J Comp Physiol B (1984) 154:35–46

Journal of Comparative Physiology B Biochemical, Systemic, and Environmental Physiology

Mechanisms of acid-base and ionoregulation in white suckers (*Catostomus commersoni*) in natural soft water

I. Acute exposure to low ambient pH

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Accepted July 21, 1983

Summary. Simultaneous measurements of whole body proton flux and both unidirectional and net ion fluxes together with assessment of the blood acid-base, respiratory gas, electrolyte and lactate status were performed in white suckers (*Catostomus commersoni*) originating from a natural soft water lake ($[Ca^{++}]=0.18$ meq·l^{-1}) in Ontario, Canada. Fish were examined under control (pH ≈ 6.8) and acidic conditions (pH ≈ 4.3) in natural soft water at 19–20 °C. Resting blood composition was similar to that previously reported for this species in natural hard water except for a marked enhancement of both plasma pH and bicarbonate levels.

Acute acid exposure promoted a significant net influx of protons (or loss of base) concomitant with a plasma acidosis of mixed origin (metabolic + respiratory) as well as whole body Na^+, Cl^-, Ca^{++} and K^+ losses. Circulating ion levels in plasma were partially conserved by intracellular ion depletion. Radiotracer studies showed that net body losses of Na^+ and Cl^- ensued largely through stimulation of efflux components and, to a lesser extent, inhibition of inward transport. Cl^- loss eventually exceeded Na^+, suggesting transport of an unmeasured substance to maintain electroneutrality. A markedly reduced blood P_{O_2}, enhanced plasma P_{CO_2}, elevated blood lactate levels and significant hemoconcentration were also observed. Thus, disturbances in acid-base regulation, ionoregulation and respiratory function may all contribute to acid toxicity in white suckers in natural soft water.

Introduction

The physiological responses of adult fish to low environmental pH have been the topic of a number of recent papers due to a growing concern over the impact of atmospheric acidification ("acid rain") on freshwater fish populations (reviewed by Fromm 1980; Haines 1981; Spry et al. 1981; Wood and McDonald 1982; Booth et al. 1982). It is currently thought that tissue hypoxia is the primary cause of adult fish mortality at ambient pH levels below 4.0, whereas both impaired acid-base balance and ionoregulatory failure seem to predominate at higher pH levels (above 4.0). The relative importance of these latter two disturbances is dependent upon water calcium concentration in the rainbow trout, *Salmo gairdneri* (McDonald et al. 1980; McDonald 1983; McDonald et al. 1983) but apparently not in the white sucker, *Catostomus commersoni* (Hõbe et al. 1983; Hõbe and McMahon, unpublished).

Most of the available literature stems from laboratory studies of fish which have either been both reared and tested in natural hard water (high $CaCO_3$) or reared in hard water and examined after short-term acclimation to artificially-prepared dilute media (low $CaCO_3$; soft water); notable exceptions include the field studies of Leivestad et al. (1976), Lockhart and Lutz (1977), and McWilliams (1980a, 1982a). Since fish populations stressed by acid input invariably inhabit natural soft waters characterized by a low buffering capacity and low ionic strength (Haines 1981), the indig-

Abbreviations: *ECF* extracellular fluid; *Hb* hemoglobin; *Ht* hematocrit; *ICF* intracellular fluid; *MCHC* mean corpuscular hemoglobin concentration; *TEP* transepithelial electrical potential

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enous origin of the fish may be important in determining its sensitivity to acid water (Brown 1981; McWilliams 1982b). Similar reasoning may apply to the physiological mechanism(s) involved in provoking fish mortality which may also be different in the field situation.

Thus, in the present study, a field investigation was conducted to ascertain the effects of acid exposure on white suckers (*Catostomus commersoni*) from a soft water lake in Ontario, Canada. Blood composition, whole body proton fluxes and both unidirectional and net ion fluxes were monitored to investigate the toxic mechanism(s) of low pH action in natural soft water.

Materials and methods

This study was conducted during August and September, 1981 at the Harkness Laboratory of Fisheries Research (Ontario Ministry of Natural Resources), located on the shores of Lake Opeongo, Algonquin Park, in the Precambrian Shield area of Ontario, Canada (latitude, 45°42′; longitude, 78°23′). Mean annual pH of precipitation in the area is currently pH 4.2 (J. MacLean, personal communication) and the region is undergoing progressive acidification. Lake Opeongo is a soft water lake (area, 51.5 km^2; mean depth 14.6 m), characterized by both a low buffering capacity (alkalinity ≈ 95 $\mu eq \cdot l^{-1}$) and a low ionic content (conductivity ≈ 35 $\mu S \cdot cm^{-1}$).

Experimental animals and test conditions. White suckers (*Catostomus commersoni*) were collected from shallow water using commercial trap nets (approx. 6.5 m × 6.5 m) and, in initial experiments, held for one week in polyethylene tanks which received a continuous flow of lake water at ambient conditions (19–20 °C; pH 6.7–6.9). Fish either held under these conditions or in trap nets for extended time periods and/or excessively handled, suffered variable degrees of skin abrasion, scale loss, and epidermal infections; they progressively lost ions and eventually died (see data in Table 2). Thus, for subsequent experimentation, freshly caught fish which appeared healthy (i.e. unmarked) were selected from trap nets, then carefully transferred to a holding tank for 5–7 h and finally placed in the experimental system (described below) for a minimum of 8 h recovery prior to initial measurements. Whole body rather than separate branchial and renal fluxes were monitored in order to avoid additional stress induced by implanting urinary catheters.

During both recovery and experimentation, fish were kept individually in 2 l lucite boxes which were contained within chambers of 10 l capacity (McDonald 1983); an air-lift pump provided water flow through the lucite box, mixed the systems, and maintained sufficient aeration ($P_{O_2} \approx 125$ Torr). With the exception of flux periods (1.5 h or 5.0 h) where the system was closed-circuited, each chamber received a continuous flow of lake water (0.2–0.3 $l \cdot min^{-1}$) either at ambient pH (≈ 6.8; control group) or acid pH (≈ 4.0; experimental group). Acidified lake water was prepared in 700 l polyethylene reservoirs by titration to pH ≈ 4.0 with H_2SO_4 and vigorous aeration to remove CO_2. Problems associated with accumulation of ammonia and other wastes which commonly occur in recirculated systems were minimized with this flow through water circuit. Temperature control (19–20 °C) was provided by water-jacketing the chambers with water pumped from the lake.

