J. exp. Biol. **133**, 87–109 (1987)
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SULPHATE ENTRY INTO SOFT-WATER FISH (*SALMO GAIRDNERI*, *CATOSTOMUS COMMERSONI*) DURING LOW AMBIENT pH EXPOSURE

BY HELVE HÖBE*

Harkness Laboratory of Fisheries Research, Ontario Ministry of Natural Resources, Box 110, Whitney, Ontario, Canada K0J 2M0 and *Department of Biology, University of Calgary, Calgary, Alberta, Canada T2N 1N4*

Accepted 18 May 1987

SUMMARY

The physiological responses of white sucker (*Catostomus commersoni* Lacépède) and rainbow trout (*Salmo gairdneri* Richardson), both reared in natural soft water, to a reduction in ambient pH were compared by simultaneous analyses of ion levels in various body compartments (plasma, muscle, whole fish) and net ion transfer rates. Following 24 h of exposure to acidified (H_2SO_4) natural soft-water, both species displayed a net influx of protons (or loss of base) and net losses of body Na^+, Cl^-, Ca^{2+}, Mg^{2+}, K^+ and phosphate. The magnitude of ion loss from plasma was twice as large in the trout as in the sucker. Shifts of fluid from the extracellular to the intracellular fluid occurred in both species. Losses of ions from epaxial white muscle were small relative to intracellular ion losses from the rest of the body in both species.

The most notable finding was the entry of sulphate into the body fluids of both species, accumulating primarily in plasma and in the intracellular compartment of sucker and trout, respectively. The possible mechanism(s) and implications of sulphate influx into fish are discussed.

INTRODUCTION

Over the past decade, considerable attention has been placed on defining the physiological mechanisms involved in the responses of fish to acid environments (see reviews by Muniz & Leivestad, 1980; Fromm, 1980; Haines, 1981; Spry, Wood & Hodson, 1981; Brown, 1982; Leivestad, 1982; Wood & McDonald, 1982; McDonald, 1983*a*; Howells, Brown & Sadler, 1983; Howells, 1984). The fish gill has been identified as the primary target of high ambient H^+ levels. Disturbances in ionoregulation, acid–base regulation and oxygen transfer have all been implicated. The specific mode of action of high ambient H^+ levels varies, however, with the severity of acid stress ($pH < 4{\cdot}0$ *versus* $pH > 4{\cdot}0$), acid type (HCl^- *versus* H_2SO_4), water hardness and carbon dioxide levels. A recent study by Höbe, Wood &

* Present address: Department of Biology, Lakehead University, Thunder Bay, Ontario, Canada P7B 5E1.

Key words: plasma, muscle and whole-body ion status, net ion flux rates, sulphate, freshwater teleosts, natural soft water, low ambient pH exposure.

McMahon (1984*b*) also found that fish reared and examined in natural soft water were much more sensitive to external acid stress than those reared in hard water and examined in artificial soft water (Hōbe, 1985; Hōbe & McMahon, 1982). It is not yet clear, however, to what extent these findings are species-specific and/or reflect differences between acidified artificial and natural soft-water media.

Thus, one of the aims of the present study was to compare the responses of white sucker (*Catostomus commersoni*) and rainbow trout (*Salmo gairdneri*), both reared in natural soft water, to acute acid (H_2SO_4) exposure. White sucker was of interest since it is native to soft-water lakes stressed by acid input (see Beamish, 1972; Hōbe *et al.* 1983, 1984*b*; Fraser & Harvey, 1984). Rainbow trout was chosen because of the extensive literature now available on artificial soft-water acid exposure for this species (see reviews by Wood & McDonald, 1982; McDonald, 1983*a*; Wood, 1987) and one report on natural soft-water acid exposure (Hōbe, Laurent & McMahon, 1984*a*). In contrast with earlier studies, this study incorporated simultaneous sampling of various body compartments (plasma, muscle, whole fish) for ionic content as well as an analysis of a full spectrum of cation and anion net transfer rates.

Acidification of water to pH 4·0 with H_2SO_4 is associated not only with increased H^+ levels but also with elevated inorganic sulphate levels. While the latter changes undoubtedly occurred in earlier studies of external acid stress (e.g. Hōbe *et al.* 1983, 1984*a*,*b*; McDonald, 1983*b*; McDonald, Walker & Wilkes, 1983; Fraser & Harvey, 1984; Lacroix, 1985; McKeon *et al.* 1985), their implications have been completely neglected. Thus, another aim of the present study was to examine the possibility of sulphate accumulating in acid-exposed fish.

MATERIALS AND METHODS

Experimental animals and media

White suckers (*Catostomus commersoni*; 200–400 g) were collected from Lake Opeongo, a natural soft-water lake situated in Algonquin Park, using commercial trap nets (see Hōbe *et al.* 1984*b*). Rainbow trout (*Salmo gairdneri*; 150–250 g) were not found in Lake Opeongo and had to be obtained from a local soft-water hatchery (Skeleton Lake Hatchery, Bracebridge, Ontario; see Spry & Wood, 1985).

Prior to use in experiments, fish were held (sucker 1–2 days; trout, 1–2 weeks) in separate wooden holding tanks (1000 l) which received a continual supply of natural soft water (NSW; 12–13 °C) pumped from below the thermocline of the lake. NSW white suckers in the present study proved to be somewhat less sensitive to handling than previously reported (Hōbe *et al.* 1984*b*), probably because of the lower temperatures used (12–13 °C *versus*, 19–20 °C). Fish were not fed during either holding or experimentation.

During recovery from handling (12 h) and experimentation, fish were kept individually in 2- to 3-l Lucite boxes which were contained within larger, black, rectangular boxes; an air-lift pump provided water flow through the Lucite box, while air lines at the circumference of the larger box mixed the two systems and provided adequate aeration (P_{O_2} = 125 mmHg; 1 mmHg = 133·3 Pa). This closed-

circuit experimental arrangement differed from that shown in McDonald (1983*b*) and described earlier by Hōbe *et al.* (1984*b*) in that larger rectangular boxes (30 l capacity) and therefore total operational water volumes (9–13 l) were used to lengthen sampling periods and to minimize the ambient pH changes resulting from ammonia excretion by the fish. Water ammonia levels did not exceed 200 μequiv l^{-1} during a closed-circuit experiment (see Cameron & Heisler, 1983).

Natural soft water (NSW, in mequiv l^{-1}; Na^+, 0·05–0·06; Cl^-, 0·02–0·03; Ca^{2+}, 0·16–0·17; K^+, 0·01–0·02; Mg^{2+}, 0·09–0·10; SO_4^{2-}, 0·15–0·20) was used in all experiments. No acclimation of fish was required. Acidified NSW was prepared in 700 l polyethylene reservoirs by titration to pH 4·0 with H_2SO_4; vigorous aeration was sufficient to remove carbon dioxide released during acidification. With the exception of flux periods during which the system was maintained on closed-circuit, each experimental flux box received a continual flow of lake water (0·2–0·3 l min^{-1}) at either ambient pH (6·8 for the control group) or acid pH (4·2 for the experimental group). This water was maintained at constant temperature (12–13 °C) by passing it through glass coils in a polyethylene tank (400 l) equipped with a cooling unit (Min-o-cool; 746 W). During closed-circuit flux periods, temperature control was provided by water jacketing the flux boxes with natural well water (9–10 °C). Acid conditions (pH $\approx$ 4·0–4·3) were maintained in the flux boxes by frequent monitoring of ambient pH (at 0·5- to 2·0-h intervals) and subsequent addition of measured amounts (10–30 ml) of 0·01 mol l^{-1} H_2SO_4 on the basis of the ratio of fish body mass to operational water volume; the level of SO_4^{2-} in the flux boxes was therefore not constant during acid exposure but ranged from initial values of 0·4 to 0·8 mequiv l^{-1} over a 24-h flux period.

Experimental protocol

Two experimental series were performed. Series I was conducted to examine whole-body net ion and acidic equivalent flux rates together with the ionic status of various body compartments (plasma, epaxial white muscle, whole fish) in NSW white suckers (uncannulated) held in near-neutral NSW (series Ia; mean mass 380 ± 13 g; $N = 6$) and during acute exposure to acidified NSW at 12–13 °C (series Ib; mean mass 243 ± 32 g; $N = 5$). Series II was conducted to measure similar variables in soft-water hatchery-bred rainbow trout (uncannulated) held in near-neutral NSW (series IIa; mean mass 160 ± 9 g; $N = 6$) and during acute exposure to acidified NSW (series IIb; mean mass 198 ± 8 g; $N = 5$). Following recovery (from handling), each fish was either held in control NSW for 24 h and then exposed to acid conditions for 24 h (acid series Ib, IIb) or maintained in control NSW for an additional 24 h (control series Ia, IIa).

