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# The effect of ration on acclimation to environmental acidity in rainbow trout

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**Synopsis**

Freshwater salmonids exposed to low environmental pH typically suffer a net loss of ions, primarily $Na^+$ and $Cl^-$, across the gills, resulting in reduced plasma and tissue ion concentrations. However, in recent experiments in our laboratory, juvenile rainbow trout, *Oncorhynchus mykiss*, fed a ration of 1% body weight $d^{-1}$ or greater showed no ionoregulatory disturbance during chronic, sublethal acidification. This raised the possibility that these fish had acclimated to low pH in that they would be better able to withstand further, more severe acidification than fish that had no prior experience of acid conditions: previous studies had concluded that such acclimation does not occur. This hypothesis was tested by measuring unidirectional ion fluxes during a 24 h acute acid challenge (pH 4.2) in juvenile rainbow trout that had previously been exposed to either ambient pH 6.2 (naive fish) or sublethal low pH 5.2 (acid pre-exposed fish) for 90 days, and fed a ration of either 1.0 or 0.25% $d^{-1}$ (wet basis). No mortalities were observed during the acute acid challenge in the fish fed the higher ration and no differences between the two groups in the response of $Na^+$ fluxes were observed. Sodium influx in both groups was significantly inhibited throughout the challenge and $Na^+$ net flux was significantly stimulated over the first 6 h. Prior to the acute acid challenge, the fish fed the lower ration that had previously been exposed to pH 5.2 had significantly lower plasma ion concentrations than those fish previously exposed to pH 6.2. Both groups suffered mortalities; those of the naive fish (22% by 24 h) being markedly lower than those of the acid pre-exposed fish (68% by 24 h). However, there were no significant differences in either $Na^+$ or $Cl^-$ fluxes between the two groups of fish during the acid challenge: both showed significant inhibition of ion influxes and significantly greater net ion losses, resulting in reduced plasma ion concentrations. These results indicate that rainbow trout are unable to acclimate to environmental acidification irrespective of the availability of dietary salts.

## Introduction

The major physiological effect of environmental acidity on freshwater salmonids is a disruption of branchial ionoregulation (reviewed in Leivestad 1982, Wood 1989, Reid 1995). During lethal acid exposure, a large net loss of $Na^+$ and $Cl^-$ occurs across the gills due to a severe inhibition of ion uptake and a stimulation of diffusional ion losses, resulting in a decrease in plasma $[Na^+]$ and $[Cl^-]$ (McDonald 1983). The drop in plasma osmolarity causes a fluid shift into the intracellular compartment, an increase in blood viscosity and arterial blood pressure, and death from circulatory failure (Milligan & Wood 1982). During sublethal acidification, the inhibition of ion influx is less severe, but the net effect is also a loss of ions and consequent decrease in plasma ion concentrations (Jones et al. 1987, Lacroix & Townsend 1987, Audet et al. 1988, D'Cruz et al. 1998).

There has been some disagreement in the literature as to whether fishes may acclimate to environmental acidification. This debate is in part due to

the use of different definitions of the term 'acclimation'. A wider definition, based on earlier studies of environmental factors, such as that of Fry (1971), is that recovery from the initial impact of acid exposure either to the pre-exposure condition or to a new steady state, would constitute acclimation. For example, McWilliams (1980) suggested that brown trout, *Salmo trutta*, were able to acclimate to low pH as they showed a gradual recovery of plasma [$Na^+$] and [$Cl^-$] following an initial decline. Balm & Pottinger (1993) found only minimal physiological impact of a gradual reduction of ambient pH from 7.1 to 4.0 in rainbow trout, *Oncorhynchus mykiss*, and concluded that this was due to acid acclimation in the fish. However, a stricter definition of acclimation has been used in recent studies of acid toxicity in freshwater fish in which acclimation requires '... an increased tolerance of an elevated, usually lethal concentration of a toxicant arising from chronic exposure to a sublethal concentration of that toxicant' (McDonald & Wood 1993). This definition is used hereafter in the present study. On this basis most studies have concluded that acclimation to low pH does not occur (Falk & Dunson 1976, Wood et al. 1988a,b). Indeed, Audet & Wood (1988) found that an 81 day sublethal exposure of adult rainbow trout to pH 4.8 resulted in reduced rather than increased tolerance to a more severe acid challenge of pH 4.0, i.e. acid sensitization occurred.