Experimental protocol. Four experimental series were performed. In the first three series, whole body net proton and ion fluxes were measured under control conditions (Series I. pH 6.7–6.9; mean wt = 326.3 ± 22.0 g; $n = 15$), during the first 5 h of acid exposure (Series II. pH 4.2–4.4; mean wt = 212.3 ± 9.4 g; $n = 16$), and following 24 h of acid exposure (Series III. pH 4.2–4.4; mean wt = 330.9 ± 21.4 g; $n = 11$). In each series, unidirectional fluxes of Na^+ and Cl^- were measured simultaneously but on separate sets of fish; all fish were terminally sampled for blood parameters. In Series IV, blood gases, acid-base status and blood composition were sequentially monitored in cannulated fish (mean wt = 413.8 ± 39.8 g; $n = 6$) under control conditions (lake water at pH 6.8; non-recirculated) and over 24 h acid exposure (acidified lake water; pH 4.1–4.3; non-recirculated).

For Series I (control), the following sampling regime was used. After water flow to each fish chamber was closed off and total volume adjusted to set levels (range 6–8 l) by drainage, isotope was added (5.0 μCi ^{22}Na or 2.5 μCi ^{36}Cl; New England Nuclear) and allowed to mix for 15 min. Water samples were taken initially ($t = 0$ h) and at 0.5 h, 1.0 h, 1.5 h, 2.0 h, 3.0 h, 4.0 h and 5.0 h and then analyzed for $[Na^+]$, $[Cl^-]$ and radioactivity. The 0 h and 5 h samples were also processed for titratable proton content, ammonia and other ions (K^+, Ca^{++}, Mg^{++}). At the end of the flux period, fish were killed rapidly by a blow on the head and blood samples withdrawn either by caudal or cardiac puncture.Hematocrit and hemoglobin were measured immediately and remaining blood centrifuged for the determination of plasma osmolality, protein, ammonia, inorganic phosphate and ionic content ($[Na^+]$, $[Cl^-]$, $[Ca^{++}]$, $[Mg^{++}]$). Plasma K^+ levels were not measured because of the possibility of slight hemolysis with these sampling techniques (Wilkes et al. 1981).

The protocol in Series II (5 h acid) and Series III (24 h acid) was similar except for the following modifications. In Series II, 30–45 min was allowed prior to the 5 h flux period in order to completely switch from lake water at pH ≈ 6.8 to acid conditions; a measured amount of 0.02 N H_2SO_4 was also added to each chamber after 2.5 h to maintain water pH. For Series III, each chamber was continuously fed with acidified lake water for 24 h and then closed off. A flux period of 1.5 h was employed since water pH could not be reliably held in the desired pH range of 4.2–4.4 for any longer; larger fish were used in this series. Fortunately, it was still possible to accurately assess changes over 1.5 h because of the low baseline levels of all ions in lake water (Table 1).

In Series IV, fish were anaesthetized with an aerated solution of tricaine methanesulfonate (MS-222; 1:10,000 dilution adjusted to pH ≈ 7.0 with 1.0 N KOH) while being fitted with a caudal arterial catheter; they were then allowed to recover in individual flux chambers under control conditions for 24 to 36 h (Hõbe et al. 1983). Blood samples were withdrawn anaerobically (700 μl) and replaced with an equal volume of Cortland saline (Wolf 1963). Samples were processed immediately for whole blood hematocrit, hemoglobin levels, blood oxygen tension and lactate concentration as well as plasma pH and total carbon dioxide levels. The remaining plasma was analyzed as described for Series I. Plasma K^+ levels were also measured since this sampling technique did not cause excessive hemolysis.

Analytical techniques. Water Cl^- levels were assessed by coulometric titration using a digital chloridometer (Buchler Model 4-2500) and a modified acid reagent which was spiked with 2 mM NaCl to enhance sensitivity. Cation levels (in water or plasma) were analyzed, after appropriate dilution, with a flame photometer (Eel; $[Na^+]$, $[K^+]$) or atomic absorption spectrophotometer (Jarrel Ash 850; $[Ca^{++}]$, $[Mg^{++}]$). Water ammonia ($NH_3 + NH_4^+$) concentration was determined by a micro-modification of the salicylate hypochlorite method of Verdoux et al. (1978).

Table 1. Mean ionic composition (meq·l^{-1}) of water in fish chambers at the beginning of each flux experiment (lake water at 19–20 °C). Values are means ± SEM; number of determinations are represented by *n*

Experimental system	*n*	$[Na^+]$	$[Cl^-]$	$[K^+]$	$[Ca^{++}]$	$[Mg^{++}]$
Control (Series I)	15	0.057 ± 0.003	0.054 ± 0.006	0.020 ± 0.001	0.191 ± 0.004	0.117 ± 0.003
5 h Acid (Series II)	16	0.074 ± 0.001	0.035 ± 0.002	0.133[a] ± 0.001	0.182 ± 0.002	0.120 ± 0.003
24 h Acid (Series IIII)	10	0.085 ± 0.003	0.033 ± 0.002	0.024 ± 0.001	0.164 ± 0.002	0.097 ± 0.001
Combined mean of the experimental systems		0.072	0.041	0.059	0.179	0.111

[a] 1.0 N KOH added during preparation of acidified lake water (700 l tank)

Titratable proton content of water samples (10 ml) was determined by acid titration using 0.02 N H_2SO_4 and an endpoint of pH 4.0, as outlined in McDonald and Wood (1981); values were corrected for 0.02 N H_2SO_4 addition during flux periods. Net flux (J_{net}) of each substance (e.g. Na^+) was calculated using the equation,

$$J_{net} = \frac{([Na^+]_i - [Na^+]_f) \times V}{(t_f - t_i) \times W}$$

where, $[Na^+]_i$ and $[Na^+]_f$ represent the initial and final concentrations (in μeq·ml^{-1}) in the external medium at the beginning (i) and end (f), respectively, of a flux measurement with the $(t_f - t_i)$ period expressed in hours. The symbol W represents the fish wet weight (kg) and V the average water volume (ml) in the fish chamber over the flux period. Net proton influx/or base efflux ($J_{net}^{H^+}$) was determined as the difference between net titratable proton flux (apparent H^+ influx or base loss) and ammonia efflux ($J_{net}^{NH_3+NH_4^+}$) (McDonald and Wood 1981).

