Ion Flux Rates, Acid–Base Status, and Blood Gases in Rainbow Trout, *Salmo gairdneri*, Exposed to Toxic Zinc in Natural Soft Water

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Spry, D. J., and C. M. Wood. 1985. Ion flux rates, acid–base status, and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water. Can. J. Fish. Aquat. Sci. 42: 1332–1341.

Exposure to 0.8 mg Zn^{2+}/L in natural soft water for up to 72 h was toxic to rainbow trout, *Salmo gairdneri*, causing an acid–base disturbance and net branchial ion losses. Mean arterial pH fell from 7.78 to 7.58. Both Pa_{CO_2} and lactate rose, indicating a mixed respiratory and metabolic acidosis, despite maintenance of high Pa_{O_2}. Net branchial uptake of Na^+ and Cl^- became a net loss immediately following exposure to Zn^{2+}, and this continued during 60 h of exposure. Net K^+ loss was exacerbated, and net Ca^{2+} uptake was abolished. Unidirectional flux measurements with $^{22}Na^+$ and $^{36}Cl^-$ indicated an increased efflux immediately following zinc exposure. Both influx and efflux of Na^+ and Cl^- were stimulated after 48–60 h in Zn^{2+}. Both net branchial ammonia excretion and net branchial uptake of acidic equivalents from the water (=base loss) were greatly stimulated, the latter contributing to metabolic acidosis. Kidney function, as measured by urine flow rate and excretion of ammonia, acidic equivalents, Na^+, Cl^-, K^+, and Zn^{2+}, was relatively insensitive to the effects of zinc. The only renal component to be affected was Ca^{2+} excretion, which decreased during a single flux period, possibly in response to the reduced entry of Ca^{2+} at the gill. We conclude that toxic concentrations of zinc are capable of altering gill function so as to cause ionoregulatory and acid–base disturbances without disturbance of Pa_{O_2}.

L'exposition de truites arc-en-ciel, *Salmo gairdneri*, à 0,8 mg de Zn^{+2} par litre d'eau douce naturelle pendant 72 h a eu une incidence toxique, c.-à-d. une perturbation de l'équilibre acide–base et des pertes nettes d'ions branchiaux. Le pH artériel moyen est passé de 7,78 à 7,58 tandis que le Pa_{CO_2} et le lactate ont augmenté, ce qui indique une acidose respiratoire et métabolique hétérogène malgré le maintien d'un Pa_{CO_2} élevé. La captation nette de Na^+ et de Cl^- au niveau des branchies est devenue une perte nette immédiatement après l'exposition à du Zu^{+2} et pendant 60 h d'exposition. La perte nette de K^+ a été aggravée et la captation nette de Ca^{+2}, supprimée. Des quantifications du flux unidirectionnel de $^{22}Na^+$ et de $^{36}Cl^-$ ont révélé un écoulement accru immédiatement après l'exposition au zinc. L'entrée et la sortie de Na^+ et de Cl^- ont été stimulées après une exposition au Zn^{+2} allant de 48 à 60 h. L'excrétion nette d'ammoniaque branchial et la captation nette d'équivalents acides du milieu au niveau des branchies (= perte de base) ont été grandement stimulées; cette dernière a contribué à l'acidose métabolique. La fonction rénale, telle que mesurée par le taux d'évacuation de l'urine et l'excrétion d'ammoniaque, d'équivalents acides, de Na^+, de Cl^-, de K^+ et de Zn^{+2}, a été relativement insensible aux effets du zinc. L'excrétion du Ca^{+2} était la seule composante rénale touchée : elle a diminué au cours d'une seule période de flux, probablement en réaction à la réduction de l'entrée de Ca^{+2} au niveau des branchies. Les auteurs formulent la conclusion que des concentrations toxiques de zinc sont capables de modifier la fonction des branchies et de causer des perturbations de l'ionorégulation et de l'équilibre acide–base sans modifier le Pa_{O_2}.

Received October 18, 1984
Accepted April 15, 1985
(J7986)

Reçu le 18 octobre 1984
Accepté le 15 avril 1985

Concentrations of waterborne zinc that are rapidly lethal to trout severely disrupt gill tissue (Skidmore and Tovell 1972) and hence oxygen transfer by imposing a diffusion limitation for oxygen. The result is hypoxia (Skidmore 1970) and acidemia (Sellers et al. 1975; Spry and Wood 1984). The effects upon ion regulation are less clear. Skidmore (1970) found small but significant changes in plasma osmotic pressure in rainbow trout, *Salmo gairdneri*, exposed to 40 mg Zn^{2+}/L, but discounted these as being unimportant in the rapidly developing lethality. Spry and Wood (1984) reported no significant changes in major blood electrolytes in dying trout which showed hypoxemia and acidosis in 1.5 mg Zn^{2+}/L when compared with controls. This might have been obscured by the observed hemoconcentration. However, at a lower concentration (0.8 mg Zn^{2+}/L), where acute hypoxemic death did not occur, plasma Ca^{2+} levels decreased over a 3-d exposure but other plasma ions were unaffected. Lewis and Lewis (1971) noted a decrease in the serum osmolality of channel catfish (*Ictalurus punctatus*) exposed to lethal Zn^{2+} solutions (12–30 mg/L). When they added NaCl to the water to create an external

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osmotic pressure of 235 mosmol/L, mortality was delayed. This latter study suggests that regulation of Na^+ and/or Cl^- might indeed be affected, and possibly be of primary importance, under conditions where oxygen delivery was not clearly limiting.

Zinc might exert such an effect upon ion regulation by altering ATPase activities. Watson and Beamish (1981) showed in vitro inhibition of various ATPases in freshwater-adapted rainbow trout, although an earlier study (Watson and Beamish 1980) showed increased activity in vivo after 30 d in Zn^{2+}. This increased activity was suggested to be secondary to a Zn^{2+}-induced increase in gill permeability even though serum osmolality and electrolytes were unchanged. Zinc also inhibited chloride transport across the isolated opercular epithelium of seawater-adapted *Fundulus heteroclitus*, possibly as a consequence of its inhibitory effect in vitro upon Na^+,K^+-ATPase (Crespo and Karnaky 1983).

It is well known that zinc toxicity increases with decreasing hardness and alkalinity (Spear 1981). In the very soft water of Ontario Precambrian Shield lakes, zinc enrichment accompanying acidification may become a problem (Spry et al. 1981). The effects of zinc exposure on rainbow trout in *artificial* soft water have been reported earlier (Spry and Wood 1984). To compare these results with exposure in *natural* soft water of the Shield area, we completed a series of experiments at a field site which, in addition, examined the effects of a low level of Zn^{2+} (0.8 mg/L) upon ionoregulation. Our objectives were firstly, to measure acid–base, ionic, blood gas, and other blood variables in rainbow trout fitted with dorsal aortic cannulae and secondly, to separate branchial and renal net ammonia and acidic equivalent fluxes, as well as net and unidirectional ion fluxes, in trout with bladder catheters. We chose a zinc concentration close to the 96-h LC50 for comparison with the previous study (Spry and Wood 1984). Branchial and renal flux rates have not previously been measured in fish exposed to Zn^{2+}.