During recent studies in our laboratory of chronic (90 d) exposure to sublethal acidity (pH 5.2) in softwater, juvenile rainbow trout fed to satiation or fed a ration of 1.0% body weight $d^{-1}$ showed no evidence of any ionoregulatory disturbances (Dockray et al. 1996, 1998, D'Cruz et al. 1998). However, fish exposed to a similar acid regime, but fed a much lower ration of 0.25% $d^{-1}$ had lower whole-body ion concentrations than controls (pH 6.2) (D'Cruz et al. 1998). This difference was attributed to the contribution of dietary salts to mineral balance, with acid exposed fish having a greater appetite than controls when food was not limited (Dockray et al. 1996, D'Cruz et al. 1998). In a follow-up study, D'Cruz & Wood (1998) independently manipulated the salt content and the energy content of the diet, and confirmed that the former was of prime importance. Sadler & Lynam (1986) also found that diet had a profound effect on acid toxicity; starved fish showing decreased muscle and plasma ion concentrations whilst fed fish were unaffected. These results raised the possibility, not previously considered, that the ability of fish to acclimate to increased acidity may be dependent upon diet. In the study of Audet & Wood (1988) which reported acid sensitization, the fish were fed to satiation only once per week (i.e. a low ration), whilst Falk & Dunson (1977) deprived the fish of food throughout the study period.

Therefore, the present study was designed to examine the effect of ration on the ability of juvenile rainbow trout to acclimate to sublethal acidification, i.e. to be better able to deal with a subsequent, more severe acidic episode. Fish were exposed for 90 d to either ambient pH (6.2 = naive fish) or reduced pH (5.2 = acid pre-exposed fish) in softwater and then subjected to a pH 4.2 challenge for 24 h. In the initial experiment (experiment 1), the effect of these procedures on $Na^+$ and ammonia fluxes in fish fed 1% body wt $d^{-1}$ was determined. In the second, more detailed experiment (experiment 2), the ration was reduced to 1% every four days and $Cl^-$ fluxes and plasma parameters were also measured.

## Materials and methods

### *Animal holding and chronic acid exposures*

Juvenile rainbow trout, *Oncorhynchus mykiss*, of 4–5 g initial weight were exposed to sublethal acid conditions in softwater under naturally fluctuating temperatures for 90 days, as described in full by Dockray et al. (1996). Briefly, the fish were initially allowed to acclimatize to dechlorinated Hamilton tapwater ([$Ca^{2+}$] $\approx$ 1.0 mM, [$Na^+$] $\approx$ 0.5 mM, titratable alkalinity to pH 4.0 $\approx$ 1.0; pH 7.8–8.0) for at least two days and then the hardness of the water was slowly decreased over 7 d by increasing the ratio of artificially-softened Hamilton tapwater (generated by reverse osmosis) to achieve final concentrations of [$Ca^{2+}$] $\approx$ 25 µM and [$Na^+$] $\approx$ 75 µM (pH 6.2). The fish were allowed to acclimate to the softwater conditions for a further 3–4 weeks before exposure for 90 days to either ambient (6.2) or low (5.2) pH, the latter achieved by automatic titration with 0.1 M $H_2SO_4$. Photoperiod was controlled and adjusted to mimic the natural photoperiod throughout the acclimation and experimental periods. Two such exposures were carried out with different feeding regimes. In the first experiment (April–September 1994), the fish were fed a higher ration of 1% of body weight $day^{-1}$ (wet basis) of Zeigler Trout Starter no. 3 (protein 50%; lipid 15%; water 12%). In the second experiment (April–September 1995), the fish were fed with food of the same quality but at a lower ration of

1% body weight every four days. The frequency, rather than the amount of food given at one time was reduced in experiment 2 in order to minimize the establishment of dominance feeding hierarchies.