The sampling regime in each medium was as follows. The control series (Ia, IIa) were held in closed-circuit flux boxes (water volume 12–13 l) for 48 h to assess the effect of confinement. For net flux rate determinations, water samples (65 ml) were collected at 12 h intervals and analysed for ion concentration (Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}), titratable proton content (TA) and ammonia (NH_3+NH_4^+) levels. Water sulphate (SO_4^{2-}) levels were also measured but only in the 0, 24 and 48 h samples. At

the end of the 48-h period, fish were killed rapidly and weighed. Terminal blood samples were removed by caudal puncture (Höbe *et al.* 1984*b*). Epaxial white muscle samples (1–3 g, in duplicate) were excised just posterior to the dorsal fin in all cases because of the known variation in muscle ion content between different regions of the fish (Love, 1970). Blood, muscle and whole fish were processed later (as described below) for cation (Na^+, K^+, Ca^{2+}, Mg^{2+}) and anion (Cl^-, SO_4^{2-}, phosphate) analyses. No measurements of plasma [K^+] or [phosphate] and muscle [SO_4^{2-}] or [phosphate] were made. In the acid series (Ib, IIb) after 24 h in control NSW (no measurements) each flux box (water volume 9–10 l) was flushed (70–100 l) for 30 min to switch completely from control to acidified NSW. Water samples were collected at 0, 6, 12, and 24 h and measured for [Na^+], [Cl^-] and [K^+]. Other procedures for collecting water samples for ion analyses and terminal sampling (at 24 h of acid exposure) were identical to those described above for series Ia and IIa.

Analytical techniques and calculations

Epaxial white muscle samples (1–3 g; in duplicate) were prepared for tissue water content and ion analyses using methodology similar to that used by McDonald & Wood (1981). Total muscle water (TMW; ml kg^{-1}) was determined in muscle following oven-drying (48–72 h; 90°C). Ground muscle samples (200 mg, in duplicate) were diluted with 1·0 mol l^{-1} nitric acid (10 ml) and left for 36–48 h in a 37°C oven to ensure solubility. Following centrifugation, each supernatant was analysed for ion levels, as described below, and total muscle tissue concentrations (mequiv kg^{-1} tissue wet mass) calculated using the equation:

$$[\chi]_m = \frac{[\text{supernatant}] \times (V_a + M_d)}{M_w}, \quad (1)$$

where M_d refers to the mass of the dry tissue sample (g), M_w to the corresponding wet mass (g) and V_a to the volume of acid added (ml). Intracellular ion levels (mequiv kg^{-1} cell water) were subsequently calculated using the equation:

$$\text{muscle } [\chi]_{ICF} = \frac{[\chi]_m - \text{muscle } [\chi]_{ECF}}{TMW - \text{muscle ECFV}} \times 1000, \quad (2)$$

where the extracellular ion content ($[\chi]_{ECF}$) of muscle was taken as the product of plasma ion concentration $[\chi]_p$ and muscle extracellular fluid volume (ECFV; ml kg^{-1}). The latter was estimated as the chloride–potassium space ($Cl–K_{sp}$) using the equation given in Conway (1957). Houston & Mearow (1979) found that $Cl–K_{sp}$ gave ECFV values which were closely comparable to those measured directly with a radiolabelled ECF marker ([^{14}C]polyethylene glycol).

Intact fish were prepared for the analysis of total body water and ion content using procedures similar to those outlined above for muscle but with the following modifications. Whole fish were oven-dried (1–2 months; 90°C) and total body water (TBW; ml kg^{-1}) was calculated. The dried fish were macerated to a fine powder with a blender. Four subsamples were dissolved in either concentrated (16 mol l^{-1}; 5 ml for cations) or dilute (0·2 mol l^{-1}; 10 ml; for anions) nitric acid and left for at least

1 week in a 37°C oven to ensure solubility; following filtration, each sample was analysed for ion levels, as described below. Total whole-body ion concentrations (mequiv kg^{-1} body water; $[\chi]_{wb}$) were calculated using the equation given above for $[\chi]_m$. Values were subsequently partitioned into intracellular (whole-body $[\chi]_{ICF}$) and extracellular concentrations (whole-body $[\chi]_{ECF}$) using the equation given above for muscle $[\chi]_{ICF}$ and respective body compartmental volumes (ICFV, ECFV; estimated using $Cl\text{–}K_{sp}$; Conway, 1957).

The concentrations of cations (mequiv l^{-1}) in samples of plasma, epaxial white muscle supernatant, whole fish filtrate and water were measured, after appropriate dilution, using atomic absorption spectrophotometry (Perkin Elmer 5000) and an air/acetylene flame. Ionization interferences on Na^+ and K^+ analyses were eliminated by swamping samples and standards with 1 g l^{-1} caesium chloride. Chemical interferences were eliminated from Ca^{2+} and Mg^{2+} measurements by swamping with 1 g l^{-1} lanthanum chloride.

The concentrations of anions (mequiv l^{-1}) in various samples were determined by different techniques, depending upon availability of instrumentation. Cl^- levels were measured either directly (undiluted plasma; Radiometer Model CMT 10) or indirectly (water and muscle; Buchler Model 4-2500); for the latter, a modified acid reagent (spiked with 2 mmol l^{-1} NaCl) and calibration standards (range 0·01–2·0 mequiv l^{-1} Cl^-) were used (Hõbe *et al.* 1984*b*).

Total sulphate concentration was determined indirectly using the residual barium method of Dunk, Mostyn & Hoare (1969) and modifications by Logan, Morris & Rankin (1980). The method was further modified as follows. To 2 or 3 ml of sample [undiluted water; diluted (30-fold) plasma and diluted (80-fold) whole-body preparations spiked with 500 μequiv l^{-1} SO_4^{2-}], 1 ml of 800 μmol l^{-1} $BaCl_2$ was added and the mixture was allowed to stand for 48–72 h at room temperature. Following centrifugation (3000–4000 ***g***), residual barium in the supernatant was measured by atomic absorption spectrophotometry (Perkin Elmer 5000) using a nitrous oxide/acetylene flame. Calibration was by magnesium or ammonium sulphate standards and found to be linear within the range 100–1000 μequiv l^{-1} SO_4^{2-}. Samples and standards were swamped with 1 g l^{-1} caesium chloride to prevent ionic interferences. Some standards were also supplemented with dilute nitric acid (<0·02 mol l^{-1}) to match the amount present in whole-body preparations. Preliminary analyses revealed that samples containing high levels of nitric acid (>0·05 mol l^{-1}) gave spurious data.

An ion chromatograph (Dionex Model 10), equipped with concentrator (Model 30825), anion separator (Model 30827) and cation suppressor columns (Model 30829), was also used for the analysis of anions (Cl^-, PO_4^{3-}, SO_4^{2-}) in samples of water and whole-body preparations. Eluent (0·0043 mol l^{-1} $NaHCO_3$ and 0·0034 mol l^{-1} Na_2CO_3) flowed through the columns at a rate of 80 ml h^{-1}. Columns were regenerated after 6–8 samples with 0·5 mol l^{-1} H_2SO_4 and distilled water. Filtered samples (2 ml), diluted with eluent (concentration as above), were measured. Calibration was with a standard containing 0·0282 mequiv l^{-1} Cl^-, 0·0316 mequiv l^{-1} PO_4^{3-} and 0·1041 mequiv^{-1} SO_4^{2-}. This technique provided

results which were similar to those obtained with other methods (described above). Whole-body and intracellular phosphate (phosphate) levels (see Figs 6, 7) were converted from mmol l^{-1} to mequiv l^{-1} by assuming that 78 % of body phosphate was in bone as PO_4^{3-} in combination with calcium (ratio 1·75 mmol Ca^{2+} per mmol PO_4^{3-}; Weiss & Watabe, 1978) with the remainder in the form of PO_4^{2-}.

Water ammonia ($NH_3 + NH_4^+$) concentration (mequiv l^{-1}) was determined by a micromodification of the salicylate hypochlorite technique of Verdoux, van Echteld & Dekkers (1978). Titratable proton content (μequiv ml^{-1}; or titratable alkalinity) of water samples (10 ml), collected at the beginning and end of a flux period, were determined by acid titration (0·01 mol l^{-1} H_2SO_4; end point pH 4·0; Höbe *et al.* 1984*b*). Water pH measurements were made with a combination pH electrode (Radiometer Model G 2402C) coupled to a digital acid–base analyser (Radiometer PHM 72).

