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Author(s): Michael P. Wilkie and Chris M. Wood
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Nitrogenous Waste Excretion, Acid-Base Regulation, and Ionoregulation in Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Extremely Alkaline Water

Michael P. Wilkie
Chris M. Wood
Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada

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Abstract

*Rainbow trout (*Oncorhynchus mykiss*) survived in alkaline fresh water (pH = 9.50) for 72 h, although the exposure rendered the fish more susceptible to mortality from other causes. At pH 9.50 ammonia excretion (J_{Amm}) was initially blocked, and total plasma ammonia levels increased. However, J_{Amm} steadily recovered thereafter; by 48 h control rates were reestablished and plasma total ammonia stabilized at six times the control level. The initial blockade of J_{Amm} was associated with a reversal of the blood to bulk water P_{NH_3} gradient. Paradoxically, the continued depression of J_{Amm} until 48 h occurred despite the presence of favorable blood-to-water gradients for passive NH_3 and NH_4^+ diffusion. An increase in urea excretion helped sustain waste N excretion in the face of inhibited J_{Amm}. A respiratory alkalosis (decreased arterial P_{CO_2}, increased arterial pH) occurred initially but was partially counteracted by a metabolic acidosis (decreased plasma HCO_3^-), which stabilized arterial pH at about 8.0 throughout the exposure. Increases in blood lactate, without marked changes in arterial O_2 tension, suggested that an activation of glycolysis occurred that was not caused by hypoxemia. Plasma Na^+ and Cl^- levels decreased by about 7% during the first 24 h of exposure but stabilized thereafter.*

Introduction

Most investigations of environmental pH effects on the physiology of fish have focused on increased acidity, reflecting the ongoing problem of environmental acidification (reviewed by McDonald 1983*a*; Wood 1989). Only a few have examined the effects of an alkaline environment (Jordan and Lloyd 1964; Johansen, Maloiy, and Lykkeboe 1975; Eddy, Bamford, and Ma-

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loiy 1981; Wright and Wood 1985; Heming and Blumhagen 1988; Playle and Wood 1989; Randall et al. 1989; Wood et al. 1989; Lin and Randall 1990). Studies on the rainbow trout (*Oncorhynchus mykiss*) have suggested that this species is unable to tolerate high pH (9.0–10.0) for more than a few hours or days (Jordan and Lloyd 1964; Heming and Blumhagen 1988; Randall and Wright 1989). However, in the wild, the related cutthroat trout (*Oncorhynchus clarki henshawi*) thrives in highly alkaline lakes of comparable pH (Galat et al. 1985). The goals of the present study were to determine whether or not the rainbow trout can survive an acute exposure to pH = 9.50 for a 3-d period and to identify the associated physiological mechanisms that either permit adaptation or result in mortality. Our study focused on three areas: nitrogenous waste excretion, acid-base balance, and ionoregulation.

Acute exposure to alkaline pH is known to inhibit branchial ammonia excretion (Wright and Wood 1985; Randall and Wright 1989; Lin and Randall 1990). This phenomenon is probably due to a reduction of the diffusion gradient for NH_3 (ΔP_{NH_3}) across the gills when the environmental pH approaches the negative logarithm of the dissociation constant for ammonia (pK_{Amm}; about 9.50 at 15°C; Cameron and Heisler 1983), and to an inhibition of branchial Na^+ influx/NH_4^+ efflux exchange (Wright and Wood 1985). However, fish are still able to excrete some ammonia N in the face of opposing ΔP_{NH_3}'s (Maetz 1972, 1973; Payan 1978; Cameron and Heisler 1983; Wright and Wood 1985; Cameron 1986; Claiborne and Evans 1988). This raises the possibility that recovery of Na^+/NH_4^+ exchange or induction of other mechanisms (see Evans and Cameron 1986) might support adequate rates of ammonia N excretion over the longer term. A more unusual strategy has been identified in a tilapia (*Oreochromis alcalicus grahami*) that thrives in the highly alkaline water (pH $\simeq$ 10) of Lake Magadi, Kenya. This fish excretes all waste nitrogen as urea rather than ammonia (Randall et al. 1989; Wood et al. 1989). To address these possibilities, the present study examined rates of ammonia N (J_{Amm}) and urea N excretion (J_{Urea}) and plasma levels of these two waste products in trout throughout the exposure to alkaline pH.

At high pH, the water outside the gills becomes an essential "vacuum" for P_{CO_2} because of diffusion trapping of CO_2 as HCO_3^- or CO_3^{2-} (Johansen et al. 1975). Decreases in arterial CO_2 tension (Pa_{CO_2}) and resulting increases in arterial pH (pHa) ("respiratory alkalosis") are known to occur during the first few hours of exposure (Wright and Wood 1985; Lin and Randall 1990). Over the longer term, increases of plasma HCO_3^- and additional increases in pHa ("metabolic alkalosis") might result from losses of H^+ and/or entry of HCO_3^-, OH^-, or CO_3^{2-} at the gills. Alternately, some form

of metabolic compensation might be implemented. Heming and Blumhagen (1988) reported elevated plasma HCO_3^- and pHa levels after 1 d of high pH exposure, which were apparently corrected on subsequent days in surviving trout. These possibilities were evaluated by following arterial acid-base status throughout the exposure in the present study.