Materials and Methods

Rainbow trout underyearlings were procured from Skeleton Lake Hatchery or Milford Bay Trout Farm in late summer. Both hatcheries had soft water (Ca^{2+} = 0.2 mequiv/L). Fish were moved by truck to Harkness Laboratory in Algonquin Park (latitude 45°42′; longitude 78°23′) where they were kept in tanks supplied with flowing Lake Opeongo water at 17–22°C. A commercial pelleted diet (Martin Feed Mills, Elmira, Ont.) was fed daily. This was the same diet as used in our previous study (Spry and Wood 1984). Trout were acclimated to 15°C for at least 4 d in a flow-through system chilled with Min-o-cool units (Frigid Units). Food was withheld 2 d prior to any surgery and throughout the experimental period.

Blood Measurements

To assess the effects of Zn^{2+} exposure on arterial blood composition, we cannulated the dorsal aorta (Smith and Bell 1964) of rainbow trout (141–267 g) under MS222 anesthesia and placed each fish in one of eight individual compartments of a black acrylic box, allowing them to recover for 36–48 h. Cannulae were periodically flushed with heparinized (ammonium heparin, 100 IU/mL) Cortland saline (Wolf 1963). Water was circulated to the fish from a common head tank at 200 ± 16 mL·min^{-1}·fish^{-1}, collected in a sump tank, and then cooled, aerated, and returned to the head tank. Total volume of the system was 70–115 L, and there were no metal parts in contact with the water. A continuous slow input to the recirculating system of fresh lake water, or lake water plus toxicant, provided a 90% replacement time of 12–14 h (Sprague 1973). Water quality characteristics representative of the "best" and "worst" cases (i.e. start of the experiment, and in one trial where the system was closed for 24 h) are given in Table 1; typical experimental values lay between these extremes.

TABLE 1. Some water quality measurements under control conditions, means ± SE (n).

Variable	Lake Opeongo water prior to start of experiment	Lake Opeongo water from test battery after 24 h with fish in place, without replacement
Na^+ (mequiv/L)[a]	0.067±0.003 (5)	0.089±0.011 (6)
Cl^- (mequiv/L)[b]	0.028±0.003 (5)	0.075±0.029 (6)
K^+ (mequiv/L)[a]	0.020±0.001 (5)	0.056±0.018 (6)
Ca^{2+} (mequiv/L)[a]	0.170±0.001 (5)	0.170±0.012 (6)
NH_4^+ (mequiv/L)[c]	0.040±0.004 (4)	0.190±0.050 (4)
NO_2^- (mequiv/L)[d]	—[e]	0.001±0.005 (4)
Al (μg/L total)[f]	6.46	10.66
Zn (μg/L total)	<50	<50
Conductivity (μS/cm)	46.90±0.48 (8)	75.25±6.19 (8)
Temperature (°C)	15.5±0.3 (12)	15.5±0.3 (12)
pH[g]	6.78±0.27 (6)	7.32±0.05 (8)
Alkalinity (μequiv/L)[h]	95	282

[a]Measured by flame photometry.
[b]Measured by coulometric titration.
[c]Method of Verdouw et al. (1978).
[d]Standard methods (APHA et al. 1975).
[e]Not measured.
[f]Lumogallion fluorescence method of Playle et al. (1982).
[g]Statistics performed on [H^+] and converted to pH.
[h]Single measurement, inflection point titration.

Blood variables were measured daily for 4 d. After the first sample (control), reagent-grade $ZnSO_4 \cdot 7H_2O$ was added to the head tank to give 0.8 mg/L. The blood sampling protocol consisted of drawing 0.6 mL of arterial blood into gas-tight Hamilton syringes for determinations of arterial pH (pHa), total CO_2 (Ca_{CO_2}), oxygen partial pressure (Pa_{O_2}), hematocrit (hct), and concentrations of hemoglobin ([Hb]) and lactate. An additional 0.3 mL of sample was centrifuged for 5 min at 8000 × g and plasma removed for analysis of Na^+, Cl^-, K^+, Ca^{2+}, and total protein (C_{Pr}). The pellet of red blood cells (rbc) was resuspended in heparinized Cortland saline and the rbc returned to the fish to reduce loss due to sampling. Hemolysis occurred rarely, and these samples were discarded. Here, saline alone was infused to maintain extracellular fluid volume. On average, the total whole blood removed at each sample time was 0.6 mL, of which the rbc from 0.3 mL were returned. Data from fish whose hct fell below 5% were discarded, since anemia itself can provoke an acidosis (Wood et al. 1982). In fact, only 2 of 12 final hct values were <9%, and the mean was 13.5 ± 1.7% (12).

Branchial and Renal Flux Rates

To separately measure branchial and renal flux rates of ions and "acidic equivalents" under control conditions and subsequent exposure to 0.8 mg Zn^{2+}/L, we implanted only bladder catheters (Wood and Randall 1973b), using MS222 anesthesia, in a second group of fish. Each fish was then placed in a flux box (McDonald 1983a), consisting of a small inner box of clear

acrylic which confined the fish and a larger outer box of black acrylic which held 6–8 L of water. Boxes were placed on a wet table flooded with cool well water to maintain 15–17°C. An airlift pump at the rear of the inner box, together with perimeter aeration within each outer box, ensured circulation and maintained $Pw_{O_2} > 120$ Torr. Urine was collected in 50-mL flasks outside the boxes. Thus, changes in the composition of the water during the flux period reflected net branchial fluxes.

Following 3–4 d of recovery, three control fluxes were monitored, followed by five treatment (0.8 mg Zn^{2+}/L) fluxes of 12 h each. Variation within the three control periods was minimal, and composite control means are reported. Boxes were flushed with fresh solution (lake water or toxicant) at 12-h intervals, providing 70–80% replacement, based upon ammonia dilution. Since this procedure took ~2 h, the branchial fluxes were actually measured over 10 h, whereas the renal collections were throughout the entire 12-h period. Samples for water titratable alkalinity or urine titratable acidity minus bicarbonate ([TA − HCO_3^-]) were stored at 4°C and analyzed within 24 h. The remainder were frozen (−20°C) for later analysis.

Unidirectional branchial fluxes of Na^+ and Cl^- were partitioned after Kirschner (1970) during the first control flux period, flux period 1 (step change to Zn^{2+}), and flux period 5 (2 d of Zn^{2+} exposure). One hundred and eighty five kilobequerels (5 μCi) of $^{22}Na^+$ and 92.5 kBq (2.5 μCi) of $^{36}Cl^-$ (New England Nuclear) were added to each flux box and mixed thoroughly, and water samples were taken at 0, 0.5, 1, 1.5, 2, 3, 4, and 5 h.