The net flux of a given ion (J^{χ}_{net}; μequiv kg^{-1} h^{-1}) between the external medium and body fluids was calculated using the equation:

$$J^{\chi}_{net} = \frac{([\chi]_i - [\chi]_f) \times V}{(t_f - t_i) \times W}, \quad (3)$$

where i and f refer to the initial and final concentrations (μequiv ml^{-1}) of the ion (χ), V is the average operational water volume (ml) in the fish box over the flux period, with ($t_f - t_i$) expressed in hours. Net ion losses from the fish have a negative sign, net gains, a positive sign. The net flux rate of acidic (or basic) equivalents (J^{H}_{net}) was calculated by taking the difference between the apparent titratable proton flux (J^{TA}_{net}) and ammonia efflux (J^{Amm}_{net}) (Höbe *et al.* 1984*b*; Wood, Wheatly & Höbe, 1984). A positive J^{H}_{net} reflects net proton influx (or base efflux) while a negative value implies net proton efflux (or base influx). Both J^{H}_{net} and net flux rate of sulphate (J^{S}_{net}) were corrected for the addition of 0·01 mol l^{-1} H_2SO_4 during flux periods.

Statistical data analyses

Calculations were performed on individual fish for each variable but data have been expressed throughout as means (±S.E.M.) unless otherwise stated. In all series, between-group and within-group statistical comparisons were performed using unpaired and paired Student's two-tailed *t*-tests, respectively. Significant differences ($P < 0·05$) between control and acid groups are indicated in each figure by an asterisk. Within-group comparisons have only been described in the text.

RESULTS

Net ion flux rates

During the 48 h in control NSW (series Ia, IIa), patterns in J_{net} over each of the four 12-h flux periods were not significantly different for any of the ions measured (data not shown). Thus, the flux periods for each ion were averaged and grand means presented as control rates (Figs 1, 2). Note that both white sucker and trout were virtually in net whole-body ion balance in near-neutral NSW. Thus, confining fish in

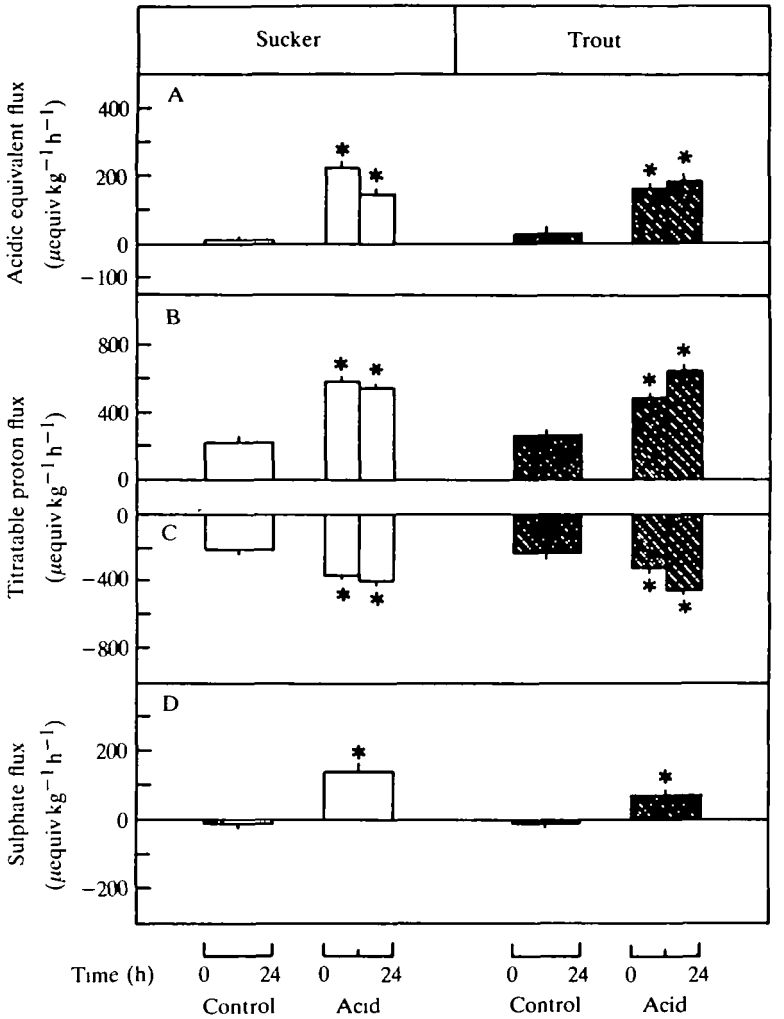

Fig. 1. A comparison of whole-body net acidic equivalent (J^{H}_{net}) (A) and sulphate flux rates (J^{S}_{net}) (D) between white sucker and rainbow trout held in near-neutral (control) and acidified (pH 4·2; 24 h) natural soft water at 12–13°C. Data for titratable proton flux (J^{TA}_{net}) (B) and ammonia efflux (J^{Amm}_{net}) (C) are also illustrated. Rates are means (±S.E.M.) for either 12- or 24-h flux periods. Asterisks denote significant differences ($P < 0{\cdot}05$) between the control ($N = 6$) and acid ($N = 5$) groups (unpaired t-test) for each fish species.

the experimental chambers for 48 h seemed to have no measurable effect on any of the variables.

During 24 h of exposure to acidified NSW (series Ib, IIb), there was a significant net influx of acidic equivalents (or loss of basic equivalents) in both species but the pattern of response differed (Fig. 1A). In the white sucker, J^{H}_{net} reached a maximum rate [216·86 ± 13·78 (5) mequiv kg^{-1} h^{-1}] over the first 12 h; this arose mainly from an elevation in J^{TA}_{net} (175%; Fig. 1B) and to a lesser extent J^{Amm}_{net} (77%; Fig. 1C).