*Acute acid challenges*

*Experiment 1 (higher ration)*
At the end of the chronic acid exposures the fish were exposed to an acute acid challenge of pH 4.2 for 24 h. The whole-body fluxes of $Na^+$ (influx, efflux, net flux) and of total ammonia ($T_{Amm}$, $NH_3/NH_4^+$) were measured immediately prior to (referred to as control fluxes) and at 0–1 h (1 h), 6–7 h (7 h) and 23–24 h (24 h) of the acid challenge. The fish were placed individually in 11 darkened chambers within a large, recessed wet table. The chambers were initially supplied with flowing, aerated water of the appropriate pH from the chronic acid exposures (6.2 or 5.2) and allowed to acclimatize to these conditions for 24 h. The control fluxes of $Na^+$ and ammonia were initiated by stopping the water flow to each chamber and adding 1 µCi $^{22}Na$. Four 5 ml water samples were taken after a 10 min mixing period and again one hour later for measurement of $^{22}Na$ radioactivity, and $Na^+$ and total ammonia concentrations.

For 5 min after the control, and continuously between the subsequent flux determinations, the chambers were flushed with water of pH 4.2. The pH was maintained during all (control and challenge) flux determination periods by manual titration with 0.1 M $H_2SO_4$; typically pH was within 0.1 units of the desired level. Water temperatures within the chambers were maintained during the flux periods by running water of the appropriate temperature around each chamber within the wet table.

*Experiment 2 (lower ration)*
The fish were exposed to an acute challenge of pH 4.2 for 24 h as described for experiment 1. However, in addition to measurements of whole-body $Na^+$ and ammonia fluxes, whole body $Cl^-$ fluxes were also measured in experiment 2 using $^{36}Cl$ as a radiotracer. Moreover, all fluxes in experiment 2 were measured more frequently following the control period – after 0–1 h, 3–4 h, 9–10 h and 23–24 h (referred to as 1 h, 4 h, 10 h and 24 h, respectively).

At the end of the 24 h acute acid challenge, fish were killed by a blow to the head and blood was collected by caudal severance into ammonium heparinized capillary tubes. Haematocrit was determined following centrifugation at 10 000 g, and plasma protein was measured using a hand-held refractometer (American Optical). The remaining plasma was frozen at −70°C for subsequent analysis of $[Na^+]$ and $[Cl^-]$.

*Calculations and statistical analysis*

Water samples taken at the beginning and end of flux determinations, and terminal plasma samples were analysed for $^{22}Na$ radioactivity by $\gamma$-counting (Canberra-Packard Miniaxi $\gamma$); $^{36}Cl$ radioactivity by $\beta$-scintillation counting (LKB Rackbeta) after correction for $^{22}Na$ $\beta$-radioactivity; $[Na^+]$ by atomic absorption spectrophotometry (Varian AA 1275); $[Cl^-]$ by the mercuric thiocyanate method (Zall et al. 1956); and total ammonia ($T_{Amm}$, $NH_3/NH_4^+$) using the indophenol method of Verdouw et al. (1978).

The unidirectional fluxes of $Na^+$ and $Cl^-$ (µmol $kg^{-1}$ $h^{-1}$) were calculated as follows:

$$\text{Influx, } J_{in}^{ion} = \frac{(cpm_i \text{ ion} - cpm_f \text{ ion})}{MSA} \cdot \frac{V}{W \cdot t},$$

where $cpm_i$ ion and $cpm_f$ ion = initial and final radioactivity respectively (counts $min^{-1}$ $l^{-1}$) of $^{22}Na$ or $^{36}Cl$; MSA = mean specific activity of $Na^+$ or $Cl^-$ (see below); V = volume of the flux chamber (l); W = weight of fish (kg); and t = time (h). The mean specific activity was calculated from:

$$MSA = \frac{\left(cpm_i \text{ ion}/[ion]_i\right) + \left(cpm_f \text{ ion}/[ion]_f\right)}{2},$$

where $[ion]_i$ and $[ion]_f$ are the initial and final concentration of $Na^+$ or $Cl^-$ (µM).