^{22}Na and ^{36}Cl radioactivity in water were measured by liquid scintillation counting (5 ml sample + 10 ml PCS; Amersham) in a Mark III Liquid Scintillation System (Tracor Analytic Model 6881). Unidirectional influx (J_{in}) of Na^+ (or Cl^-) was determined from the disappearance of ^{22}Na (or ^{36}Cl) from the bathing medium over each flux interval (e.g. 0–0.5 h, 0.5–1.0 h) using the arithmetic equation (Cuthbert and Maetz 1972),

$$J_{in} = \frac{(C_i - C_f) \times V}{SA \times (t_f - t_i) \times W}$$

where, $(C_i - C_f)$ represents the change in radioactivity (in cpm·ml^{-1}) in the bathing medium and SA, the average specific activity (cpm·μeq·l^{-1}) during the flux period in question. Preliminary analyses by Kirschner's (1970) logarithmic equation gave virtually identical results. Unidirectional effluxes (J_{out}) were calculated for each flux period using the conservation equation,

$$J_{net} = J_{in} - J_{out}.$$

Plasma samples, counted for Na^+ and Cl^- radioactivity in Series I (control), verified that backflux correction was not necessary since the internal specific activity in plasma never exceeded more than 4% of the values in the external bathing medium. Radiospaces (ml·kg^{-1}) were calculated according to the equation of Mayer and Nibelle (1969),

$$\text{Radiospace} = \frac{(C_i - C_f) \times V}{C_p \times W}$$

where C_p represents the radioactivity per ml of plasma (in cpm·ml^{-1}) at the end of the flux experiment; the remaining symbols have been previously defined. Whole body exchangeable content (meq·kg^{-1}) of Na^+ (or Cl^-) was estimated from calculated radiospaces and measured plasma concentrations (Bath and Eddy 1979).

Hematocrit (Ht), hemoglobin (Hb), plasma pH and total carbon dioxide levels (C_{CO_2}) were measured as previously described (McDonald et al. 1980; Höbe et al. 1983). Mean corpuscular hemoglobin concentration (MCHC) was calculated as the ratio of Hb/Ht (gm·ml^{-1}). Plasma bicarbonate levels (HCO_3^-) and carbon dioxide tensions (P_{CO_2}) were estimated using the Henderson-Hasselbalch equation with pH and temperature corrected pk' values (Severinghaus et al. 1956) and temperature corrected αCO_2 values (Severinghaus 1965). Blood oxygen tension (P_{O_2}) was measured with a Radiometer microelectrode. Blood lactate was assessed on deproteinized samples (8%, w/v perchloric acid) using an enzymatic method for L-lactate (Sigma Technical Bulletin no. 826). Plasma chloride (Cl^-), osmolality and protein were analyzed without dilution using a chloridometer (Radiometer CMT-10), osmometer (Wescor Vapor pressure Osmometer, Model 5100C) and refractometer (Goldberg, American Optical), respectively. Plasma ammonia ($NH_3 + NH_4^+$) was measured by an enzymatic procedure based on the conversion of α-ketoglutarate to glutamate using Sigma reagents (Sigma Technical Bulletin no. 170-UV). Inorganic phosphate was determined in deproteinized plasma (20%, w/v trichloracetic acid) according to the method of Fiske and SubbaRow with Sigma reagents (Sigma Technical Bulletin no. 670).

Data from flux experiments (uncannulated fish) have been expressed throughout as means (± SEM; *n*) unless otherwise stated. Differences between mean values of control (Series I) and acid groups (Series II, III) were tested using a Student's two-tailed *t*-test (unpaired design; $P < 0.05$). Statistical treatment of data from Series IV (cannulated) was not feasible since fish were sampled at different time intervals.

Results

Control data

From the branchial and renal ion flux data given in Table 2, it was evident that surface abrasions resulting from handling and other procedures caused significant losses of Na^+, Cl^- and K^+ as well as increased $J_{net}^{NH_3+NH_4^+}$. When the holding-time was reduced and handling minimized (see Methods), suckers remained virtually in net balance with respect to all ions (Table 3). Thus, 13–16 h from the time of capture was a sufficient period for recovery from both handling and confinement in experimental chambers.

Table 2. Effects of surface abrasions on proton and ion fluxes in white sucker in natural soft water (pH ≈6.8; 19–20 °C). Fish were fitted with a urinary catheter, recovered for 36 h and branchial (J_{net}) and renal[a] (E_x) fluxes measured (10 h flux period). Values are means (±SEM; *n*) for fish without (Group I) and with (Group II) surface abrasions. The two groups were compared using an unpaired *t*-test. Significance level is listed

Variable	Group I no abrasions	Group II abrasions	Significance level
$J_{net}^{H^+}$	+34.64 ±28.68 (7)	+81.07 ±50.23 (8)	ns
$E_{\Sigma H^+}$	+ 5.19 ±1.77 (5)	+2.79 ±1.33 (5)	ns
$J_{net}^{NH_3+NH_4^+}$	−628.21 ±35.71 (7)	−771.40 ±66.82 (8)	$P<0.05$
$E_{NH_4^+}$	−4.39 ±1.12 (9)	−4.78 ±0.89 (5)	ns
$J_{net}^{Na^+}$	−47.51 ±38.35 (7)	−179.85 ±49.29 (8)	$P<0.05$
E_{Na^+}	−3.99 ±0.68 (9)	−24.44 ±6.77 (5)	$P<0.05$
$J_{net}^{Cl^-}$	−83.21 ±22.20 (7)	−170.43 ±34.45 (8)	$P<0.05$
E_{Cl^-}	−3.31 ±0.52 (9)	−13.12 ±3.05 (5)	$P<0.05$
$J_{net}^{K^+}$	−104.83 ±15.59 (7)	−136.42 ±10.20 (8)	$P<0.05$
E_{K^+}	−6.20 ±2.96 (9)	−6.65 ±0.51 (5)	ns
$J_{net}^{Ca^{++}}$	+22.38 ±5.40 (7)	+32.28 ±22.15 (8)	ns
$E_{Ca^{++}}$	−0.79 ±0.31 (9)	−1.51 ±0.55 (5)	ns
$J_{net}^{Mg^{++}}$	+3.94 ±9.87 (7)	+10.05 ±2.31 (8)	ns
$E_{Mg^{++}}$	−0.23 ±0.06 (9)	−0.62 ±0.25 (5)	ns

[a] Renal methodology in Hõbe et al. (1983)

Table 3. Resting proton and ion fluxes (μeq·kg^{-1} h^{-1}), radiospaces (ml·kg^{-1}) and calculated whole body exchangeable Na^+ and Cl^- content (meq·kg^{-1}) in *Catostomus commersoni* in natural soft water (pH ≈6.8; 19–20 °C). Values are means±SEM (*n*). Symbols are defined in text

$J_{net}^{H^+}$	− 83.15±19.65 (15)
$J_{net}^{NH_3+NH_4^+}$	−592.24± 4.29 (15)
$J_{net}^{K^+}$	− 52.86± 5.23 (15)
$J_{net}^{Ca^{++}}$	+ 55.23±10.26 (15)
$J_{net}^{Mg^{++}}$	− 6.37±11.14 (15)
$J_{net}^{Na^+}$	− 24.52±11.23 (15)
$J_{net}^{Cl^-}$	− 12.01±12.18 (15)
$J_{in}^{Na^+}$	+219.04±19.76 (10)
$J_{out}^{Na^+}$	−250.96±29.48 (10)
Na^+-space	301.83±15.2 (8)
Na^+-content	36.13±2.97 (8)
$J_{in}^{Cl^-}$	+120.90±27.43 (6)
$J_{out}^{Cl^-}$	−145.98±15.69 (6)
Cl^--space	242.39±17.35 (7)
Cl^--content	23.43± 1.61 (7)