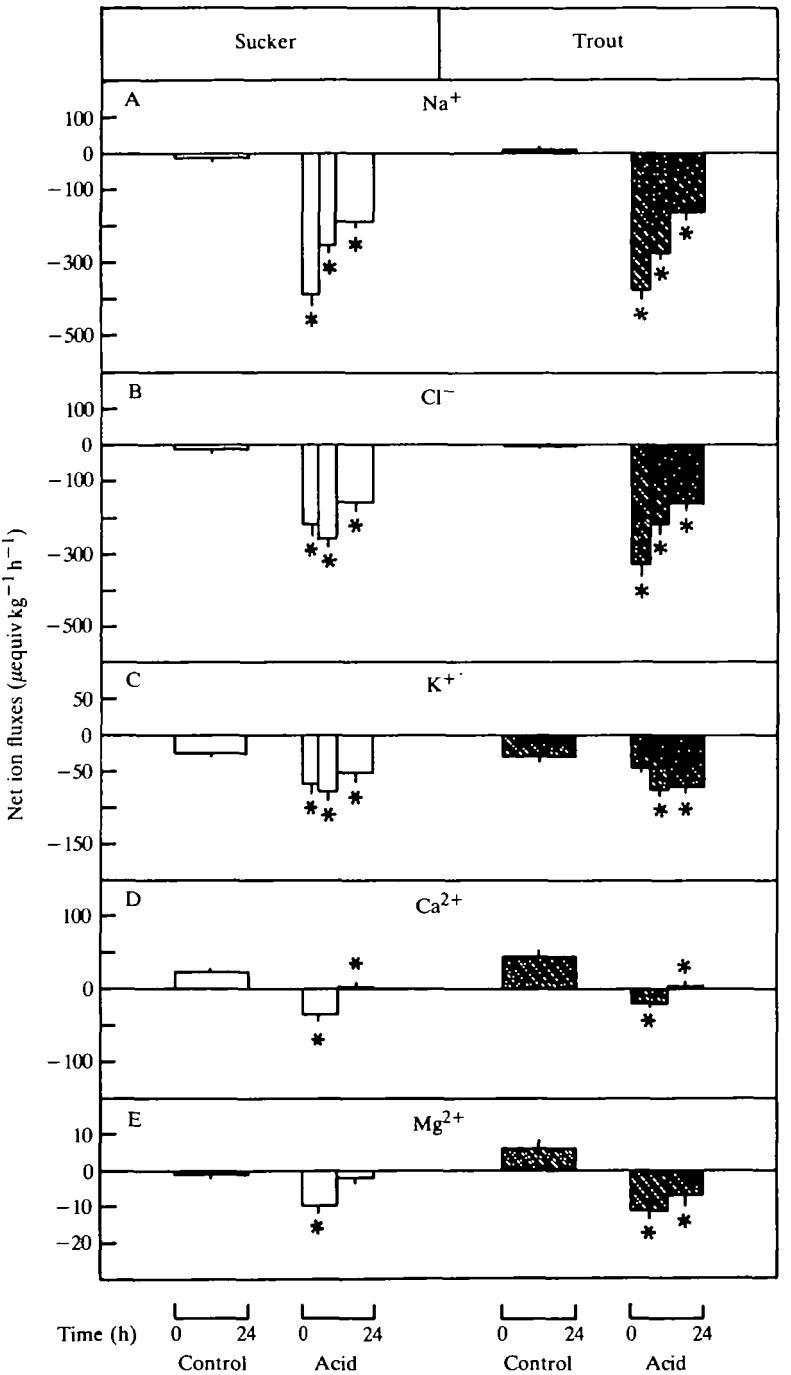

Fig. 2. A comparison of whole-body net flux rates (J_{net}) of sodium (A), chloride (B), potassium (C), calcium (D) and magnesium (E) between white sucker and rainbow trout held in near-neutral (control) and acidified (pH 4·2; 24 h) natural soft water at 12–13°C. Rates are means (±S.E.M.) for either 6-, 12- or 24-h flux periods. Asterisks denote significance as described in Fig. 1.

During the remaining 12 h, J^{H}_{net} had recovered because of a slight reduction in J^{TA}_{net} and marginal elevation in J^{Amm}_{net}. In contrast, in trout J^{H}_{net} attained a peak rate [187·57 ± 19·61 (5) μequiv $kg^{-1} h^{-1}$] over 12–24 h of acid exposure; this arose from a doubling of J^{Amm}_{net} (Fig. 1C) together with an elevation (138 %) in J^{TA}_{net} (Fig. 1B). There was also a marked net influx of sulphate over the 24-h period with J^{S}_{net} reaching 138·71 ± 25·35 (5) and 68·12 ± 14·82 (5) μequiv $kg^{-1} h^{-1}$ in acid-exposed white sucker and trout, respectively (Fig. 1D).

Net losses of other ions occurred concomitantly. J^{Na}_{net} became highly negative over the first 6 h at rates of −394·36 ± 24·63 (5) and −377·67 ± 22·00 (5) μequiv $kg^{-1} h^{-1}$ in white sucker and trout, respectively; these loss rates decreased progressively but did not return to control rates by 12–24 h (Fig. 2A). The pattern of variation in J^{Cl}_{net} differed between the two species (Fig. 2B); in trout, a peak loss rate of −339·29 ± 29·51 (5) μequiv $kg^{-1} h^{-1}$ occurred over the initial 6 h, declining thereafter, whereas 6–12 h was required for the rate of loss to reach a maximum of −260·97 ± 18·67 (5) μequiv $kg^{-1} h^{-1}$ in the white suckers. J^{K}_{net} exhibited a three-fold increase over the first 6 h in white suckers, remaining elevated thereafter, while no significant change was detected in trout until 6–12 h, when J^{K}_{net} rose to −74·11 ± 12·66 (5) μequiv $kg^{-1} h^{-1}$ (Fig. 2C). J^{Ca}_{net} reversed from positive control rates to negative rates of −30·27 ± 9·34 (5) and −20·80 ± 3·63 (5) μequiv $kg^{-1} h^{-1}$ in white sucker and trout, respectively, over the initial 12 h of acid exposure, but returned to rates not significantly different from zero over the remaining 12 h (Fig. 2D). Significant net losses of Mg^{2+} occurred in both species (Fig. 2E), mainly over the first 12 h of acid exposure.

Fluid compartmental volumes

By 24 h of exposure to acidified NSW, there was a significant redistribution of fluid from the extracellular to the intracellular compartment in white sucker (and trout), resulting in a net whole-body shift of 40–50 ml kg^{-1} (Table 1). This overall change in body fluid distribution was qualitatively reflected in epaxial white muscle which exhibited a decrease in ECFV and rise in ICFV in both species. In contrast with white sucker, no significant increase in TMW was observed in trout. Additionally, the net shift of fluid in muscle was only 20–40 ml kg^{-1}, which was not of sufficient magnitude to explain the corresponding size of the whole-body fluid shift, suggesting that other body compartments may also be involved.

Ionic status of selected body compartments

The total concentration of measured ions in plasma had also dropped significantly in both species by 24 h of acid exposure (Fig. 3; compare heights of gamblegrams), primarily as a result of alterations in $[Na^+]_p$ and $[Cl^-]_p$. In white sucker, the magnitude of decrease in $[Na^+]_p$ (14 %) was less than that found for $[Cl^-]_p$ (20 %), whereas virtually identical reduction of $[Na^+]_p$ (32 %) and $[Cl^-]_p$ (30 %) occurred in trout. In turn, $[Ca^{2+}]_p$ was reduced (5 %) and $[Mg^{2+}]_p$ raised (8 %) in white sucker,

Table 1. *Estimates of whole-body and epaxial white muscle fluid compartmental volumes (ml kg^{-1}) in white sucker and rainbow trout held in near-neutral and acidified (pH ≈ 4·2; 24 h) natural soft water at 12–13°C*

	White sucker		Rainbow trout	
	Control ($N = 6$)	Acid ($N = 5$)	Control ($N = 6$)	Acid ($N = 5$)
Whole body				
ECFV†	227·2 ± 11·8	182·5 ± 11·4*	216·4 ± 6·5	166·3 ± 9·4*
ICFV	547·6 ± 14·5	602·5 ± 13·8*	523·7 ± 8·8	565·0 ± 8·6*
TBW	775·3 ± 7·7	785·1 ± 1·9	740·0 ± 8·1	731·2 ± 4·1
Expaxial white muscle				
ECFV†	46·2 ± 2·6	21·3 ± 2·7*	53·5 ± 1·8	31·2 ± 2·2
ICFV	744·8 ± 5·3	788·2 ± 4·5*	720·6 ± 4·9	731·5 ± 4·5*
TMW	791·0 ± 4·1	809·4 ± 2·5*	774·1 ± 4·0	762·7 ± 2·9

Values are means (±S.E.M.).
Asterisks denote significance as described in the text.
† Extracellular fluid volumes (ECFV) were derived from Cl–K_{sp} (see text for details); ICFV, intracellular fluid volume; TBW, total body water.

while both variables were depressed in trout (8% and 11%, respectively). No significant change in $[SO_4^{2-}]_p$ was seen in trout, while a marked six-fold elevation occurred in white suckers. Note that the data given for $[K^+]_p$ in Fig. 3 were taken from another study on acid-exposed cannulated NSW suckers (unpublished results), in which a 2·5-fold rise in $[K^+]_p$ was shown; a similar increase in $[K^+]_p$ was assumed to occur in acid-exposed NSW trout, since this response has been reported earlier for acid-exposed hard-water trout (McDonald & Wood, 1981).

The total concentration of measured ions in epaxial white muscle was also reduced in both species by 24 h of acid exposure (Fig. 4), mainly as a consequence of changes in $[K^+]_m$ (10–12%), although the depressions in $[Na^+]_m$ (37–44%) and $[Cl^-]_m$ (27–29%) were both significant. In white sucker, significant changes in $[Mg^{2+}]_m$ (10%) and in $[Ca^{2+}]_m$ (two-fold rise) also occurred. Since modest shifts in muscle fluid compartmental volumes were found in both species (Table 1), changes in ion levels in the intracellular compartment of muscle (Fig. 5) were not necessarily representative of those in total muscle (Fig. 4), particularly for ions (Na^+, Cl^-) which have high extracellular (or plasma) concentrations. Indeed, in both species, muscle $[Cl^-]_{ICF}$ more than doubled while $[Na^+]_{ICF}$ remained unchanged. Trends in $[K^+]_{ICF}$, $[Ca^{2+}]_{ICF}$ and $[Mg^{2+}]_{ICF}$ (Fig. 5) were all similar to those seen in respective total muscle levels (Fig. 4).

