). The ammonia exposure period is indicated by the vertical lines. An unexposed group was run concurrently (filled symbols). Mean values are in μmol g^{-1} with standard error, *N*=8. Values significantly different from the controls are marked with an asterisk.

be an underestimate for the exposed population, since moribund alevins which were likely to have lost greater amounts of body ions were not selected for analysis. The experimental protocol precluded an estimate of net fluxes for individuals from water ionic content changes. Because of high background levels (15.8mmol l^{-1}), significant changes in external Cl^- level were undetectable. Throughout the experiment (Fig. 2), except during ammonia exposure, water Na^+, K^+ and Cl^- values were within 10% of those given in Materials and methods and total ammonia levels were less than 0.01mmol l^{-1}.

No mortalities were observed in any of the experiments, although 24h of exposure to 21.7 μmol l^{-1} NH_3 and above resulted in hyperexcitability, darker coloration and morbidity of rainbow trout alevins. One week after exposure, the rainbow trout alevins appeared to be almost fully recovered. Amongst the controls, no abnormalities or mortalities were observed.

Discussion

The body ammonia levels of about 0.6 μmol g^{-1} for unexposed rainbow trout late alevins is an average value for all tissues. Body ammonia levels appear to be a function of

Table 4. *Effect of 24h of exposure to various concentrations of ammonia (μmol l^{-1} NH_3) on net fluxes of Na^+, K^+, ammonia and Cl^- of rainbow trout alevins*

	External NH_3 (μmol l^{-1})					
	0	7.2	14.4	21.7	28.9	36.2
Na^+	−	−0.234±0.043	−0.252±0.010	−0.378±0.026	−0.413±0.015	−0.437±0.032
K^+	−	−0.263±0.055	−0.58±0.033	−0.64±0.045	−0.743±0.032	−0.911±0.039
Ammonia	−	0.024±0.007	0.095±0.008	0.112±0.005	0.127±0.005	0.130±0.007
Cl^-	−	0±0	−0.212±0.10	−0.225±0.063	−0.175±0.075	−0.417±0.088

Net fluxes (μmol g^{-1} h^{-1}) were calculated from changes in body ionic content (see Fig. 1); negative values indicate a net loss from the alevin.

Mean and standard error are given; *N* values as in Fig. 1.

development since, at the end of the 8-day experiment, they had increased to around 2 μmol g^{-1} (Fig. 2). These values are higher than blood plasma values of about 0.3mmol l^{-1} (Cameron and Heisler, 1983; Randall and Wright, 1987), but similar to tissue values of about 1–2mmol l^{-1} for white muscle of unexercised rainbow trout (Dobson and Hochachka, 1987; Wright and Wood, 1988) and estimated whole-body ammonia values of about 0.6mmol l^{-1} for a 1kg fish (Randall and Wright, 1987). Values of about 6mmol l^{-1} in lemon sole muscle were noted by Wright *et al.* (1988), suggesting differences between freshwater and marine fish.

Body Na^+, K^+ and Cl^- values for unexposed rainbow trout alevins were in the range reported for freshwater and seawater salmonids by Talbot *et al.* (1982, 1986) and Rombough and Garside (1984). Over the 8-day experimental period, control alevins showed an increase in values for Na^+ and K^+, but a decrease in the value for Cl^-. Such changes for cations have been previously noted (Runn and Sohtell, 1982; Rombough and Garside, 1984; McWilliams and Shephard, 1991). These changes in body ions probably relate to growth, development and metabolism.

Table 5. *Net fluxes of Na^+, K^+, ammonia and Cl^- for rainbow trout alevins exposed to ammonia (36.2 μmol l^{-1} NH_3, 15.8mmol l^{-1} ammonia, pH7, 10°C) and during post-exposure recovery in ammonia-free water*

	Time (h)		
	Exposure	Post-exposure	
	0–24 h	0–24 h	24–168 h
Na^+	−0.165±0.003	0.343±0.007	0.043±0.001
K^+	−0.61±0.012	0.063±0.002	0.151±0.003
Ammonia	0.215±0.004	−0.152±0.003	0.003±0.0001
Cl^-	−0.33±0.007	−0.125±0.003	0.029±0.0006

Values are in μmol g^{-1} h^{-1}; negative values indicate a net loss from the alevin.

Net fluxes were calculated from changes in body ionic content, see Fig. 2.