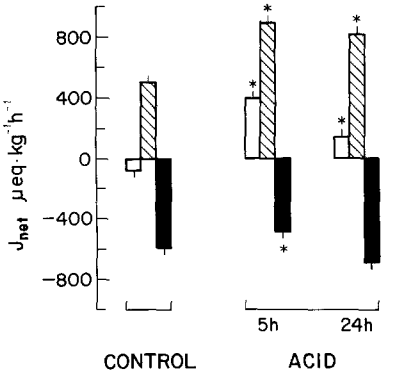

Fig. 1. Whole body net proton fluxes in uncannulated white suckers in near-neutral (pH ≈6.8; control) and acidified lake water (pH ≈4.3) at 19–20 °C. Titratable proton flux (*hatched bars*) and ammonia efflux (*shaded bars*) components of net proton flux (*unshaded bars*) are illustrated. *Asterisks* represent significance ($P<0.05$) obtained by a "between group" comparison of means (unpaired *t*-test)

To substantiate the remarkably high control plasma pH (8.15±0.03) and HCO_3^- levels (15.29±0.35) seen in cannulated fish (see Fig. 5), blood samples were also withdrawn by caudal puncture from 4 freshly caught fish. While this sampling technique is less than ideal for acid-base analysis since it causes some disturbance to the fish and thus, underestimates both plasma pH and $[HCO_3^-]$, the results obtained (i.e. pH 7.91±0.08; $[HCO_3^-]=13.77\pm0.91$) were nonetheless confirmatory.

Acid exposure (uncannulated fish)

Uncannulated fish survived ambient pH 4.3 for 24 h but in preliminary experiments, commonly succumbed by 48 h.

With the onset of acid exposure, fish exhibited some reduction in ventilatory pumping frequency and by 5 h, copious mucus covered the gills and skin; during the remaining time, mucus accumulation persisted but the breathing mode had changed to one of rapid, shallow ventilatory pumping (visual observations).

Acid exposure modified both the direction and magnitude of $J_{net}^{H^+}$ (Fig. 1). Over the initial 5 h, there was a significant net influx of H^+ (or net base efflux), predominantly due to a doubling in the titratable component since $J_{net}^{NH_3+NH_4^+}$ actually declined by 17%. By 24 h, $J_{net}^{H^+}$ had partially recovered to rates one half those attained over 5 h because of a 37% elevation in $J_{net}^{NH_3+NH_4^+}$ and 9% reduction in titratable proton flux.

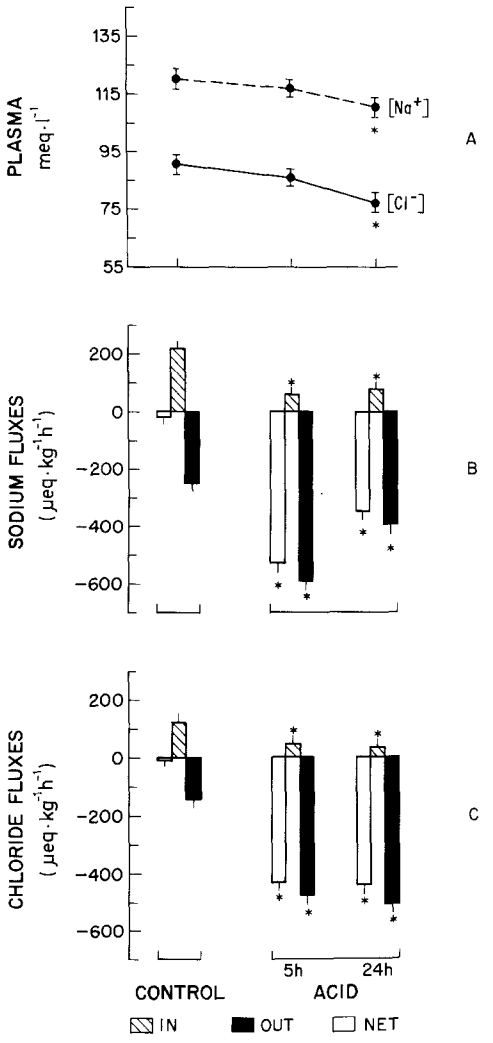

Fig. 2. Plasma levels, whole body net flux (J_{net}) and unidirectional fluxes (J_{in}, J_{out}) of sodium and chloride in uncannulated white suckers in near-neutral (pH ≈6.8; control) and acidified lake water (pH ≈4.3) at 19–20° C. Values for plasma and net fluxes are means (±SEM). Unidirectional flux data, calculated for each flux interval, were averaged for each fish and the grand mean plotted (±SEM). *Asterisks* represent signficance as described in Fig. 1

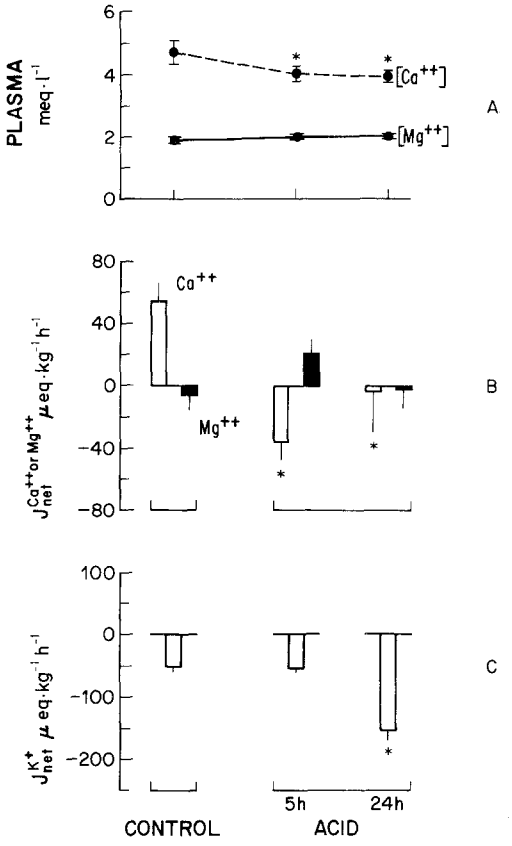

Fig. 3. Plasma divalent cation levels and whole body net fluxes (J_{net}) of calcium, magnesium and potassium in uncannulated white suckers in near-neutral (pH ≈6.8; control) and acidified lake water (pH ≈4.3) at 19–20 °C. *Asterisks* represent significance as described in Fig. 1