The total concentration of measured ions in whole fish samples was well below control levels by 24 h of acid exposure (Fig. 6). This arose from a significant reduction in both species (white sucker *versus* trout) of $[Na^+]_{wb}$ (26% *vs* 27%), $[Cl^-]_{wb}$ (27% *vs* 33%), $[K^+]_{wb}$ (7% *vs* 8%), $[Ca^{2+}]_{wb}$ (8% *vs* 9%) and $[phosphate]_{wb}$ (5% *vs* 6%). A significant decline (7%) in $[Mg^{2+}]_{wb}$ also occurred in white suckers. Superimposed on these changes was a significant elevation in

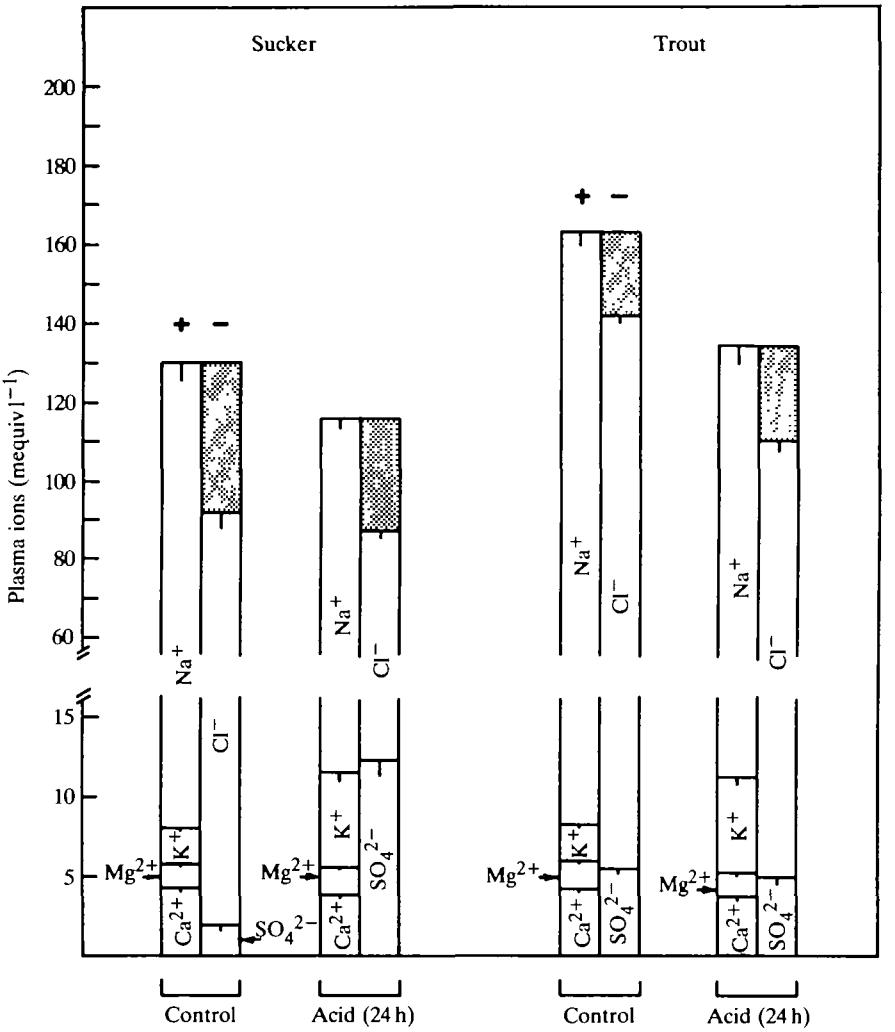

Fig. 3. A comparison of the plasma ionic status ($[\chi]_p$) in white sucker and rainbow trout held in near-neutral (control) and acidified (pH 4.2; 24 h) natural soft water at 12–13 °C. Data (means ± S.E.M.) from terminal blood samples have been plotted as gamblegrams to illustrate the relative proportion of cations and anions in plasma. Stippled areas show unmeasured ions. See Fig. 1 for N values.

$[SO_4^{2-}]_{wb}$ in both white sucker (35 %) and trout (34 %). The concentrations of ions in the intracellular compartment of whole fish also exhibited significant variation by 24 h of acid exposure (Fig. 7). Intracellular ion loss prevailed in both species as a result of a marked depression in whole-body $[K^+]_{ICF}$ (15–16 %), $[Ca^{2+}]_{ICF}$ (16–17 %) and [phosphate]$_{ICF}$ (13–14 %). In white suckers, a significant decrease in both whole-body $[Na^+]_{ICF}$ (27 %) and $[Mg^{2+}]_{ICF}$ (16 %) was also observed. Whole-body $[Cl^-]_{ICF}$ increased significantly (three-fold) to a similar extent in both species. A rise in whole-body $[SO_4^{2-}]_{ICF}$ was seen in both species but the change was only significant in trout.

DISCUSSION

Ionic status of selected body compartments in soft water fish

The measured concentrations of Na^+, Cl^-, Ca^{2+} and Mg^{2+} in plasma of both white sucker and trout (Fig. 3) correspond closely with those reported earlier for either species in NSW (Höbe *et al.* 1984*a*,*b*; Fraser & Harvey, 1984; Spry & Wood, 1985). Plasma SO_4^{2-} levels have not been previously documented for fish reared in NSW. The 2- to 3-fold lower values observed in white suckers relative to trout

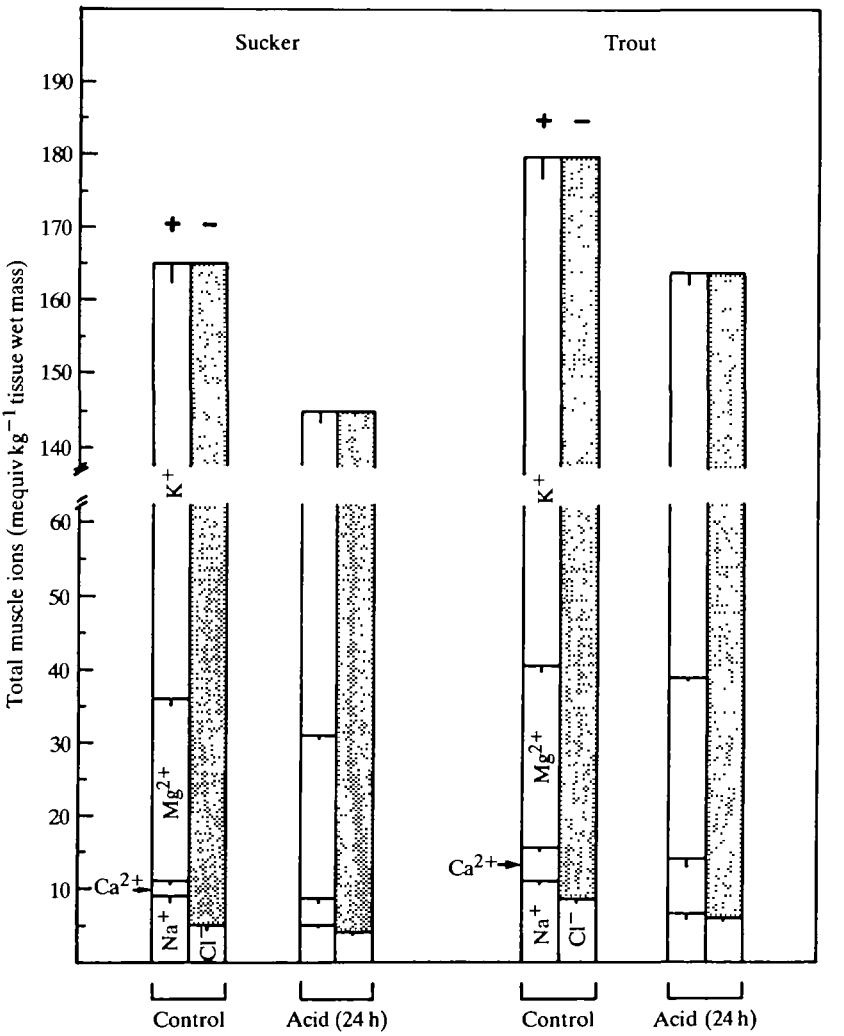

Fig. 4. A comparison of the total concentration of cations and anions in epaxial white muscle tissue ($[\chi]_m$) between white sucker and rainbow trout held in near-neutral (control) and acidified (pH 4·2; 24 h) natural soft water at 12–13°C. Data (means ± S.E.M.) from terminal samples have been plotted as gamblegrams to illustrate the relative proportion of electrolytes in muscle. Stippled areas indicate unmeasured ions. See Fig. 1 for N values.