Net fluxes and effluxes of $Na^+$ and $Cl^-$ were calculated from:

$$\text{Net flux, } J_{net}^{ion} = ([ion]_i - [ion]_f) \cdot \frac{V}{W \cdot t}$$

$$\text{Efflux, } J_{out}^{ion} = \text{net flux} - \text{influx}.$$

Corrections were made throughout for water volumes removed through sampling. Correction for 'backflux', i.e. the movement, during later flux determinations, of radioactivity accumulated during earlier flux determinations, from the fish back into the water, was not necessary as internal or plasma specific activity did not exceed 5% of the external specific activity (Maetz 1956).

The net total ammonia flux was calculated from:

$$\text{Net flux, } J_{Amm} = ([T_{Amm}]_i - [T_{Amm}]_f) \cdot \frac{V}{W \cdot t}.$$

Values of all measured parameters were expressed as mean ± SEM. Within each experiment, the effect of the challenge on ion fluxes was analysed with repeated-measures ANOVA, using time (vs. control) as the within-subjects factor and group [naive (6.2) or acid pre-exposed (5.2)] as a between-subjects factor, for influx, efflux and net flux separately. Repeated-measures ANOVA requires data at every sampling time for an individual, otherwise that individual is omitted from the entire analysis. In experiment 2, this would have resulted in only survivors at 24 h being included, which would have reduced the sample size considerably and introduced a potential 'survivor effect' into the data. Statistical analysis of ion fluxes in experiment 2 was therefore carried out up to (and including) $t = 9$ h only; no significant differences between eventual survivors and mortalities were found up to this time. Statistical comparisons of plasma parameters in experiment 2 were made using two-way ANOVA. An acceptance level of $p < 0.05$ was used in all analyses.

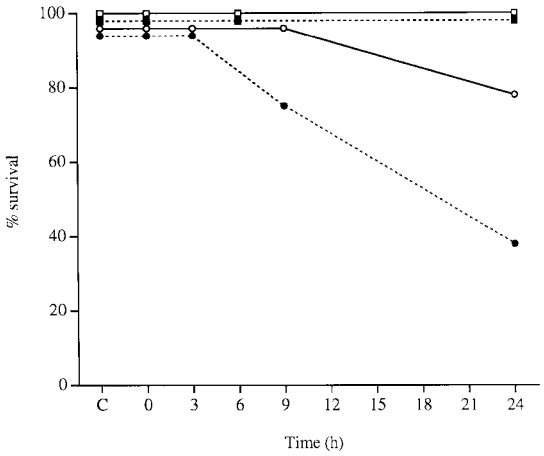


*Figure 1*. Percentage survival of juvenile rainbow trout, *O. mykiss*, during exposure to pH 4.2 for 24 h. The fish had previously been fed a ration of 1.0% body weight $d^{-1}$ (squares) or 0.25% $d^{-1}$ (circles) and been exposed to ambient pH 6.2 (open symbols) or sublethal low pH 5.2 (closed symbols) for 90 d.

## Results

*Experiment 1 (higher ration)*

No mortalities occurred in either the naive (6.2) or acid pre-exposed (5.2) fish during the acute acid challenge (Figure 1). Although the naive fish had a noticeably greater $Na^+$ efflux and (negative) $Na^+$ net flux under control (pre-challenge) conditions than did the acid pre-exposed fish, these values were not significantly different (Figure 2). There were no significant differences in any of the $Na^+$ fluxes between the two groups of fish during the acute acid challenge. Exposure to pH 4.2 resulted in an immediate, large inhibition of $Na^+$ influx for both groups which continued for the full length of the challenge. Increased acidity had no significant effect on $Na^+$ efflux but resulted in a significantly increased loss of $Na^+$ at 6 h in both groups, which recovered to control values by 24 h. Net ammonia excretion rates averaged approximately 580 µmol $kg^{-1}$ $h^{-1}$ and were not significantly affected in either of the groups at any time over the 24 h acute acid challenge (data not shown).