Concomitantly, dramatic changes occurred in whole body fluxes of major electrolytes (Figs. 2, 3). $J_{in}^{Na^+}$ and $J_{in}^{Cl^-}$ decreased initially by 72% and 62%, respectively (Fig. 2B, C). The drop in $J_{in}^{Na^+}$ (Fig. 2B) was stoichiometrically equivalent to the reduction in $J_{net}^{NH_3+NH_4^+}$ (Fig. 1). More pronounced changes occurred in $J_{out}^{Cl^-}$ (+226%) and $J_{out}^{Na^+}$ (+134%) but the elevation in $J_{net}^{Na^+}$ slightly exceeded that in $J_{net}^{Cl^-}$. By 24 h, however, $J_{out}^{Na^+}$ had partially recovered largely through a 25% decrease in $J_{out}^{Na^+}$; the rise in $J_{in}^{Na^+}$ was not significant. $J_{net}^{Na^+}$, by this time, amounted to approximately 0.9% of whole body exchangeable Na^+ per hour, given a control Na^+ content of 36.13 ± 2.97 meq·kg^{-1} (Table 3). Since there was no corresponding recovery in either $J_{in}^{Cl^-}$ or $J_{out}^{Cl^-}$, $J_{net}^{Cl^-}$ amounted to about 1.8% of whole body exchangeable Cl^- per hour relative to a control Cl^- content of 23.43 ± 1.61 meq·kg^{-1} (Table 3). This pattern of $J_{net}^{Cl^-} > J_{net}^{Na^+}$ was reflected in plasma [Na^+] and [Cl^-] which by 24 h were lowered by 8% and 15%, respectively (Fig. 2A). By contrast, $J_{net}^{K^+}$ was not significantly altered until 24 h where loss rates were three-fold higher than controls (Fig. 3C). $J_{net}^{Ca^{++}}$ switched from a pattern of net influx (control) to net loss over 5 h (Fig. 3B) whereas the reverse situation was evident in $J_{net}^{Mg^{++}}$ (Fig. 3B) though the latter response was not significant. By 24 h, $J_{net}^{Ca^{++}}$ had slightly recovered but control rates were not attained. Plasma [Ca^{++}] decreased by 14% (Fig. 3A). The slight rise in plasma [Mg^{++}] was not significant (Fig. 3A).

Other blood components were also modified over the 24 h period (Fig. 4). Plasma osmolality decreased by 6% (Fig. 4C) reflecting the reductions in both plasma [Na^+] and [Cl^-] (Fig. 2A). MCHC, a measure of the amount of hemoglobin

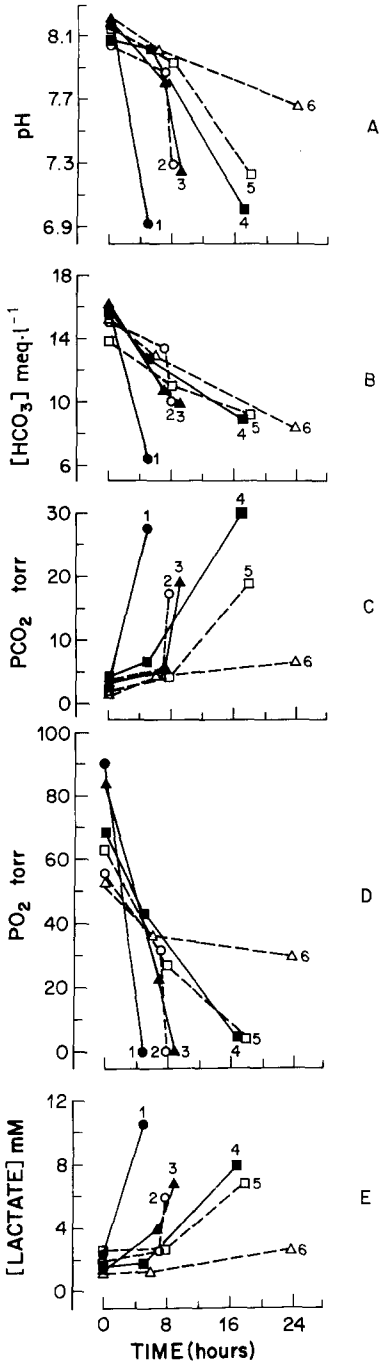

Fig. 4A–E. Blood composition of uncannulated white suckers in near neutral (pH ≈6.8; control) and acidified lake water (pH ≈4.3) at 19–20 °C. **A** Mean corpuscular hemoglobin concentration (calculated from ratio of Hb/Ht). **B** Plasma protein. **C** Plasma osmolality. **D** Plasma ammonia. **E** Plasma inorganic phosphate. A *square bracket* denotes concentration. *Asterisks* represent significance as described in Fig. 1

per ml red cell, gradually declined to levels 20% lower than controls (Fig. 4A); this was attributed to erythrocyte swelling since Ht rose while [Hb] stayed within the control range (data not included). Plasma [protein] increased initially by 16% and remained elevated thereafter (Fig. 4B).

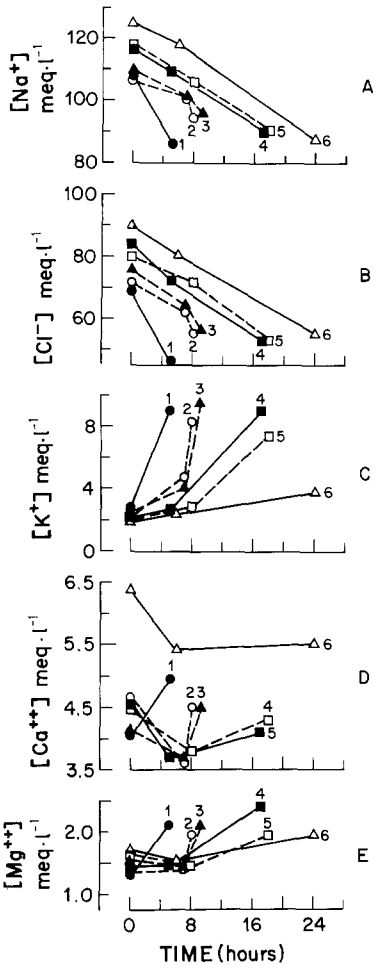

Fig. 5. Blood gas and plasma acid-base status in cannulated (caudal arterial catheter) white suckers in near-neutral (pH ≈ 6.8; control) and acidified lake water (pH ≈4.2) at 19–20 °C. Individual data points are plotted with a different symbol used for each fish (curves numbered 1–6). Fish were sequentially sampled for control values (time "0") and at various times in acid water (max. 24 h)

The transient but marked rise (50%) in plasma $[NH_3 + NH_4^+]$ (Fig. 4D) was inversely correlated with $J_{net}^{NH_3+NH_4^+}$ (Fig. 1). The increasing trend in plasma $[PO_4^-]$ was not statistically significant (Fig. 4E) but inversely correlated with plasma $[Ca^{++}]$ (Fig. 3A).