Analytical Methods

Whole blood and plasma Ca_{CO_2} (Cameron 1971), pHa, and Pa_{O_2} were measured on a Radiometer PHM 71 acid–base analyzer fitted with gas modules. The pH and O_2 electrodes were water-cooled to the experimental temperature, while the CO_2 electrode in the Cameron chamber was maintained at 37°C. The pH electrode was calibrated frequently with precision buffers. The O_2 electrode was calibrated with water-saturated nitrogen or air and the Cameron chamber by known bicarbonate addition. L-(+)-lactate was determined enzymatically (LDH/NADH, Sigma Technical Bulletin 726UV/826UV) after deproteination of whole blood in 8% $HClO_3$. Hemoglobin was measured as cyanmethemoglobin (Sigma Technical Bulletin 525) using Sigma or Hycel reagents. Hematocrit was measured by centrifuging blood in heparinized microhematocrit tubes at 5000 × *g* for 5 min. The mean cell hemoglobin concentration (MCHC) was calculated as [Hb]/hct × 100 (Dacie and Lewis 1975). Plasma total protein determination was by refractometry (American Optical). Plasma Cl^- was titrated on a chloridometer (Radiometer CMT-10). After suitable dilution and swamping to eliminate interference effects, plasma Na^+ and K^+ were measured on an EEL mk 2 flame photometer. Plasma Ca^{2+} was measured by colorimetry (Sigma Technical Bulletin 585).

Major ions (Na^+, K^+, Ca^{2+}, Cl^-) in water and urine were measured as for plasma except for water Cl^-, which at very low levels was assayed with a Buchler–Cotlove chloridometer. The acid reagent had 0.2 mmol NaCl/L added to provide a linear response. Ca^{2+} and Zn^{2+} in the water and urine were determined by atomic absorption spectrophotometry (Varian AA1275, or initially for Zn^{2+}, a Jarrel-Ash 800). Total water and urine ammonia levels were determined using a micromodification of the salicylate–hypochlorite method of Verdouw et al. (1978). For titratable alkalinity, 10 mL of water was gently aerated and titrated with 0.02 mol HCl/L from a Gilmont burette to <pH 4, and the volume of titrant required to titrate to pH 4 was interpolated (DeRenzis and Maetz 1973; McDonald and Wood 1981). Aeration throughout titration ensured CO_2 removal. Titration of urine was by the single step determination of titratable acidity minus bicarbonate ([TA − HCO_3^-]) (Hills 1973) in which sufficient 0.02 mol HCl/L was added to 500 μL of urine to drive the pH to <4. This was then aerated for CO_2 removal and titrated back through the pHa (mean day 0 value, acid–base experiment) with freshly standardized 0.02 mol NaOH/L. The volume of titrant at pHa was interpolated and the volume of the acid added was subtracted.

For unidirectional flux measurements, water samples were counted as follows. $^{36}Cl^-$ is a pure beta emitter, while $^{22}Na^+$ is a mixed beta and gamma emitter. Dual labelled water samples were prepared in duplicate, with $^{22}Na^+$ alone measured in a well-type counter (Nuclear-Chicago model 1085) and $^{22}Na^+$ plus $^{36}Cl^-$ by scintillation counting (Beckman LS 250). After correction for difference in efficiency of $^{22}Na^+$ counting by the two machines, the $^{36}Cl^-$ counts were obtained by subtraction.

Calculations

The partial pressure of CO_2 in arterial blood (Pa_{CO_2}), the HCO_3^-, and the total blood metabolic acid load (ΔH^+_m) were calculated using standard acid–base equations as described by Spry and Wood (1984). Values for α_{CO_2} (CO_2 solubility in plasma), pK_1' (apparent first dissociation constant of carbonic acid), and β (nonbicarbonate buffering capacity) at a particular blood [Hb] were taken from Severinghaus (1965), Albers (1970), and Wood et al. (1982), respectively.

Net branchial ion fluxes observed from changes in waterborne concentrations were calculated as follows:

$$(1)\quad J_{net} = \frac{[X]_i V_i - [X]_f V_f}{t \cdot W}$$

where $[X]$ is the ion concentration (microequivalents per litre), V is the box volume which decreases during the period due to sampling, subscripts i and f are initial and final, respectively, t is the duration of the flux period (hours), and W is the fish weight (kilograms). Thus, net losses by the animal have a negative sign and net gains a positive sign. For titratable alkalinity, $V_t N/V_s$ is substituted for $[X]$, where V_t is the titrant volume (millilitres), N is the acid normality (microequivalents per litre), and V_s is the sample volume (millilitres). By reversing the i and f terms, the net titratable acidity flux was calculated from the titratable alkalinities. The net acidic equivalent flux is the sum of titratable acidity flux and ammonia flux, signs considered (cf. McDonald and Wood 1981).

For unidirectional branchial fluxes, J_{in} was calculated from the natural logarithm function given by Kirschner (1970), since no measurable backflux of isotope occurred:

$$(2)\quad J_{in} = \frac{Q_{out}}{t \cdot W} \cdot (\ln Q^*_{out(0)} - \ln Q^*_{out(t)})$$

where Q_{out} is total amount of the desired ion in the medium and Q^*_{out} is the total amount of radioactivity (cpm) at time 0 and t, respectively. J_{out} (negative by convention) was calculated as $J_{net} - J_{in}$. Renal ion losses were the product of concentration and urine flow rate (UFR) whereas the renal net acidic equivalent flux is given by

$$(3)\quad J_{out} = ([TA - HCO_3^-] + [NH_4^+]) \cdot UFR,$$

total ammonia being considered equivalent to $[NH_4^+]$, as free NH_3 was negligible at urine pH.

All values are reported as mean ± 1 standard error. To assess significant differences, we used paired Student's *t*-test where possible, since each fish served as its own control. Where missing values made this impossible, unpaired *t*-tests were used, with a resultant loss of power (Steel and Torrie 1960).

Results

Exposure to 0.8 mg Zn^{2+}/L in natural soft water resulted in significant blood acidosis, a decrease in plasma Ca^{2+}, increase in plasma K^+, and altered branchial ion and acidic equivalent fluxes. In contrast, there was little effect upon renal fluxes. The exposure was toxic, and overall mortality in 31 fish was 53% over 3 d. For blood data, values for all fish, and for those that survived the 3-d exposure, are shown separately in Fig. 1–3. Differences between the two data sets were not significant. The response of the survivors was therefore representative of the experimental population as a whole. For branchial and renal fluxes, only data from surviving animals have been plotted in Fig. 4–6 and Table 2 for the sake of clarity. Flux rates in dying fish showed similar trends, but generally increased and/or became highly erratic prior to death. Due to time constraints in the field situation, parallel controls were not run. However, under similar conditions in artificial softwater, the protocol caused neither mortality nor acid–base nor ionic disturbance (Spry and Wood 1984).