*Experiment 2 (lower ration)*

Mortalities occurred in both groups of fish during the acute acid challenge (Figure 1). By 24 h, 22% (2/9) of the naive fish and 63% (5/8) of the acid pre-exposed fish had died, the large majority of the mortalities occurring between 9 and 24 h.

Sodium influx was immediately inhibited by pH 4.2 in both groups of fish (Figure 3). However, in contrast to experiment 1 (in which $J_{in}Na^+$ was significantly inhibited throughout the challenge) the inhibition of $Na^+$ influx in experiment 2 was significant only at 0 and 3 h in both groups of fish. Once again however, there was no overall difference in the effect of increased acidity on sodium influx between the two groups. Sodium efflux was stimulated by pH 4.2 in both groups, but the change was smaller than for $J_{in}Na^+$, and was only significant at $t = 3$ h in the acid pre-exposed fish (Figure 3b). These changes in unidirectional $Na^+$ fluxes resulted in a significantly greater net loss of $Na^+$ at 0 and 3 h in both groups of fish. The effects of the acute acid challenge on unidirectional $Cl^-$ fluxes were similar to those on $Na^+$ fluxes in that influx was affected to a greater extent than efflux (Figure 4). Chloride influx was significantly inhibited in both groups of fish for the first 9 h of the exposure to pH 4.0 but $Cl^-$ efflux was not significantly different to control values throughout. Both naive and acid pre-exposed fish suffered significantly increased net losses of $Cl^-$ at 0 and 3 h, but not thereafter. Net ammonia excretion rates averaged approximately 630 µmol $kg^{-1}$ $h^{-1}$ and were not significantly affected in either of the groups at any time over the 24 h acute acid challenge (data not shown).

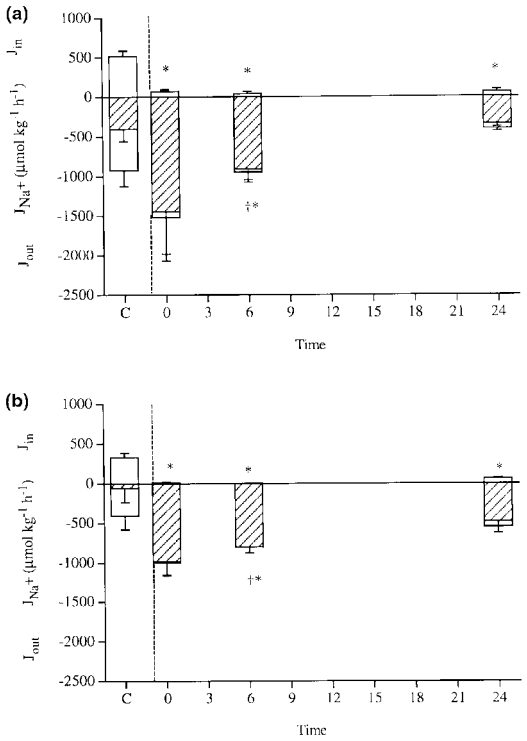


*Figure 2.* Unidirectional $Na^+$ fluxes during exposure to pH 4.2 for 24 h in juvenile rainbow trout, *O. mykiss*, that had previously been exposed to (a) ambient pH 6.2 or (b) sublethal low pH 5.2 for 90 d, and fed a ration of 1.0% body weight $d^{-1}$. Values are means ± SEM (N = 6). Positive values represent movement into the fish ($J_{in}$), negative values represent movement out of the fish ($J_{out}$) and hatched bars represent the net movement of the ion ($J_{net}$) (*significantly different from the control (C) for $J_{in}$ and $J_{out}$, †significantly different from the control for $J_{net}$ ($p < 0.05$)).