Acid exposure (cannulated fish)

While behavioral patterns in cannulated fish were similar to those described for uncannulated fish (see above), these fish were much more sensitive to acid water (Figs. 5, 6, 7). Of the six fish measured, one survived 24 h (curve no. 6) and the rest succumbed either after 5 h (curve no. 1), 8 h (curve no. 2), 8.5 h (curve no. 3), 17 h (curve no. 4) or 18 h (curve no. 5). Other cannulated fish maintained under control conditions (lake water at pH ≈6.8) survived the length of the experiment

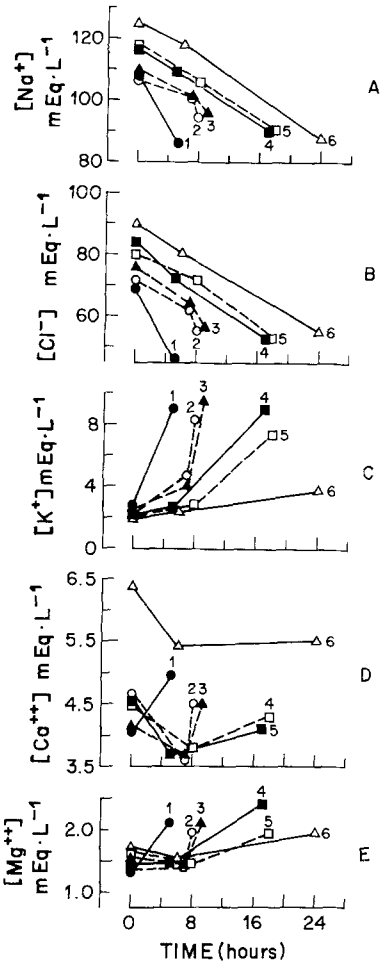

Fig. 6. Blood plasma ion concentrations (represented by square brackets) in cannulated (caudal arterial catheter) white suckers in near-neutral (pH ≈6.8; control) and acidified lake water (pH ≈4.2) at 19–20 °C. Individual data points for each fish are plotted (curves 1 to 6), as described in Fig. 5

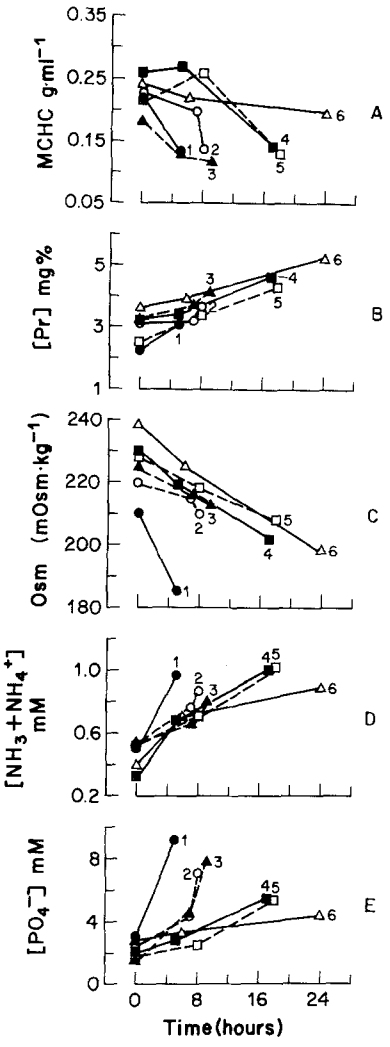

Fig. 7. Blood composition of cannulated (caudal arterial catheter) white suckers in near-neutral (pH ≈6.8; control) and acidified lake water (pH ≈4.2) at 19–20 °C. A *square bracket* denotes concentration. Symbols as in Fig. 4. Individual data points for each fish are plotted (curves 1 to 6), as described in Fig. 5

(i.e. 24 h). Since pre-exposure plasma [Na^+] and [Cl^-] (Fig. 6B, C) were slightly lower than in uncannulated fish (Fig. 2A), some combination of acid and cannulation stress probably accounted for this high mortality.

Trends in these fish were nonetheless instructive in characterizing the overall toxic syndrome. A substantial plasma acid-base disturbance was observed (Fig. 5). The average values of pH (Fig. 5A), [HCO_3^-] (Fig. 5B) and P_{CO_2} (Fig. 5C) near death were 7.22 ± 0.11 pH units, 8.75 ± 0.55 meq·l^{-1} and 19.8 ± 3.6 Torr ($n=6$), respectively. Some form of respiratory distress also prevailed since blood P_{O_2} declined from control levels of 70.7 ± 7.4 Torr to 6.7 ± 4.7 Torr (Fig. 5D) concomitant with elevated blood lactate levels which reached as high as 10.47 mM in one fish (Fig. 5E).

The plasma ionic disturbance (Figs. 6, 7) mimicked that in uncannulated fish (Figs. 2, 3, 4) but changes in [Na^+] (Fig. 6A), [Cl^-] (Fig. 6B), [K^+] (Fig. 6C), [Mg^{++}] (Fig. 6E), ammonia (Fig. 7D) and [PO_4^-] (Fig. 7E) were all much more pronounced. Terminal MCHC (Fig. 7A) and plasma osmolality (Fig. 7C) were markedly reduced and plasma [protein] (Fig. 7B) considerably enhanced from controls, indicating that a progressive loss of plasma water probably ensued as death approached.

Discussion

Sensitivity to handling and acid stress

Catostomus commersoni collected from natural soft water were extremely sensitive to handling and

other procedures which caused epidermal infections and consequent physiological disturbance (Table 2). These fish (particularly cannulated animals) were also very sensitive to acid stress, commonly dying by 24–48 h at an ambient pH of ≈4.3 whereas suckers collected from natural hard water and tested after acclimation either in hard water (Hõbe et al. 1983) or artificial soft water (Hõbe and McMahon, unpublished), survived cannulation for up to 13 days at near-neutral pH as well as exposure to pH ≈4.3 for 96 h. The etiology of this general sensitivity is unknown but may have a genetic basis and/or may involve some characteristic(s) of the natural soft water such as its complement of microorganisms, organic substances, or trace elements. Whatever the cause, these findings emphasize the importance of examining fish in their natural environment with appropriate methodology in studies of this nature.