Mean and standard errors are given and *N* values are as in Fig. 2.

Table 6. *Na^+, K^+ and ammonia concentrations of the water at time 0 and after 24h of exposure of rainbow trout late alevins to 36.2 μmol l^{-1} NH_3 (15.8mmol l^{-1} ammonia), pH7, 10°C*

Time (h)	Na^+ (mmol l^{-1})	K^+ (mmol l^{-1})	Ammonia (mmol l^{-1})	NH_3 (μmol l^{-1})
0	0.18	0.02	15.8	36.2
24	0.32	0.34	15.8	36.2

See Fig. 2, Table 3 and Materials and methods for further details.

Exposure of rainbow trout alevins to 36.2 μmol l^{-1} NH_3 (15.8mmol l^{-1} ammonia) for 24h increased the body ammonia levels to about 4 μmol g^{-1} (Fig. 1). The response was not linear with lower ammonia levels, e.g. 14.4 μmol l^{-1} NH_3 produced nearly the same increase as the higher levels (Fig. 1). The reason for this is unknown, but a threshold value for body ammonia build up may be involved.

The diffusion gradients for both ionised and unionised species of ammonia are from water to body fluids, but it is not possible to conclude whether ammonia loading is through uptake from the water or through the build up of metabolic ammonia, or a combination of both. Diffusive uptake of NH_3 down its partial pressure gradient is believed to be the primary route for ammonia entry into the fish (WHO, 1986; Randall and Wright, 1987; Wilson and Taylor, 1992). This would result in intracellular alkalisation through titration of protons (Avella and Bornancin, 1989; Wilson and Taylor, 1992). Although ammonia loading may stimulate Na^+/NH_4^+ exchange (Cameron and Heisler, 1983; Wright and Wood, 1985), intracellular alkalisation might reduce proton excretion and thus Na^+ influx *via* the Na^+ channel, which is the primary route for Na^+ uptake (Avella and Bornancin, 1989; Lin and Randall, 1991), thus contributing to further Na^+ loss. External NH_4^+ may also contribute to ammonia loading and Na^+ imbalance, since it is a competitive inhibitor of Na^+ uptake (Twitchen and Eddy, 1993).

Ammonia exposure of both rainbow trout and catfish resulted in increased blood plasma ammonia levels, which reached a steady state despite the net inward ammonia gradient, explained by export of NH_4^+ in exchange for Na^+ or H^+ (Cameron and Heisler, 1983; Cameron, 1986; Wilson and Taylor, 1992). However, in exposed alevins, ammonia loading of body tissues as well as blood plasma might be expected, since in many membrane processes NH_4^+ is known to displace cellular K^+ (Binstock and Lecar, 1969), and ammonia distribution between tissues has been shown to be a function of membrane potential (Wood *et al.* 1989; Tang *et al.* 1992). It seems likely that the body tissues of ammonia-exposed alevins also reach a steady state (Fig. 2).

After exposure, NH_3 would rapidly diffuse down its partial pressure gradient from fish to water, resulting in intracellular acidification. Stimulation of proton excretion may be expected and hence Na^+ influx *via* Na^+ channels (Avella and Bornancin, 1989; Lin and Randall, 1991), so facilitating recovery of Na^+ balance (Fig. 2). However, intracellular acidification may reduce Cl^-/HCO_3^- exchange contributing to the accelerated chloride loss immediately after exposure (Fig. 2).

After 24h of ammonia exposure, rainbow trout alevins showed a net cationic loss of

18 μmol g^{-1} (4 μmol g^{-1} Na^+ + 14 μmol g^{-1} K^+, Fig. 2), which exceeded the measured anionic loss (8 μmol g^{-1} Cl^-) and cation gain (4 μmol g^{-1} NH_4^+); i.e. a net anionic loss (or cationic gain) of 6 μmol g^{-1} would be required for electroneutrality. A similar trend was observed in Fig. 1, although the differences were greater, principally on account of greater K^+ losses. A possible explanation is a gain of hydrogen ions *via* H^+/NH_4^+ exchange (Wilson and Taylor, 1992), though the H^+ concentration of the external medium was low and the proton pump in gills is directed outwards (Avella and Bornancin, 1989). Another possibility that may partly account for the deficit is loss of bicarbonate in exchange for external Cl^- (Maetz and Garcia-Romeu, 1964; McDonald *et al.* 1989), i.e. Cl^- influx should be operating at its maximum rate since the external Cl^- concentration was at a saturating level of 15.8 mmol l^{-1} (Williams and Eddy, 1986) and cellular alkalisation, together with increased hydration rates of metabolic CO_2 to HCO_3^-, could provide an additional source of HCO_3^-. Immediately after exposure, Cl^- loss accelerated prior to recovery, possibly because exposure to water of relatively low Cl^- content (0.3 mmol l^{-1}) would significantly decrease Cl^- influx but might leave Cl^- efflux unaltered. The Cl^- loss during exposure might have been greater had another ammonium salt been used.