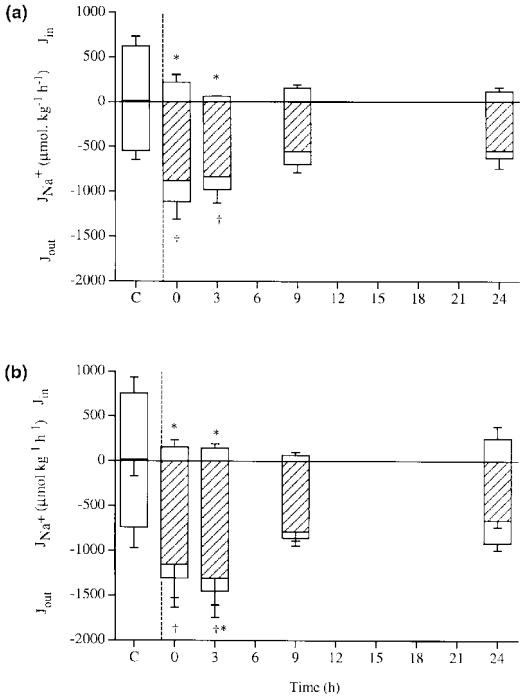


*Figure 3.* Unidirectional $Na^+$ fluxes during exposure to pH 4.2 for 24 h in juvenile rainbow trout, *O. mykiss*, that had previously been exposed to (a) ambient pH 6.2 or (b) sublethal low pH 5.2 for 90 d, and fed a ration of 0.25% body weight $d^{-1}$. Values are means ± SEM (N = 3–10) (see Figure 2 caption for other details).

Under control (pre-challenge) conditions, the concentrations of plasma sodium ($[Na^+]_P$) and chloride ($[Cl^-]_p$) were significantly lower, and the haematocrit and concentration of plasma protein significantly higher, in the acid pre-exposed fish than in the naive fish (Table 1). The net losses of $Na^+$ and $Cl^-$ during the acute acid challenge resulted in a decrease in plasma ion concentrations in both groups of fish despite a fluid shift away from the plasma, as indicated by the increase in blood haematocrit.

## Discussion

Exposure to chronic, sublethal, environmental acidification has been reported to have deleterious effects on behaviour (Jones et al. 1987), growth (Menendez 1976, Johnston et al. 1984, Lacroix et al. 1985) and reproductive success (Menendez 1976) in freshwater salmonids. However, the most commonly reported effect of low pH is ionoregulatory disturbance, as seen in reduced plasma or tissue ion concentrations (Lacroix et al. 1985, Sadler & Lynam 1986, Lacroix & Townsend 1987). Few studies have examined the physiological mechanisms of this disturbance but Audet et al. (1988) found that the initial ionoregulatory effects of sublethal acid exposure (pH 4.8) in rainbow trout were similar to those of acute acid stress, i.e. an inhibition of branchial $Na^+$ and $Cl^-$ influx and a net loss of ions. Although net ion fluxes recovered after approximately one month of exposure, both influx and efflux continued to be significantly inhibited compared to control (pre-exposure) values and plasma ion concentrations remained suppressed (Audet et al. 1988). Reduced plasma or tissue ion concentrations have therefore been seen as a characteristic feature of chronic acid exposure in freshwater salmonids.