Control blood composition and ion fluxes

Despite the low concentration of ions in natural soft water (Table 1), plasma levels of major electrolytes (Fig. 2) were similar to those measured in suckers acclimated to natural hard water with a 6–10 fold greater $[Ca^{++}]$ and up to 15 fold greater $[Na^+]$ and $[Cl^-]$ (Wilkes et al. 1981). The most notable feature was the unusually high plasma pH (8.15) associated with elevated plasma $[HCO_3^-]$ (15.3 meq·l^{-1}). Based on a coefficient of temperature-variation for this species of 0.016 in hard water ($[Ca^{++}] \approx 1.8$–2.0 meq·l^{-1}; Hõbe, unpublished results), and the relative alkalinity concept (Reeves 1977), a pH of 7.90 would be anticipated at the high temperature of the present study. Similar atypically high blood pH values have been reported in tropical freshwater fish which live at 30–35 °C in dilute Amazonian waters (Cameron and Wood 1978; Heisler 1982). If the branchial Cl^-/HCO_3^- exchange process thought to modulate Cl^- dynamics (De Renzis 1975) is reduced in suckers relative to the Na^+/NH_4^+ or H^+ exchange because of a limited supply of chloride in soft water (Table 1), then this may explain the observed plasma bicarbonate retention. Internal acid-base status has been shown to reflect ambient $[Cl^-]$ in both fish (Dejours 1969) and crayfish (Dejours et al. 1982).

Resting proton and ion fluxes have not been previously documented for suckers (Table 3). Ammonia efflux almost completely balanced titratable proton flux, resulting in a modest net extrusion of H^+. Most endogenously generated ammonia production is thought to traverse the fish gill in the unionized form (Heisler 1980) but in suckers, a significant contribution from the branchial Na^+/NH_4^+ exchange process (Maetz 1973) cannot be entirely discounted (see below). The high concentration gradients for sodium and chloride movement (eg. plasma $[Na^+]$/external $[Na^+]$) of 1663 and 2213, respectively, together with a $J_{in}^{Na^+}/J_{in}^{Cl^-}$ ratio of 1.6, demonstrated the presence of highly efficient and independent transporting systems for these electrolytes in suckers, as found in other freshwater fish species (eg. Maetz 1973; Wood and Randall 1973; Cameron 1976). Suckers were also capable of sustaining a net influx of calcium (Table 3), suggesting that inward movement of this cation may entail an active process. Branchial influx of Ca^{++} has been demonstrated in other freshwater forms using isolated-perfused gills (eg. Payan et al. 1981).

Effects of acid exposure

Acid-base regulation

The large H^+ concentration gradient generated by a decrease in ambient pH would tend to favor inward diffusion of H^+ since the gill is thought to be highly permeable to this ion (McWilliams and Potts 1978). Indeed, this seemed to be the situation, as shown by the significant net influx of H^+ (Fig. 1, Table 4). However, it must be noted that

Table 4. Estimated changes in whole body ion and proton fluxes (in μeq·kg^{-1}) partitioned into amounts lost or gained by the extracellular and intracellular compartments in white suckers by 24 h of acid exposure

Ion	Whole body[a] fluxes	Estimated ECF[b] loss or gain	Estimated ICF loss or gain
Na^+	− 7687	−1976	−5711
Cl^-	−10191	−2824	−7367
K^+	− 2585	+ 342	−2927
Ca^{++}	− 1427	− 149	−1278
Mg^{++}	+ 117	+ 16	+ 101
H^+	+ 5370		

Whole body net ion charge = $(Na^+ + K^+ + Ca^{++} + Mg^{++} + H^+) - Cl^- = -3979$

[a] Estimated by calculating the difference between control rates and those attained at 24 h of acid exposure. Since the total time interval was not monitored and whole body electrolyte loss rates tended to fall with time, these values are probably underestimates of the actual net change in each ion which occurred

[b] Plasma ion concentrations were assumed to represent those in extracellular fluid (*ECF*). An ECF volume of 200 ml·kg^{-1} was used with the assumption that a 17% reduction in ECF occurred in white suckers as observed in rainbow trout (Milligan and Wood 1982)

the present analytical techniques do not differentiate between H^+ entry and HCO_3^- loss, though fortunately, the two events are indentical in terms of net acid-base balance. By 24 h of acid exposure, the effective net H^+ influx was somewhat ameliorated, suggesting some compensatory ability.

The marked depression in arterial pH seen in cannulated fish (Fig. 5A) reflected a plasma acidosis of mixed "metabolic" and "respiratory" origin, since plasma [HCO_3^-] was depressed (Fig. 5B) while P_{CO_2} was elevated (Fig. 5C). In turn, the metabolic component presumably resulted from both net H^+ entry from the water and endogenous lactic acid production (Fig. 5E). The respiratory component (CO_2 accumulation) probably resulted from both acid titration of the bicarbonate buffer system and impaired gas exchange at the gills (see below). These findings confirm recent laboratory studies on this species in artificial soft water where a similar, though less pronounced, plasma acidosis of mixed origin was observed (Hõbe and McMahon, unpublished). In contrast, at comparable ambient pH and calcium levels (artificial soft water) in the rainbow trout (*Salmo gairdneri*), net H^+ entry rate was much lower, plasma acid-base status exhibited little modification, and blood lactate elevation was negligible (McDonald et al. 1980; McDonald 1983).

The dramatic elevation in plasma K^+ levels in cannulated suckers (Fig. 6C) suggested that H^+ may have invaded the intracellular compartment (see Ladè and Brown 1963). Since an intracellular metabolic acidosis would catabolize intracellular organic phosphate compounds, generating PO_4^- which would subsequently move into the extracellular space (Guest and Rapoport 1939), this may explain the observed elevation in plasma PO_4^- levels (Figs. 4E, 7E).

Ionoregulation

Since whole body ion fluxes virtually reflected branchial phenomena in suckers at near-neutral pH (Table 2), the gill was probably the major route of ion loss at pH ≈4.3 in this species, as in the rainbow trout (McDonald and Wood 1981; McDonald 1983). A significant hemoconcentration and erythrocyte swelling also occurred (Figs. 4, 7), probably as a consequence of ion depletion and fluid volume redistribution, perhaps abetted by the plasma acidosis (Milligan and Wood 1982). From the calculated ion budget in Table 4, it was evident that circulating ion levels in plasma were partially conserved by redistribution between intra- and extracellular compartments. In fact, all of K^+, and over 70% of Na^+ and Cl^- net losses seemed to have originated from the intracellular compartment. Net calcium loss also arose primarily through depletion of intracellular stores (Table 4), a finding which is supported by the recent studies of Fraser and Harvey (1982) who found low levels of bone calcium in the hypurals, trunk centra and caudal centra of white suckers from an acid lake. There was, however, a significant movement of net charge unaccounted for (i.e. $-3{,}979\ \mu eq \cdot kg^{-1}$; Table 4), suggesting entry of an unmeasured anion (or loss of an unmeasured cation).