Examination of the acid–base status of fish that survived to the end of the experiment (Fig. 1a–1c) revealed a gradual and progressive decline in pHa which became significant on the final day. It was primarily respiratory in nature as shown by the rise in Pa_{CO_2}, with no significant change in plasma bicarbonate. Analysis of means plotted on a pH–bicarbonate diagram (Davenport 1974, not shown) indicated that the acidosis was mixed respiratory and metabolic, with the respiratory component becoming significant on the last day (Fig. 1c). The small but significant rise in blood lactate (Fig. 1e) was consistent with a metabolic component although the blood metabolic acid load was not significantly increased (Fig. 1f). The Pa_{O_2} remained uniformly high (Fig. 1d), a surprising finding in light of the increase in both Pa_{CO_2} and lactate. While ventilation was not directly assessed, there was no noticeable hyperventilation that might be associated with hypoxemia.

Additional blood parameters (Fig. 2) all showed significant declines with time. Similar declines were noted for hct, Hb, and C_{Pr} under control conditions (Spry and Wood 1984) which were attributable to the sampling protocol. This indicates that no significant hemoconcentration occurred in the present study, in contrast with that seen in more acutely lethal exposures at higher zinc levels (Spry and Wood 1984). However, MCHC did fall significantly (Fig. 2c), suggesting either that rbc swelling accompanied the developing acidosis or that a mobilization of Hb-poor rbc (e.g. reticulocytes) occurred.

Of the major plasma ions, Na^+ and Cl^- showed some fluctuation (Fig. 3a, 3b) but were essentially unchanged. However, K^+ rose significantly on the last day (Fig. 3c), while Ca^{2+} fell significantly after only 1 d (Fig. 3d), a trend that became even more pronounced over the following days.

Under control conditions, branchial ammonia excretion was ~200–250 $\mu equiv \cdot kg^{-1} \cdot h^{-1}$ (Fig. 4), or about 20-fold higher than renal ammonia efflux (Table 2). This was approximately balanced by the titratable acidity "uptake" (Fig. 4), such that

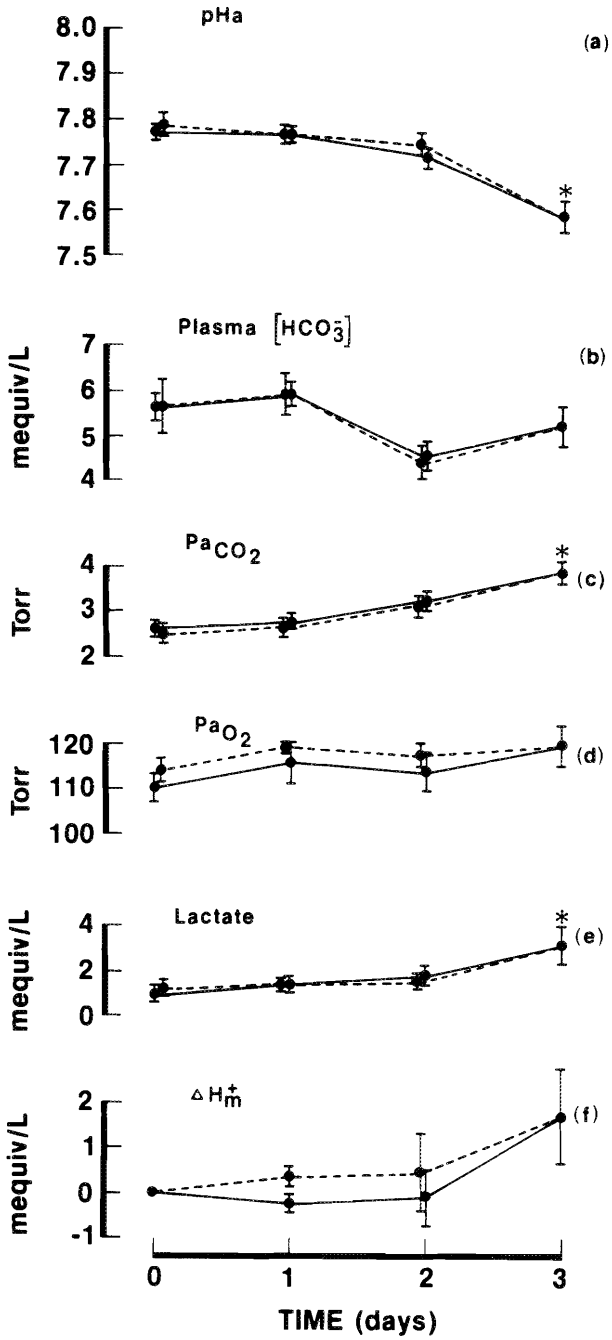

FIG. 1. Arterial blood measurements (a) pHa, (b) plasma $[HCO_3^-]$, (c) Pa_{CO_2}, (d) Pa_{O_2}, (e) lactate, and (f) metabolic acid load in rainbow trout under control conditions (day 0) and subsequently exposed to 0.8 mg Zn^{2+}/L. Means ± SE are shown for survivors to day 3 ($n = 11$, solid line) or all fish ($n = 30$, 24, 22, and 11 for day 0 to day 3, respectively). Asterisks denote means significantly different from control values ($p < 0.05$).

the net branchial acidic equivalent flux was either close to or slightly above zero (Fig. 4). Upon Zn^{2+} exposure, both components slowly increased over the following 3 d. However, the titratable acidity "uptake" increased to a greater extent than the ammonia efflux, so that an increasingly positive net acidic equivalent flux occurred, which reached approximately +200 $\mu equiv \cdot kg^{-1} \cdot h^{-1}$ by the final period.