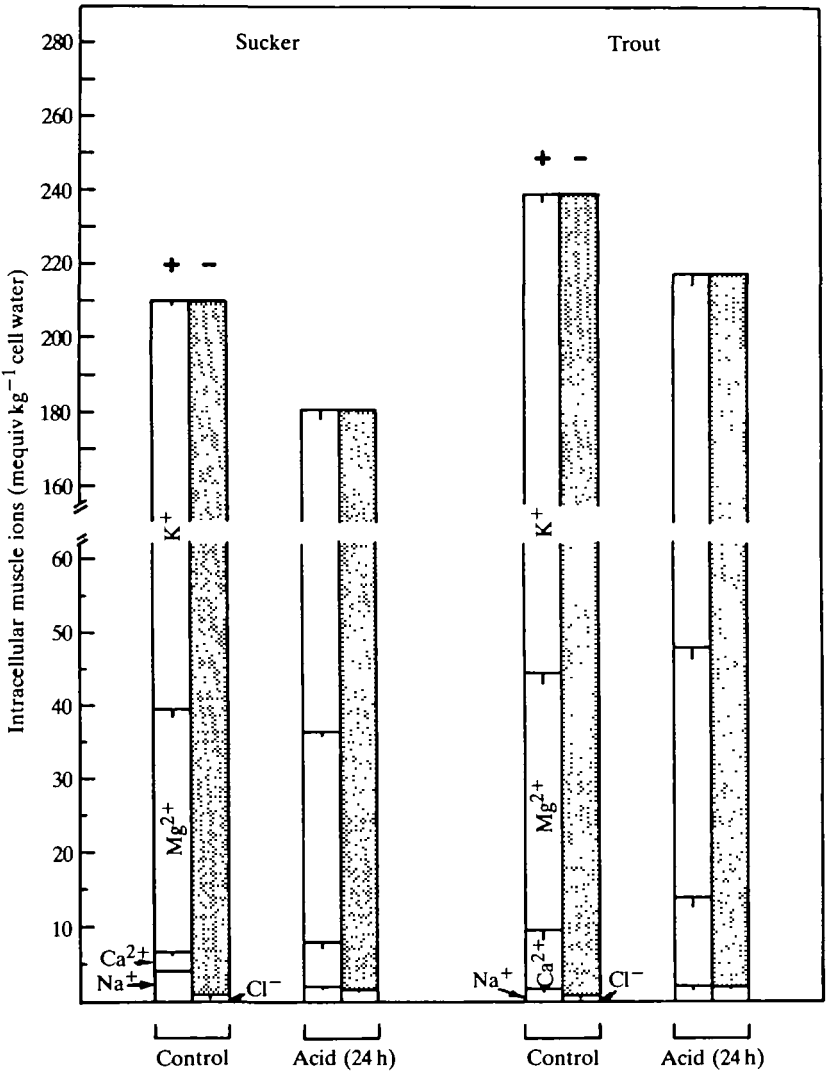

Fig. 5. A comparison of estimated intracellular concentrations of cations and anions in epaxial white muscle tissue ($[\chi]_{ICF}$) between white sucker and rainbow trout held in near-neutral (control) and acidified (pH 4·2; 24 h) natural soft water at 12–13 °C. Values were calculated from total muscle concentrations (Fig. 4) and muscle fluid compartmental volumes (Table 1) for individual fish. Mean values (±S.E.M.) have been plotted as gamblegrams. Stippled areas indicate unmeasured ions. See Fig. 1 for *N* values.

(Fig. 3) were not surprising since other plasma ion levels were consistently lower in the former. Measurements on fish reared in hard water (HW) are scanty in the literature; reported values range from 0·8 mequiv l^{-1} in the freshwater tarpon, *Megalops atlantica* (Urist, 1963) to 5·3 mequiv l^{-1} in the freshwater whitefish, *Coregonus clupeoides* (Robertson, 1954; Urist, 1963). Note also that the observed levels of monovalent ions in epaxial white muscle tissue for either white sucker or trout (Fig. 4) were at least 2- to 5-fold lower than those reported earlier for either

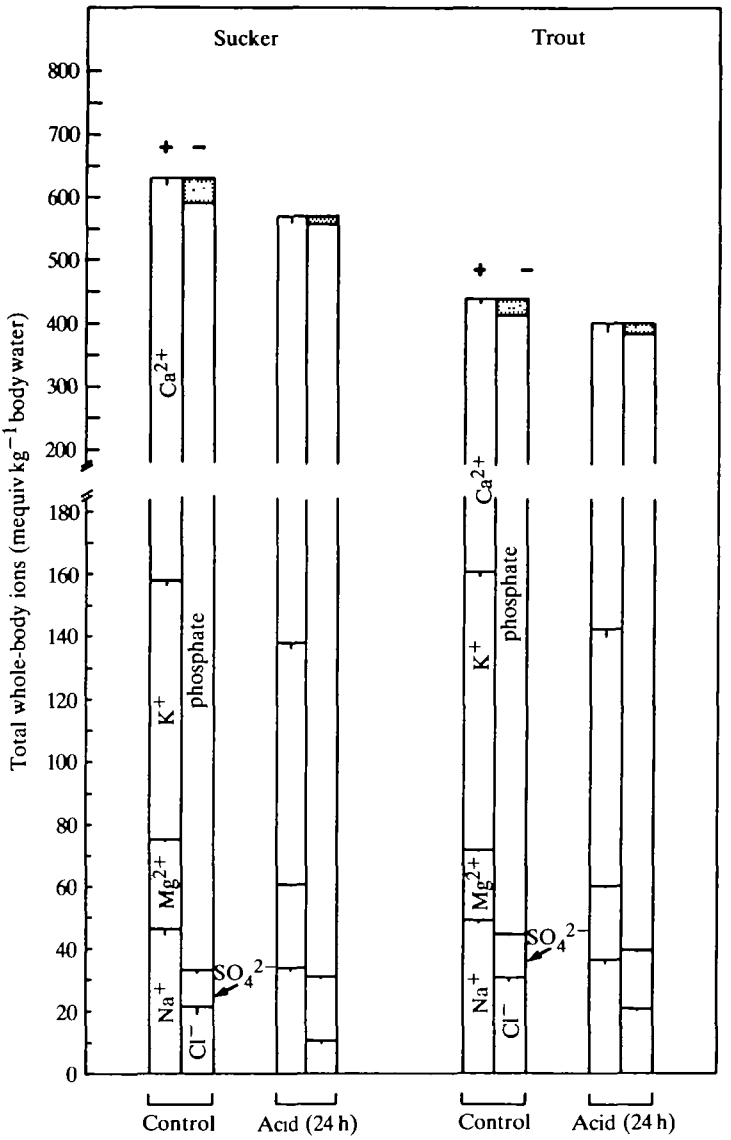

Fig. 6. A comparison of total whole-body concentrations of cations and anions ($[\chi]_{wb}$) between white sucker and rainbow trout held in near-neutral (control) and acidified (pH 4·2; 24 h) natural soft water at 12–13°C. Data (means ± S.E.M.) have been plotted as gamblegrams to illustrate the relative proportions of electrolytes in whole fish preparations. Stippled areas show unmeasured ions. See Fig. 1 for N values.

species in HW (Eddy & Bath, 1979; McDonald & Wood, 1981; Wilkes, 1984). Perhaps the most notable feature of the whole-body ion data (Fig. 6) was the low levels of Cl^- observed in both species compared with reported values for HW trout (Eddy & Bath, 1979) and just recently for NSW white sucker (Fraser & Harvey, 1984). The reason for these discrepancies, particularly with the NSW white sucker, are not known. The marked difference in whole-body Ca^{2+} and phosphate levels