The observed pattern of disturbance in Na^+ and Cl^- fluxes (Fig. 2) differed substantially from those reported in most studies of trout. In the latter, exposure to pH ≈4 initially caused complete blockage of $J_{in}^{Na^+}$ (Packer and Dunson 1970; McWilliams 1980a, b), partial blockage of $J_{in}^{Cl^-}$ (McDonald et al. 1983), and at least in artificial soft water, stimulation of both $J_{out}^{Na^+}$ and $J_{out}^{Cl^-}$ so that $J_{net}^{Na^+}$ and $J_{net}^{Cl^-}$ became highly negative and approximately equal (McDonald 1983; McDonald et al. 1983). These equimolar losses of Na^+ and Cl^-, combined with the constraints of electroneutrality, were thought to explain the minimal net H^+ entry in rainbow trout in artificial soft water. By contrast, in the sucker there was only partial inhibition of both $J_{in}^{Na^+}$ and $J_{in}^{Cl^-}$, yet stimulation of both $J_{out}^{Na^+}$ and $J_{out}^{Cl^-}$ to the extent that the highly negative $J_{net}^{Cl^-}$ eventually exceeded $J_{net}^{Na^+}$. Despite this excess of Cl^- loss over Na^+ loss, significant net H^+ entry occurred (Table 4), a situation certainly not predictable from the trout studies, where H^+ entry (at higher water calcium levels) was associated with Na^+ loss in excess of Cl^- loss (McDonald and Wood 1981). These differences, while unexplained, emphasize the dangers in extrapolating from one species to another and perhaps from artificial media to natural soft water. Certainly, the effects of low pH on sodium fluxes in different species and even different races of the same species, may be different (McWilliams 1980a, 1982a).

Traditionally, carrier-mediated Na^+ influx at the gills is thought to occur in exchange for NH_4^+ and/or H^+ and Cl^- influx in exchange for HCO_3^- and/or OH^-, though a variable amount of both fluxes may also occur by exchange diffusion (Maetz 1972, 1973; Wood and Randall 1973; DeRenzis 1975). The stoichiometrically equivalent reduction in both $J_{in}^{Na^+}$ (Fig. 2) and $J_{net}^{NH_3+NH_4^+}$ (Fig. 1) which was initially observed suggested a reduction in the active Na^+/NH_4^+ exchange. The concomitant rise in plasma ammonia levels (Fig. 4) supported this contention. The mechanism involved may either be direct H^+ competition with

the carrier or H^+ titration of negative charges on the membrane channels serving the carrier. The reduction in $J_{in}^{Cl^-}$ was more difficult to explain but may have been a consequence of the internal metabolic acidosis which would reduce HCO_3^- availability for Cl^-/HCO_3^- exchange. The partial persistence of both $J_{in}^{Na^+}$ and $J_{in}^{Cl^-}$ may represent either exchange diffusion or H^+ resistant active exchanges.

Net Na^+ and Cl^- loss predominantly arose from a marked stimulation of J_{out} rather than inhibition of J_{in} (Fig. 2), suggesting that the mechanism of H^+ action on these two exchange components may be different (cf. McDonald et al. 1983). These changes in J_{out} may be envisaged as a consequence of either a change in gill permeability (McWilliams 1982b) and/or an alteration in the transepithelial electrical potential (TEP) across the gill (McWilliams and Potts 1978). In support of the former, other epithelia (e.g. gall bladder) have been shown to be more permeable to anions than cations at low pH, presumably through a reduction of the negative charges on acidic groups in the diffusional pathway of ion flow (Diamond 1975). This may explain the observed enhancement of $J_{out}^{Cl^-}$ relative to $J_{out}^{Na^+}$ in suckers. However, the contribution of the TEP cannot be entirely discounted without experimental verification since low ambient pH has been shown to modify the TEP from a normally negative value (inside) to a positive one both in the brown trout *Salmo trutta* (McWilliams and Potts 1978) and goldfish *Carassius auratus* (Eddy, personal communication).

The partial correction of $J_{net}^{H^+}$ (Fig. 1), $J_{out}^{Na^+}$ (Fig. 2B) and $J_{net}^{Ca^{2+}}$ (Fig. 3B) by 24 h acid exposure probably did not result simply from a reduction in branchial permeability because $J_{out}^{Cl^-}$ was not similarly adjusted (Fig. 2C). Prolactin, a hormone with pronounced effects on sodium and calcium regulation (Bentley 1980; Pang et al. 1980) but minimal involvement in chloride dynamics (Fortner and Pickford 1982), may be implicated. Notter et al. (1976) have reported a delayed secretion of prolactin in *Salvelinus fontinalis* at pH 4.0. Again, the pattern in suckers was different from that in *Salmo gairdneri* where complete correction of both $J_{out}^{Na^+}$ and $J_{out}^{Cl^-}$ was seen after 40 h acid exposure, though net ion losses persisted indefinitely due to incomplete recovery of $J_{in}^{Na^+}$ and $J_{in}^{Cl^-}$ (McDonald 1983; McDonald et al. 1983).

Gas exchange

While oxygen uptake was not directly measured, there was some indirect eivdence of respiratory distress and tissue hypoxia. These included an increase in ventilatory frequency, dramatic reduction of P_{O_2}, elevation of P_{CO_2} and rise in blood lactate concentration (Fig. 5C, D, E). The cause may have been a combination of epithelial damage to the branchial lamellae (Daye and Garside 1976) and inhibition of respiratory gas diffusion across the gills by copious mucus accumulation (Ultsch and Gros 1979). The large accompanying acidosis (Fig. 5A) probably exacerbated the hypoxemia by interference with hemoglobin oxygen transport via the Bohr and Root effects (cf. Packer 1979). The magnitude of these disturbances was surprising (Fig. 5) since such effects have commonly been observed in other fish species only under more acidic (pH $\approx$3.5) conditions (e.g. Ultsch et al. 1981).

Conclusion

The present findings establish that in natural soft water, fish are very sensitive to external acid stress. The physiological consequences of exposure to pH $\approx$4.3 included acid-base and ionoregulatory failure as well as impaired respiratory gas exchange. The relative importance of these three related phenomena in the toxic syndrome is not yet clear. It remains to be seen whether these findings are species-specific and/or simply reflect differences between artificial and natural soft water.

Acknowledgements. We thank the Director, Dr. J. MacLean, and the staff of the Harkness Laboratory of Fisheries Research for their invaluable advice, assistance and hospitality. D.J. Spry, from McMaster University, helped greatly with the experiments. The field expedition was supported by grants to C.M.W. from the Canadian National Sportsmen's Fund and the Strategic Program of NSERC, and to B.R.M. from NSERC. H.H. was supported by an NSERC Postgraduate Scholarship and a Research Allowance from the Alberta Heritage Fund for Medical Research.

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