The branchial net fluxes for Na^+, Cl^-, and Ca^{2+} (Fig. 5a, 5b, 5d) were positive under control conditions, representing a net

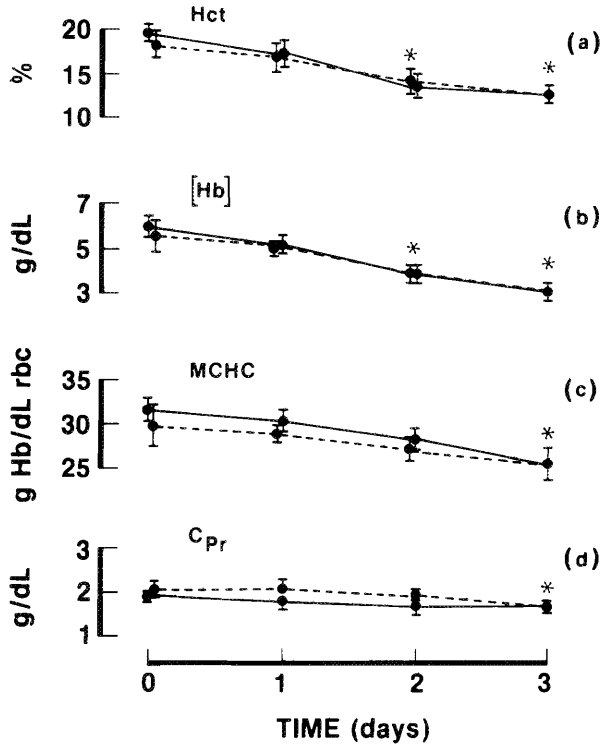

FIG. 2. Arterial blood measurements (a) hematocrit, (b) hemoglobin, (c) mean cell hemoglobin concentration, and (d) plasma total protein in rainbow trout under control conditions (day 0) and subsequently exposed to 0.8 mg Zn^{2+}/L in natural soft water. Means ± SE are shown for survivors to day 3 (n = 12, solid line) or all fish (n = 29, 24, 22, and 12 for day 0 to day 3, respectively). Statistics as in Fig. 1.

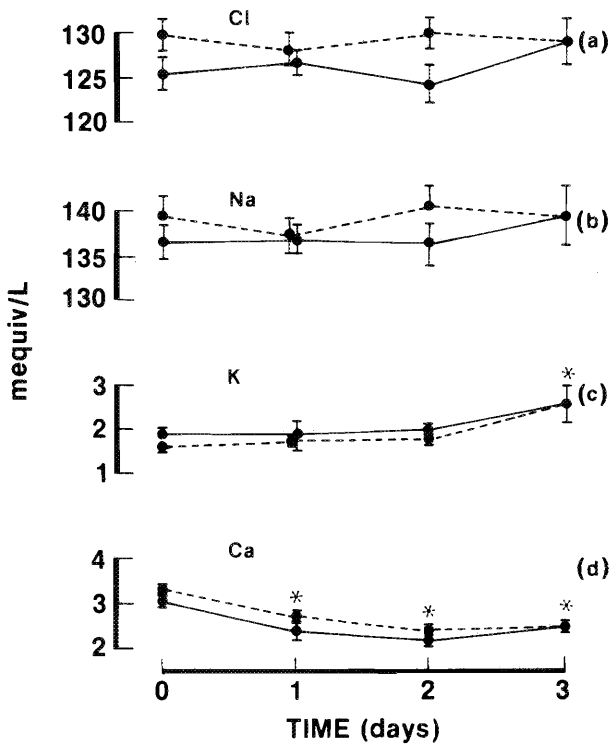

FIG. 3. Plasma ions (a) Cl^-, (b) Na^+, (c) K^+, and (d) Ca^{2+}. Legend as for Fig. 1 except for K^+ (survivors, solid line, n = 14; all fish, broken line, n = 30, 24, 22, and 14) and Ca^{2+} (survivors, n = 3; all fish, n = 13, 9, 6, and 3, on day 0 to day 3, respectively). Statistics as in Fig. 1.

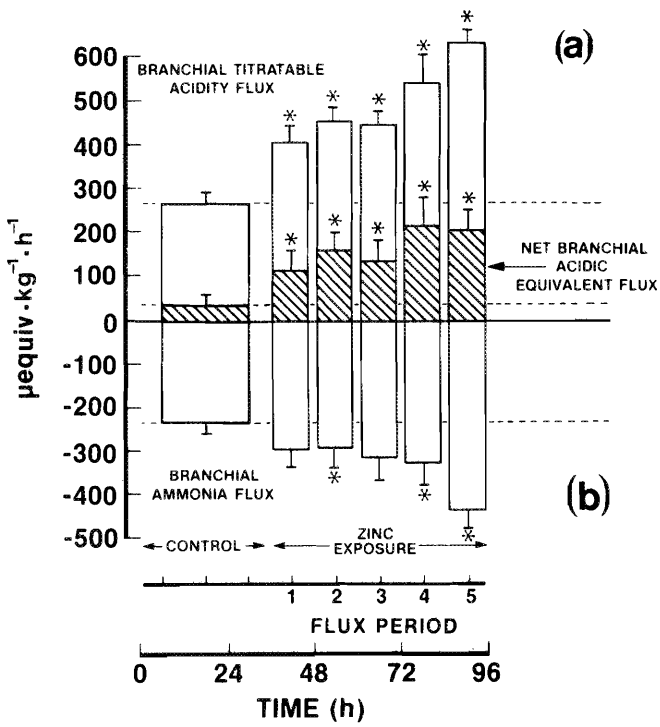

FIG. 4. Branchial net acidic equivalent flux components (a) titratable acidity flux, (b) ammonia flux, and the sum of (a) plus (b), the net acidic equivalent flux, in rainbow trout under control conditions (composite means) and during five subsequent fluxes in 0.8 mg Zn^{2+}/L, means ± SE, n = 13. Asterisks denote means significantly different ($p < 0.05$) from the composite control means (broken lines).

uptake from the water of approximately 20–40 μequiv·kg^{-1}·h^{-1}. K^+ (Fig. 5c) was an exception, exhibiting small but uniform losses. Exposure to Zn^{2+} immediately induced net losses in Na^+ and Cl^- and abolished Ca^{2+} uptake, but K^+ flux was not immediately affected. During continued Zn^{2+} exposure, the losses of Na^+ and Cl^- showed some sign of recovery after 36 h but then increased again in the last two flux periods. Overall Cl^- losses were greater than those of Na^+. K^+ losses only became significantly larger than the control mean during the last two flux periods. Net Ca^{2+} flux fluctuated near zero throughout the Zn^{2+} exposure. Two fish that were followed for an additional flux period (not shown) showed the same response for Na^+, Cl^-, and K^+ but interestingly, a positive net Ca^{2+} uptake indicating some potential for recovery.

Unidirectional flux measurements to partition Na^+ and Cl^- fluxes into efflux and influx components were performed in the first control flux period, flux period 1 (abrupt change to Zn^{2+}), and flux period 5 (2 d exposure) although not in all fish (Fig. 6). The abrupt change to Zn^{2+} increased Na^+ efflux alone, while continued exposure resulted in increases of both influx and efflux components. Effects upon Cl^- fluxes were similar, but with both components increasing immediately following Zn^{2+} exposure. In neither case were net fluxes significantly altered, in contrast with the pooled results above (Fig. 5a, 5b) although the overall trends were similar. This may have been due to the higher variability, smaller sample size, or the fact that the fluxes were only measured over 6 h instead of 12 h.