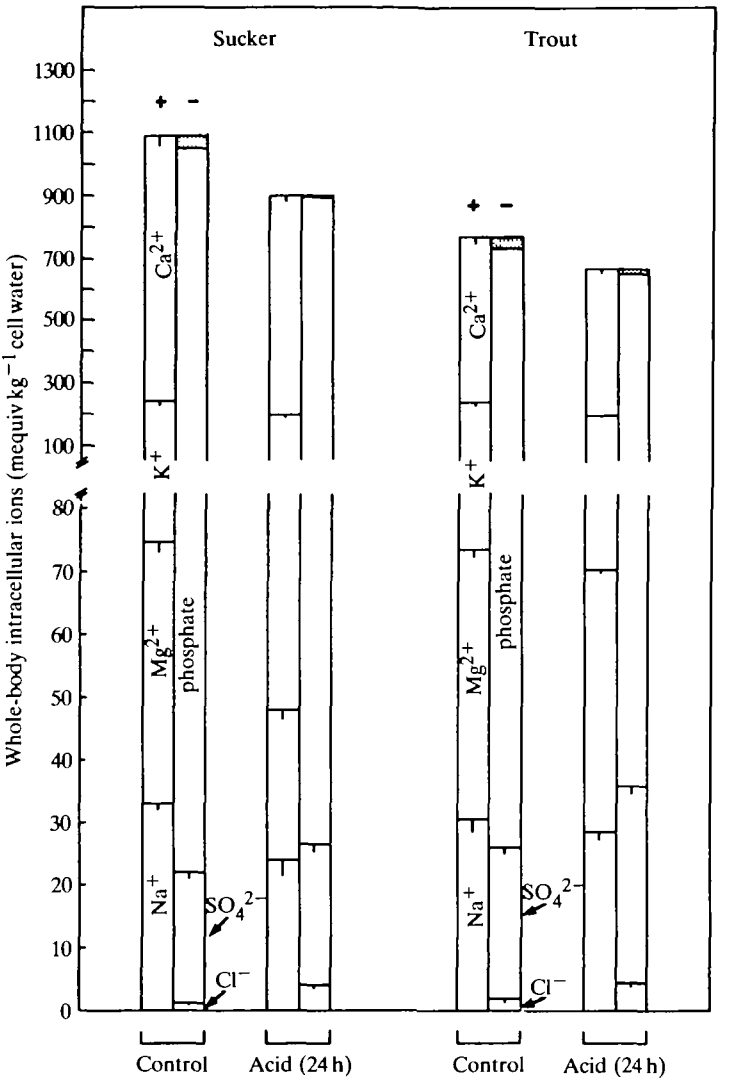

Fig. 7. A comparison of estimated intracellular concentrations of cations and anions in whole-body preparations ($[\chi]_{ICF}$) of white sucker and rainbow trout held in near-neutral (control) and acidified (pH 4·2; 24 h) natural soft water at 12–13°C. Values were calculated from total whole-body concentrations (Fig. 6) and whole-body fluid volumes (Table 1) for individual fish. Mean values (±S.E.M.) have been plotted as gamblegrams. Stippled areas show unmeasured ions. See Fig. 1 for N values.

between white sucker and trout (Fig. 6) was also interesting. This cannot be attributed to differences in body water content because these data were consistently higher rather than lower in white sucker (Table 1). The proportion of bone relative to body mass has been shown to vary between fish species (see Weiss & Watabe, 1978, 1979; Cameron, 1985) which probably explains the present findings since fish bone consists mostly of hydroxyapatite [$Ca_{10}(PO_4)_6(OH_2)$; Dacke, 1979]. Direct comparisons with other studies of body Ca^{2+} (Wood & McDonald, 1982; Fraser & Harvey,

Table 2. *A comparison of the total amount of ions and acidic equivalents lost or gained (in μequiv kg^{-1}) by white sucker (S) and rainbow trout (T) during 24 h of exposure to acidified natural soft water at 12–13°C.*

Ion	Fish	Whole-body* flux	Estimated† ECF loss or gain	Estimated ICF loss or gain
Na^+	S	−5929	−8790	+2861
	T	−6056	−13130	+7075
K^+	S	−945	+550	−1495
	T	−755	+470	−1225
Ca^{2+}	S	−811	−240	−571
	T	−1241	−270	−971
Mg^{2+}	S	−107	−50	−57
	T	−357	−110	−247
Cl^-	S	−4540	−7480	+2940
	T	−5144	−11510	+6366
SO_4^{2-}	S	+3950	+1800	+2150
	T	+1869	+410	+1459
H^+	S	+4575		
	T	+3316		

Net ion charge = $(Na^{2+} + K^+ + Ca^{2+} + Mg^{2+} + H^+) - (Cl^- + SO_4^{2-})$
sucker = −2627
trout = −3265

* Estimated by calculating the total transfer of ions over 24 h of acid exposure relative to control conditions.

† Plasma ion concentrations were assumed to represent those in the extracellular fluid volume (ECFV). Values were calculated using data in Table 1. ICF, intracellular fluid.

1984) and phosphate levels (Phillips *et al.* 1960) were not feasible because of differences in age and/or size of fish. Whole-body SO_4^{2-} levels have not been previously documented for either species (Fig. 6). The only other known measurement is for brook trout, *Salvelinus fontinalis*, by Phillips *et al.* (1960); reported values were 1·5–2·0 times higher, perhaps because of the smaller fish (5 g) used in their study.

Acid exposure

The results of this study on the effects of acute exposure to acidified NSW on net ion transfer rates in both white suckers and rainbow trout (Figs 1, 2) qualitatively confirm earlier studies on these species in acidified NSW (Höbe *et al.* 1984*a*,*b*) and artificial soft water (Höbe, 1985; McDonald, 1983*b*; McDonald *et al.* 1983). These include a net influx of H^+ (or loss of base) concomitant with net losses of body Na^+, Cl^-, K^+ and Ca^{2+}. New findings include a reduction in body Mg^{2+} and phosphate levels, together with an elevation in body SO_4^{2-} levels (Figs 1, 2, 6).

A comparison of the calculated ion budgets (total body net transfers, extracellular fluid and intracellular fluid losses or gains) for acid-exposed NSW white suckers between the present study (see Table 2) and the one by Höbe *et al.* (1984*b*) was informative. The observed net influx of SO_4^{2-} (Fig. 1D), for instance, probably

accounts for most of the net ion charge discrepancy found in the study by Hōbe *et al.* (1984*b*). Preliminary results also indicate that a net loss of phosphate occurs in acid-exposed fish at a rate which would explain the net charge deficit calculated in Table 2. Furthermore, ambient temperature appears to modify the effects of acid exposure. At low temperature (12–13°C; present study), the net gain of H^+ (or loss of base) is slightly less, while changes in plasma ion levels (particularly Na^+ and Cl^-) are more severe but, because some ion redistribution occurs from the extracellular to the intracellular fluids, total net body ion losses are up to one half those at high temperatures (19–20°C; Hōbe *et al.* 1984*b*). The pattern of net ion loss also differs such that at low temperature net Na^+ loss exceeds net Cl^- loss while the reverse trend is seen at a higher temperature.

The observed changes in the levels of Na^+ and Cl^- in epaxial white muscle cells were rather small (Fig. 4), indicating that other sites known to have high concentrations of these ions (i.e. liver, heart, brain; Murphy & Houston, 1977) probably accounted for the corresponding changes seen in whole-body intracellular levels of Na^+ and Cl^- (Fig. 7). Similar findings have been reported for acid-exposed rainbow trout in hard water (McDonald & Wood, 1981). The majority of the observed whole-body NaCl losses (Fig. 6), however, arose from the extracellular (i.e. plasma), but not the intracellular, compartment (see Hōbe *et al.* 1984*b*). In contrast, other body ion losses originated largely from the intracellular compartment. Epaxial white muscle cells were the main source of both K^+ and Mg^{2+} losses (Fig. 4). Shifts of these ions from muscle to plasma would explain the elevations in plasma K^+ and Mg^{2+} levels previously reported for acid-exposed white suckers in NSW (Hōbe *et al.* 1984*b*). The source of Ca^{2+} and phosphate losses was probably the intracellular compartment of bone (Hōbe *et al.* 1984*b*).

The marked haemoconcentration found earlier in white suckers exposed to acidified NSW (Hōbe *et al.* 1984*b*) can now be attributed to a redistribution of body fluid from the extracellular fluid to the intracellular fluid (see Table 1) rather than to water loss from the body, as reported for hard-water acid-exposed rainbow trout by Milligan & Wood (1982). This shift of fluid volume may have been a consequence of the loss of plasma ions (Fig. 3) across the gills, which would generate osmotic and ionic gradients favouring the entry of water into the intracellular fluid space and electrolyte flux in the opposite direction (McDonald & Wood, 1981; Milligan & Wood, 1982). The magnitude of this shift, however, did not differ between species (Table 1) over 24 h of exposure to acidified NSW. In fact, it was equivalent to that reported for hard-water rainbow trout after 72 h of acid exposure (Milligan & Wood, 1982).