After 24h of ammonia exposure, body K^+ values of rainbow trout alevins had decreased by some 14 μmol g^{-1}, but recovery to normal values of about 70 μmol g^{-1} was slow and incomplete even after a week in ammonia-free water (Fig. 2). These trends are reflected in Table 5, which shows a high rate of K^+ loss during ammonia exposure with low rates of uptake during recovery. This could be accounted for by the low levels of K^+ in the water and its relatively slow uptake from the water compared with uptake of Na^+ and Cl^- (Eddy, 1985). Since alevins were not feeding, dietary K^+ was unavailable. The build up of K^+ in the closed system during ammonia exposure may have reduced further K^+ loss (Table 6).

The mechanisms of K^+ loss from alevins during ammonia exposure are unclear, but may be connected with NH_4^+ entry to cells from the extracellular fluid *via* $Na^+/K^+(NH_4^+)$-ATPase and subsequent deprotonation of NH_4^+ (Evans *et al.* 1989; Lin and Randall, 1991), which may induce loss of cellular K^+. Also exercise caused increased muscle ammonia in lemon sole (Wright *et al.* 1988) and rainbow trout (Dobson and Hochachka, 1987) as well as loss of muscle cell K^+ to the plasma in rainbow trout (Nielsen and Lykkeboe, 1992). Thus, during ammonia exposure, the primary source of K^+ loss from alevins would appear to be from the intracellular compartment to the blood plasma, then to the exterior *via* body surface epithelia or paracellular routes.

Increased plasma ammonia levels occurred within an hour in freshwater rainbow trout exposed to 21.6 μmol l^{-1} NH_3 (995 μmol l^{-1} total ammonia, pH7.85, 15°C), but there were no changes in plasma Na^+, K^+, Cl^-, Ca^{2+} or Mg^{2+} levels (Wilson and Taylor, 1992). However, in our experiments at a comparable NH_3 level (Fig. 1), the total ammonia concentration in the water was about ten times higher because of lower pH and temperature. Therefore, the disturbed ionic regulatory patterns observed in our experiments may be due to both NH_3 and NH_4^+. Meade (1985), Erickson (1985) and WHO (1986) reviewed several studies where NH_4^+ toxicity has been implicated. Further evidence for the role of NH_4^+ in ammonia toxicity was demonstrated by Twitchen and

Eddy (1993), who showed that, at approximately 30 μmol l^{-1} NH_3, juvenile rainbow trout lost 19% of their body Na^+ in 24h at pH7 compared with only 5% at pH8, because the NH_4^+ levels were ten times higher at pH7.

Lloyd and Orr (1969) observed no mass changes in juvenile rainbow trout exposed to ammonia, suggesting that the increased urine flow was balanced by increased branchial water permeability. Net fluxes of Na^+ (Tables 4 and 5), calculated from changes in body content, were similar to the values for rainbow trout alevins obtained from radio tracer studies in which unidirectional Na^+ influx and efflux where determined (Twitchen and Eddy, 1993). Such comparisons indicate an additional effect, i.e. a net loss of body Na^+, K^+ and Cl^-, some of which may be urinary and the remainder from the body surface, again without mass change.

These findings have important implications for alevins exposed to intermittent or cycling ammonia levels. Body ammonia levels rise and fall reasonably rapidly in response to external ammonia, but Na^+ levels show a delay of about 4–5h before changes are detectable upon exposure and immediately after exposure, although both Na^+ and ammonia levels recover simultaneously (Fig. 2). Far more significant is the fall in body K^+ values, which decreased by more than 20%, then showed a very slow recovery which was incomplete even after a week. A second ammonia exposure during the recovery period could be extremely serious for alevins.

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