Under control conditions, renal losses of Na^+, Cl^-, and net acidic equivalents approximately balanced the net branchial uptake rates of these ions, while renal Ca^{2+} losses were only about 35% of the uptake rate at the gills (Table 2 vs. Fig. 4 and

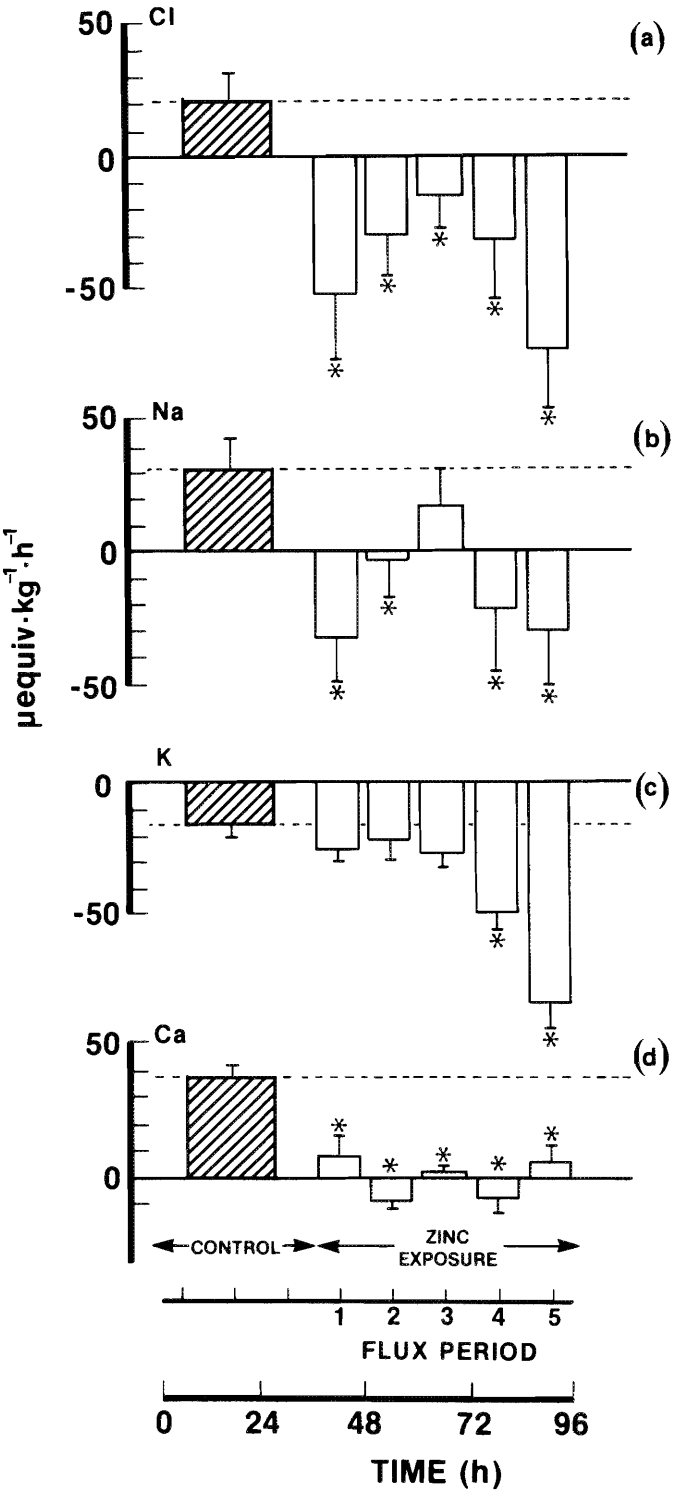

FIG. 5. Net branchial flux rates for (a) Cl^-, (b) Na^+, (c) K^+, and (d) Ca^{2+} in rainbow trout under control conditions (composite means) and subsequently exposed to 0.8 mg Zn^{2+}/L, means ± SE, n = 13 except for Ca^{2+} fluxes 3 and 5 where n = 12. Asterisks denote means significantly different ($p < 0.05$) from the control composite mean (broken lines).

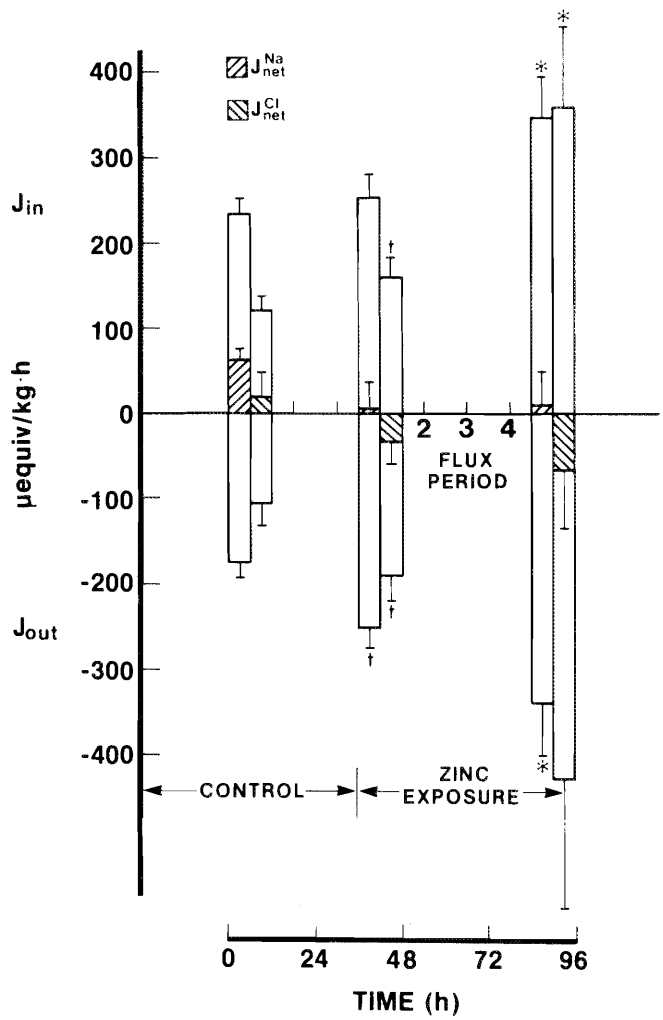

FIG. 6. Unidirectional (J_{in}, J_{out}) and net (J_{net}) branchial flux rates for Na^+ and Cl^- in rainbow trout under control conditions and subsequently exposed to 0.8 mg Zn^{2+}/L, means ± SE. For Na^+, n = 13, 13, and 7 for control flux 1, flux period 1, and flux period 5, respectively; for Cl^-, n = 9, 9, and 3. Significant differences ($p < 0.05$) from the control fluxes are denoted by daggers for paired t-test and asterisks for unpaired t-test.