Some of the effects of acid exposure differed between the two species tested. First, the magnitude of plasma ion loss in white suckers was at least half that in trout, confirming earlier findings on both species in acidified artificial soft water (Hōbe & McMahon, 1982; McDonald, 1983*b*). Second, the pattern of plasma ionic disturbance differed, probably as a consequence of the constraints of electroneutrality, in face of the rise in plasma SO_4^{2-} levels in white sucker but not in trout (Fig. 3). Finally, the decrease in plasma strong ion difference (SID; sum of cations minus

sum of anions; shaded areas of histogram in Fig. 3; Stewart, 1978) observed in white sucker but not in trout would correlate with the blood acidosis known to develop in white sucker (Höbe & McMahon, 1982; Höbe *et al.* 1984*a*) but not in trout (McDonald, 1983*b*) during exposure to acidified soft-water conditions.

Importance of sulphate

The present study is the first to establish that SO_4^{2-} penetrates into freshwater teleost fish during acute exposure to low ambient pH, as indicated by both the highly positive J_{net}^{S} (Fig. 1D) and the significant elevation in whole-body SO_4^{2-} levels (Fig. 6). This permeation was not a transient response, but was maintained with prolonged acid exposure since a nine-fold increase in plasma SO_4^{2-} levels has also been observed in white suckers at 84 h (Höbe, 1985) as opposed to a six-fold rise at 24 h (Fig. 3). The appearance of radiosulphate in blood plasma of acid-exposed white sucker and trout following its addition to the external medium (unpublished results) provides further support. Accumulation of radiosulphate in other body compartments (e.g. bone, muscle) at neutral pH has also been reported earlier in both the freshwater brook trout, *Salmo fontinalis* (Phillips *et al.* 1960), and the tropical guppy, *Lebistes reticulatus* (Rosenthal, 1961, 1963). A new interpretation of many ion transport studies in the fish literature which have assumed sulphate impermeability (e.g. Garcia-Romeu & Maetz, 1964), may therefore now be needed.

The site of SO_4^{2-} entry was not examined, but the gill epithelia may be implicated since other divalent ions (i.e. Ca^{2+}) have been shown to enter body fluids *via* this pathway (Payan, Mayer-Gostan & Pang, 1981; Mayer-Gostan *et al.* 1983; Höbe *et al.* 1984*a*; Perry & Wood, 1985). Indirect support comes from Rosenthal (1961) who found that the magnitude of [^{35}S]sulphate influx into the body of *Lebistes*, and the logarithmic relationship between isotope influx rate and external sulphate levels, were both similar to the observations on ^{45}Ca flux in this species (Rosenthal, 1957).

The mechanism involved in inward SO_4^{2-} transport is uncertain. In neutral pH media, active entry would be predicted from the prevailing concentration gradient ($= 10$; $[ion]_{blood}/[ion]_{medium}$). The absorption of SO_4^{2-} at neutral ambient pH has been reported to double in brook trout following a 10°C rise in ambient temperature (Phillips *et al.* 1960), also suggesting an active process. However, the mechanism during acid exposure may be different since the positive gill transepithelial electrical potential (McWilliams & Potts, 1978; McWilliams, 1982) and an increase in gill permeability (unpublished results), which are known to develop in acid-exposed fish, would both tend to favour passive SO_4^{2-} influx. Another possibility is that in acidified media, the unionized acid may diffuse into blood and subsequently dissociate. This was suggested from the observed changes in J_{net}^{H} and J_{net}^{S} (Fig. 1) which were parallel in both pattern (i.e. net influx) and magnitude during acid exposure. Gutknecht & Walter (1981) have recently implicated such a mechanism in their studies of molecular acid transport through artificial lipid bilayer membranes. Unfortunately, this argument is difficult to prove experimentally because the present analytical techniques do not allow differentiation between H^+ influx and base efflux.

Ion efflux in freshwater fish is known to involve two independent outflows with part of the loss occurring across the gills and part *via* the kidney (Kirschner, 1979). Although these compartments were not analysed in the present study, the maximal excretory rates of SO_4^{2-} reported for the marine teleost kidney (i.e. approximately 300 μequiv $kg^{-1} h^{-1}$; Renfro & Pritchard, 1982) would be more than adequate to remove a sulphate-load equivalent to the one occurring in acid-exposed fish. This was not the case, however, as indicated by the marked retention of SO_4^{2-} in plasma (white sucker; Fig. 3) and/or in the intracellular body compartment (white sucker and trout; Fig. 7). Net renal SO_4^{2-} secretion has not been demonstrated for vertebrates other than marine teleost fish (Berglund & Forster, 1958; Hickman, 1968*a*,*b*; Renfro & Dickman, 1980; Renfro & Pritchard, 1982). Freshwater teleosts probably regulate plasma SO_4^{2-} levels by intestinal and, to a lesser extent, renal reabsorptive processes, as in mammals (Lotspeich, 1947). Some support for this argument comes from a study on the freshwater teleost, *Gambusia affinus*, at neutral ambient pH (Ahuja, 1966); there were no renal responses when this fish was exposed to sulphate-enriched media but, instead, excess SO_4^{2-} accumulated in the intestine. However, a more recent study on the freshwater rainbow trout, at neutral ambient pH (Oikari & Rankin, 1985), reported that the kidney of this species was capable of excreting excess SO_4^{2-} following an infusion of magnesium sulphate. These conflicting data, while they cannot be explained without a complete analysis of sulphate dynamics, suggest that the role of the kidney in sulphate homeostasis in freshwater fish may be species-specific and/or dependent on the mode and/or extent of an imposed sulphate load.

Importance of the acid anion relative to protons

To what extent is mortality in acidified media a consequence of penetration of the accompanying anion rather than the hydrogen ion? If gill function was disrupted simply by the presence of a high concentration of external H^+, then at a given pH, all strong acids (e.g. H_2SO_4, HCl, HNO_3) would be expected to have the same toxicity. Experimental evidence does not support this contention, since H_2SO_4 has been shown to be less toxic than HCl in both HW and ASW, at least at ambient pH values below 4·0 (Beamish, 1972; Packer & Dunson, 1972; Graham & Wood, 1981). This difference in toxicity can only be attributed to a change in the accompanying anion. Graham & Wood's (1981) suggestion that elevated external SO_4^{2-} levels may have an ameliorative effect in acidified media by retarding H^+ entry is not very convincing in the light of the present work, because it stems from the assumption that fish gills are impermeable to SO_4^{2-} which is certainly not the case. In fact, if SO_4^{2-} ions play an ameliorative role, it is probably through some unknown internal rather than external mechanism. This argument, however, would not explain the paradoxical reversal of HCl and H_2SO_4 toxicities reported by Graham & Wood (1981) in rainbow trout in ASW at ambient pH values above 4·0. Other studies on the effects anion-supplemented media on freshwater fish are inconclusive; some (Daye & Garside, 1976; Graham & Wood, 1981; P. Laurent, personal communication) have reported that fish survive indefinitely in various sulphate salt solutions at neutral pH, while

others have not (DeRenzis & Maetz, 1973). Another complication is the study by Ahuja (1970) which showed differences between fish species in their tolerance of sulphate- and chloride-enriched media at neutral pH. Unfortunately, no physiological mechanisms were examined in any of the studies outlined above.

In the absence of definite information, one can only speculate on the consequences of sulphate entry in to acid-exposed fish. It is possible that sulphate has no direct effect on gill function but its movement may indirectly alter the rate and/or direction of other ion transfers, simply through the demands of electroneutrality.

The present data, in combination with earlier observations (Hōbe & McMahon, 1982; Hōbe *et al.* 1983, 1984*a*,*b*; Hōbe, 1985; McDonald & Wood, 1981; McDonald, 1983*a*,*b*; McDonald *et al.* 1983; Wood & McDonald, 1982; Wood, 1987), indicate that the mechanisms of action of low pH differ more markedly between species than between artificial and natural soft water. In the white sucker, failure of acid–base regulation, ionoregulation and probably oxygen uptake and transport all appear to be important contributors to mortality, but in rainbow trout failure of ionoregulation seems to be the main factor involved. The additional data on the entry of the acid anion provided in the present study, however, do not allow one to define the sequence of events that ultimately lead to fish death, since it is not possible to characterize the primary *versus* secondary effects of acid exposure.

I wish to thank the Director, Dr J. MacLean and the staff of the Harkness Laboratory of Fisheries Research for their invaluable assistance and hospitality. Dr B. R. McMahon, my Ph.D. supervisor, is thanked for his support throughout this research project. D. J. Spry, C. L. Milligan and G. Hogan are thanked for their technical assistance. Dr C. M. Wood of McMaster University is thanked for the loan of equipment and supplies. This research was supported by grants to Dr B. R. McMahon from NSERC and to HH from the Alberta Heritage Fund for Medical Research.

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