The absence of such symptoms in recent studies on fed fish in our laboratory (Dockray et al. 1996, 1998, D'Cruz et al. 1998) led to the conclusion that dietary salts may be sufficient to replace any branchial ion losses (D'Cruz & Wood 1998). This raised the possibility that the fish had acclimated to low pH in that they would be better able to resist further, more severe acidification than fish that had not previously been exposed to acidity. However, the results of experiment 1 did not support this hypothesis: fish that had been previously exposed to ambient pH (6.2) or sublethal low pH (5.2) in softwater, but had shown no chronic ionoregulatory disturbance, showed a similar and typical response to exposure to pH 4.0 for 24 h. Both groups of fish suffered a severe inhibition of $Na^+$ influx throughout the challenge, and a transitory increase in $Na^+$ net flux over the first 6 h which had recovered by 24 h. These results are similar to those of both Audet & Wood (1988) and Wood et al. (1988b) who found that prior exposure to sublethal pH did not ameliorate the ionoregulatory disturbance of an acute acid challenge compared to naive fish in adult rainbow trout or brook charr, *Salvelinus fontinalis*, respectively.

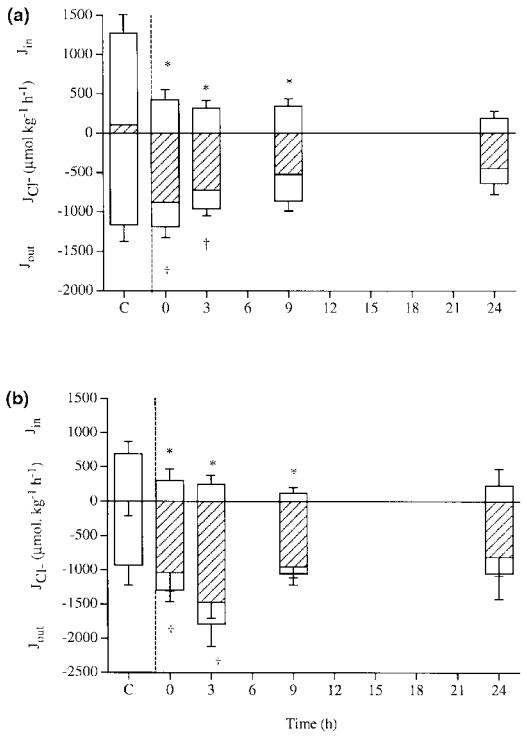


*Figure 4*. Unidirectional $Cl^-$ fluxes during exposure to pH 4.2 for 24 h in juvenile rainbow trout, *O. mykiss*, that had previously been exposed to (a) ambient pH 6.2 or (b) sublethal low pH 5.2 for 90 d, and fed a ration of 0.25% body weight $d^{-1}$. Values are means ± SEM (N = 6) (see Figure 2 captions for other details).

Experiment 2 confirmed the results of Sadler & Lynam (1986), D'Cruz et al. (1998) and D'Cruz & Wood (1988) that the ionoregulatory impact of chronic, sublethal acidification is affected by diet. Prior to the acute acid challenge of the present study, plasma $[Na^+]$ and $[Cl^-]$ were significantly lower, and plasma protein concentration and heamatocrit significantly higher, in acid exposed fish than in naive fish that had been fed a ration of 0.25% body weight $d^{-1}$, whereas no such difference had been seen in the fish fed 1% $d^{-1}$ in experiment 1 (Dockray et al. 1998). However, despite this difference, the results of the acute acid challenge confirmed that exposure to sublethal low pH conferred no acclimatory advantage. Both acid pre-exposed and naive fish showed inhibition of $Na^+$ and $Cl^-$ influxes and large net losses of ions over the first 9 h of the acute acid challenge, resulting in significantly lower

*Table 1*. The effect of acute acid challenge (pH 4.2, 24 h) following chronic (90 d) exposure to ambient pH 6.2 or sublethal low pH 5.2, on blood haematocrit and plasma $Na^+$, $Cl^-$ and protein concentrations in juvenile rainbow trout, *O. mykiss*. Values are mean ± SEM, N = 3–10. The fish were fed a ration of 0.25% body weight $d^{-1}$.