5). Renal K^+ losses were only about 10% of renal Na^+ and Cl^- excretion rates, while renal Zn^{2+} excretion was about three orders of magnitude lower (Table 2). All the renal flux rates were relatively insensitive to Zn^{2+} exposure (Table 2). The only significant change was a decrease in Ca^{2+} efflux during flux period 5, possibly in response to decreasing entry of Ca^{2+} at the gill (Fig. 5d) and declining plasma Ca^{2+} levels (Fig. 3d). Notably there was no detectable change in the very low excretion of Zn^{2+} through the kidney, even over 60 h of exposure.

Discussion

The generally accepted consequence of exposure to acutely toxic waterborne Zn^{2+} (e.g. 1.5–40 mg/L) is an irreversible interruption of oxygen transfer across the gills caused by tissue damage. The resultant severe hypoxemia (Skidmore and Tovell 1972) with concurrent high lactate accumulation is fatal (Burton et al. 1972; Hodson 1976; Spry and Wood 1984). In the present study at 0.8 mg Zn^{2+}/L, significant mortality still occurred, although the Pa_{O_2} was unaffected (Fig. 1d) and the rise in blood lactate was rather small (Fig. 1e). This implies the presence of other toxic mechanisms which may be masked by the effects of higher concentrations of waterborne Zn^{2+}.

There are several possible explanations for the eventual small rise in blood lactate in the face of unchanged Pa_{O_2}. These

TABLE 2. Urine flow rates, and renal flux parameters in rainbow trout under control conditions and subsequently exposed to 0.8 mg Zn^{2+}/L in natural soft water. Values are means + SE(n). Units are $\mu equiv \cdot kg^{-1} \cdot h^{-1}$ except where noted. *Significantly different from composite control mean ($p < 0.05$).

Variable	Composite control	Flux period 1	2	3	4	5
UFR ($mL \cdot kg^{-1} \cdot h^{-1}$)	6.59	5.77	6.06	6.77	5.40	7.70
	1.24	1.33	1.70	1.38	0.96	2.79
	9	9	9	9	9	9
[TA − HCO_3^-]	−1.76	−3.31	−2.92	−4.42	−1.08	2.10
	1.29	2.30	3.45	2.01	1.46	6.47
	7	7	6	7	4	4
Ammonia	7.82	5.60	7.12	6.69	4.75	10.57
	1.84	1.27	1.94	2.14	1.61	3.79
	7	7	6	7	4	4
Net acidic equivalents	6.50	2.28	4.20	2.27	3.67	12.66
	2.49	2.32	3.10	1.15	2.01	7.68
	7	7	6	7	4	4
Sodium	43.36	34.88	31.08	43.82	35.88	35.45
	11.60	11.71	8.72	11.75	9.48	9.42
	9	9	9	9	9	9
Chloride	39.33	27.69	26.11	30.24	21.72	24.22
	12.02	10.73	8.76	9.80	6.16	8.19
	9	9	8	9	9	9
Potasssium	4.34	3.40	3.95	4.18	4.20	5.92
	0.88	0.93	1.14	0.79	1.14	1.76
	9	9	8	9	9	9
Calcium	11.66	8.17	11.00	8.14	4.31*	10.34
	3.08	3.08	4.56	2.80	1.19	6.01
	9	9	8	9	9	9
Zinc ($nequiv \cdot kg^{-1} \cdot h^{-1}$)	16.15	19.92	21.01	18.37	11.65	15.83
	5.29	8.99	9.08	8.53	4.25	4.95
	9	7	8	7	7	7

include a diffusive limitation at the gill, decreased O_2 delivery to the tissues through a decreased arterial O_2 content or cardiac output, and finally, decreased utilization by the tissues. The latter is supported by evidence in the literature (Hiltibran 1971; Zaba and Harris 1978), although the experimental conditions are not necessarily comparable. The high Pa_{O_2} argues against a diffusive limitation, especially when compared with the dramatic fall in Pa_{O_2} reported in the other studies where Zn^{2+} exposure was rapidly lethal (Skidmore 1970; Sellers et al. 1975; Spry and Wood 1984) and structural gill damage clearly occurred (Skidmore and Tovell 1972). However, the constancy of Pa_{O_2} is not conclusive evidence against diffusive limitation, because blood O_2 capacity and/or flow rate could be simultaneously reduced to such an extent that a high Pa_{O_2} occurred despite a diffusive limitation. Indeed, blood O_2 capacity was undoubtedly reduced both by sampling-induced reduction in [Hb] and the progressively developing acidosis (i.e. Root effect) in our experiments. Definitive separation of these possible explanations will require simultaneous measurements of O_2 uptake, cardiac output, inspired and expired water P_{O_2}, and both arterial and venous blood O_2 contents and P_{O_2} levels.

The rise in Pa_{CO_2} (Fig. 1c) is unlikely to be due to simple diffusion limitations across the gill, since CO_2 is about 30-fold more soluble in water than is oxygen (Dejours 1975), and the Pa_{O_2} was unaffected. Branchial and/or erythrocytic carbonic anhydrase is thought to play a critical role in the excretion of CO_2 (Maetz 1971; Perry et al. 1981) and is known to be inhibited in vitro by Zn^{2+} (Christensen and Tucker 1976). Such inhibition by Zn^{2+} as a cause of the apparent decreased excretion of CO_2 in Zn^{2+}-exposed trout needs to be examined. Neither the acid–base disturbance nor the lactate accumulation was the cause of mortality, since severely exercised trout routinely experience much more severe acidosis and blood lactate elevation from which they usually recover (Wood et al. 1983).

The trout in this study were raised in natural soft water of the Ontario Precambrian Shield area and were exposed to 0.8 mg Zn^{2+}/L in this medium. In our previous study (Spry and Wood 1984), hardwater-reared trout were acclimated to artificial soft water and then exposed to the same level of Zn^{2+}. There was considerable similarity of the test conditions in terms of methodology, major water electrolytes, temperature, and pH. Although fish were of different stocks, both were thoroughly domesticated hatchery trout, fed identical diets. The two studies were similar in showing negligible effects on Pa_{O_2} and plasma Na^+ and Cl^-, significant increases in Pa_{CO_2} and decreases in plasma Ca^{2+}, and similar overall mortality. There were, however, some subtle but important differences. The fish in artificial

soft water developed an alkalosis rather than an acidosis, and increases in blood lactate and K^+ did not occur. These differences may have a genetic basis and/or may involve some property of natural soft water such as its complement of trace elements, other metals, or organic components. Whatever the explanation, these findings emphasize the importance of examining fish in their natural environment in studies of this nature. Hōbe et al. (1984) reached similar conclusions with respect to the effects of environmental acid stress in natural versus artificial soft water.