| | Pre-challenge | | Post-challenge | |
|---|---|---|---|---|
| | 6.2 | 5.2 | 6.2 | 5.2 |
| Haematocrit (%) | 31.6 ± 1.1 | 36.2 ± 1.2* | 44.6 ± 3.2† | 45.2 ± 8.3 |
| $[Na^+]$ (mM) | 130.5 ± 1.5 | 108.3 ± 4.0* | 97.4 ± 5.9† | 94.5 ± 7.4† |
| $[Cl^-]$ (mM) | 125.6 ± 1.4 | 106.8 ± 3.9* | 104.4 ± 2.5† | 86.2 ± 13.7† |
| [Protein] g l$^{-1}$ | 24 ± 2 | 30 ± 2* | 29 ± 4.0 | 31 ± 4.0 |

*indicates a significant effect of chronic pH.

†indicates a significant effect of acute pH ($p < 0.05$).

plasma [$Na^+$] and [$Cl^-$]. Indeed the acid pre-exposed fish suffered a much greater mortality rate (5/8) over the acute acid challenge than did the naive fish (2/9), which indicates that rather than having any acclimatory advantage, fish exposed to sublethal low pH are sensitized to further acid challenge. Audet & Wood (1988b) also found that acid pre-exposure rendered rainbow trout more rather than less susceptible to more severe acidification and that this was due to a greater stimulation of ion efflux in acid pre-exposed fish. Although the differences were not statistically significant, $Na^+$ and $Cl^-$ effluxes showed a greater and more persisitent increase relative to controls, in acid pre-exposed fish compared to naive fish in experiment 2 of the present study.

McDonald & Wood (1993) suggested that acclimation to a waterborne toxicant requires a phase of compensation and repair following initial cellular damage of the gills. Environmental acidification has been shown to inhibit gill protein turnover (Wilson et al. 1996, Reid et al. 1997) which may constitute a damage phase, but no subsequent stimulation of protein turnover corresponding to damage repair occurred, as was observed following exposure to waterborne zinc (Hogstrand et al. 1995) and aluminium (Wilson et al. 1996). Moreover, the changes in branchial morphology of salmonids during exposure to sublethal acidification have generally been very mild (Mueller et al. 1991). For example, Laurent & Perry (1990) exposed rainbow trout to pH 5.5 for one week and observed an increase in the number of branchial mucus cells, and in the development, but not the number, of branchial chloride cells. Similarly, Audet & Wood (1993) recorded some changes in mucus cell but not chloride cell numbers in the gills of rainbow trout exposed to pH 4.8 for 81 days. In comparison, the changes in branchial morphology during metal exposure occur on a much grosser scale, e.g. (reviewed by Mallat 1985). Therefore, it is likely that the lack of acclimatory ability of salmonids exposed to sublethal acidification may be due to the minimal impact of such exposure on the structure of the gill epithelium.

One other aspect of the results of the present experiments is worthy of some discussion: namely that the acute acid challenges had no effect on ammonia excretion despite pronounced effects on $Na^+$ fluxes, particularly $Na^+$ influx. The existence of a link between branchial $Na^+$ influx and ammonia efflux, via a directly-coupled $Na^+/NH_4^+$ transporter, has been debated at great length (for reviews see Heisler 1990, Wood 1993, Wilkie 1997) since first proposed by Krogh (1939). The details of this debate are beyond the scope of the present study, but the prevailing view appears to be that the majority of branchial ammonia excretion in teleosts occurs via diffusion of non-ionic $NH_3$ and that $Na^+/NH_4^+$ exchange occurs only under specific conditions such as elevated internal ammonia concentrations (Wilson et al. 1994, Salama et al. 1999). The absence of any link between $Na^+$ influx and ammonia excretion in the present study adds further evidence to support this hypothesis.

In conclusion, the results of the present study suggest that freshwater salmonids are unable to acclimate to sublethal environmental acidification. Acid-exposed fish are no better equipped to survive acute acid episodes than are fish with no previous experience of acid exposure; both groups showing the typical ionoregulatory disturbances associated with such episodes. This similarity in response is not affected by the dietary history of the fish.

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