In addition to CO_2 excretion and acid–base status, branchial ion flux rates (Fig. 5) were also affected by exposure to Zn^{2+}, Cl^- apparently more so than Na^+. The unidirectional flux measurements (Fig. 6) indicated that initially, only the Cl^- influx component was stimulated, while the efflux component for both Na^+ and Cl^- increased. Longer exposure significantly elevated both Na^+ and Cl^- influx. The pooled net flux data showed that net losses of both ions clearly occurred at the gills (Fig. 5a, 5b). These losses were probably due to increased permeability of the gills to ions, possibly by opening paracellular channels. This potential for ionic losses exists for other waterborne toxicants such as copper (Sellers et al. 1975; Laurén and McDonald 1985), mercury (Lock et al. 1981), and environmental acid (Leivestad and Muniz 1976; McDonald 1983b).

After continued exposure, influx of both Na^+ and Cl^- as well as Na^+ efflux were stimulated. Watson and Beamish (1981) found Zn^{2+} to be generally inhibitory to a variety of ATPases in vitro, but a 30-d in vivo exposure had stimulatory effects (Watson and Beamish 1980). Whether the in vitro response was purely pharmacological while the in vivo response was part of a homeostatic mechanism is not known. The K^+ fluxes in the present study were not affected until near the end of the Zn^{2+} exposure when plasma K^+ levels were elevated (Fig. 3c) and mortality was high. It may thus represent general release from the intracellular fluids in response to acidosis (e.g. Ladé and Brown 1963) or specific release from the intracellular space of the branchial tissue due to damage of the apical (water facing) membrane. Histological condition of the gills was not examined.

The abrupt abolition of net Ca^{2+} uptake by Zn^{2+} exposure (Fig. 5d) and associated fall in plasma calcium levels (Fig. 3d) is particularly interesting. Since unidirectional fluxes were not measured, we can only speculate as to whether this resulted solely from reduced influx, increased efflux, or some combination of the two. If the former is true, then Zn^{2+} might be displacing Ca^{2+} as a substrate for the Ca-ATPase which has been isolated from the gill (Ma et al. 1974; Fenwick 1976). Such "accidental active uptake" was suggested for uptake into a freshwater amphipod (Wright 1980) for both Zn^{2+} and Cd^{2+}. The apparent recovery of Ca^{2+} uptake by two fish also raises important questions as to the cause of inhibition, and the potential of the gill to recover its transport function or reduce its permeability.

Table 3 summarizes the cumulative branchial and renal fluxes (relative to the control condition) over the 60 h of Zn^{2+} exposure. Clearly, the gills were the major sites of ion loss and net acidic equivalent uptake. Compensation by the kidney, in the form of reduced ion losses and elevated acid excretion, was minimal. The tabulation shows a significant movement of net charge (+4887 μequiv/kg) unaccounted for, almost entirely at the gills, requiring entry of an unmeasured anion or loss of an unmeasured cation to maintain electroneutrality. Hōbe et al. (1984) observed a very similar discrepancy in white suckers (*Catostomus commersoni*) exposed to acid stress in the same natural soft water although they used an opposite sign convention to express it.

TABLE 3. Total fluxes of ions and acidic equivalents, relative to control levels, in rainbow trout exposed to 0.8 mg Zn^{2+}/L in natural soft water for 60 h. All units are in μequiv/kg, corrected for control rates. For each ion species, sign represents gain (+) or loss (−) from the animal. For the net charge, sign represents gain or loss of positive charge.

	Branchial	Renal	Total
Na^+	−2789	+429	−2360
Cl^-	−3751	+800	−2951
Ca^{2+}	−2314	+196	−2118
K^+	−1502	0	−1502
H^+ [a]	+7831	+85	+7916
Net charge[b]	+4977	−90	+4887

[a]Net acidic equivalents.
[b]Net charge = $Na^+ + K^+ + Ca^{2+} + H^+ - Cl^-$.

The role of the gills in both acid–base and ion regulation is intimately linked via the $Na^+/H^+(NH_4^+)$, Cl^-/HCO_3^- exchanges (Maetz 1971; Maetz et al. 1976; Girard and Payan 1980; Wood et al. 1984). The stimulation of net acidic equivalent uptake (base excretion) during Zn^{2+} exposure (Fig. 4) was the opposite of expected, since the fish were acidotic and net excretion of acidic equivalents both at the gill (McDonald et al. 1983) and kidney (McDonald and Wood 1981) normally occurs in the face of an acid load. We suggest that Zn^{2+} interfered with normal exchanges at the gill. Possible effects include stimulation of base excretion (increased Cl^- influx relative to Na^+ influx, as was seen upon initial Zn^{2+} exposure, Fig. 6), inhibition of acid excretion (decreased Na^+ uptake relative to Cl^- uptake), and elevated passive proton entry, which would be favoured by the pH gradient between soft water (pH ~ 6.7–7.3) and blood (pH ~ 7.8). All these would contribute to the metabolic component of the observed blood acid–base disturbance.

Although ammonia is a base, its loss from the fish occurs either as NH_3, in which case it does not affect the acid–base status of the fish, or as NH_4^+, which carries out a proton, and is therefore acidic equivalent excretion. Thus, although ammonia excretion increased due to Zn^{2+} exposure (Fig. 4), its contribution to acid excretion (currently under some debate, cf. Cameron and Heisler 1983; Wright and Wood 1985) was not sufficient to counteract the net base loss.

The lack of response by the kidney (Table 2) (with the exception of decreased Ca^{2+} excretion during one period) in the face of increased branchial ion losses indicated net whole body ion depletion (Table 3). Exchangeable NaCl in freshwater trout is about 48 mequiv/kg (Wood and Randall 1973a; McDonald 1983a). The observed Na^+ and Cl^- losses were thus about 5% of the exchangeable pool over 60 h. From the constancy of the plasma ions (Fig. 3a, 3b) we suggest an isosmotic loss, or replenishment from the intracellular compartment. However, such a rate of loss clearly could not be sustained. The decreased losses of Na^+ and Cl^- in flux period 3 (Fig. 5a, 5b) may represent compensation by the gill for these losses. The subsequent renewed loss suggests damage to gill tissue which precluded effective compensation, either through permeability changes or transport mechanisms.

In summary, exposure to waterborne Zn^{2+} altered both acid-base and ionoregulation in rainbow trout. Neither the acidemia

nor the ion disturbance alone or in combination appeared sufficient to cause the observed mortality within the time period of the experiment. As well, the Pa_{O_2} was unaffected. The primary lethal mechanism may well operate at the cellular level with the most likely effects either on oxygen delivery and/or utilization, or calcium homeostasis.

Acknowledgements

We are particularly grateful to Dr. J. A. MacLean and the entire staff of the Harkness Fisheries Laboratory for their hospitality and technical support. Some of the trout were a gift of the Ontario Ministry of Natural Resources, Skeleton Lake Hatchery. Dr. H. Hōbe, Lakehead University, is thanked for her assistance and discussion. This work was supported by grants from the Department of Fisheries and Oceans and the Strategic Program in Environmental Toxicology of the Natural Sciences and Engineering Research Council of Canada to C.M.W.

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