Ionoregulatory failure is the major cause of death in fish acutely exposed to acidic environments (pH = 4.0–4.5) because of inhibition of active influx and stimulation of diffusive efflux of Na^+ and Cl^- at the gills (reviewed by McDonald 1983*a*; Wood 1989). Similar effects have been observed during acute exposure to alkaline pH (Wright and Wood 1985; Heming and Blumhagen 1988; Wood 1989), which raises the possibility that considerable ionoregulatory disturbance might occur during longer-term exposure. Accordingly, the levels of plasma Na^+ and Cl^- were monitored in the rainbow trout exposed to high pH in the present study.

Material and Methods

Experimental Animals and Setup

Adult rainbow trout (*Oncorhynchus mykiss* = *Salmo gairdneri*, 346.5 ± 8.6 g, n = 105) of both sexes were obtained from Spring Valley Trout Farm, New Dundee, Ontario, held in moderately hard dechlorinated Hamilton city tap water (Ca^{2+} = 1.0, Mg^{2+} = 0.15, Na^+ = 0.6, Cl^- = 0.8, titration alkalinity = 2.1 mmol · L^{-1}; hardness = 140 mg · L^{-1} as $CaCO_3$) at 6°–15°C and were fed twice weekly with commercial trout pellets (Martin Feed Mills). One to two weeks prior to experiments, batches of 20 fish were transferred to a 560-L "Living Stream" (Frigid Units) that was supplied on a flow-through basis with the same water at 15° ± 1°C. During this acclimation period, the fish were starved to remove the influence of feeding on nitrogen metabolism (Fromm 1963).

Approximately 48 h prior to experimentation, the fish were fitted with dorsal aortic catheters (Soivio, Westman, and Nyholm 1972) while under MS-222 anesthesia (1:10,000 dilution; Sigma) and then transferred to one of seven darkened Plexiglas flux boxes in a 200-L, continuously flowing recirculating system (15° ± 0.2°C). Each box received a flow of about 2 L · min^{-1} and contained one fish. Water in the reservoir tank of the system was continually replaced at approximately 2 L · min^{-1}, which led to water total ammonia levels (T_{Amm}) of about 10 μmol · L^{-1}. The flux boxes, described by McDonald (1983*b*), comprised an inner chamber containing the fish and an outer chamber containing most of the water volume (approx. 5 L). Vigorous aeration of the outer chamber maintained circulation during

periods when the box was operated as a closed system for flux determinations.

The head tank of the recirculating system was fitted with a Radiometer GK2401C combination pH electrode connected to a PHM 82 meter and TTT80 autotitrator. This pH-stat maintained the desired water pH in the face of continual acidification due to CO_2 production by the fish and the replacement addition of fresh water. Activation of the titrator opened an electromagnetic valve (Nacon Industries), which allowed 1.0 N NaOH to flow in a dropwise fashion into the vigorously aerated head tank. Control water pH was 8.15 ± 0.04, and the experimental pH was kept at 9.50 ± 0.03 as measured in the boxes by an independent Radiometer GK2401C electrode and PHM 72MK2 meter. Addition of NaOH by the titrator during the pH = 9.50 experimental period resulted in an elevation of water Na^+ from the control level of 0.6 mmol · L^{-1} to approximately 4.2 mmol · L^{-1}. Simultaneously, the water Ca^{2+} declined from 1.0 mmol · L^{-1} to about 0.15 mmol · L^{-1}, associated with an obvious precipitate that accumulated in the system. When the flux boxes were closed systems for flux determinations, the initial pH was set by the pH-stat, but thereafter each box was manually titrated at 15-min intervals with 1.0 N NaOH, by means of the independent electrode and meter.

Experimental Protocol

Flux determinations (3 h) for J_{Amm} and J_{Urea} were performed under control conditions (pH = 8.15, after 48 h recovery from surgery) and at 0–3 h, 8–11 h, and approximately 24 h, 48 h, and 72 h exposure to pH = 9.50. At the start of the 0–3-h flux, we flushed the boxes with pH 9.50 water for 10 min to quickly raise the pH from 8.15 to 9.50 and then closed them. Water samples (15 mL) were taken at 0 h, 1 h, 2 h, and 3 h of each flux period and analyzed for T_{Amm} and urea. For J_{Amm}, fluxes were calculated from changes in water T_{Amm} over the first hour only, in order to minimize the known influence of elevated water T_{Amm} on the measured rate (Wright and Wood 1985). Typically, water T_{Amm} rose to no more than 40 μmol · L^{-1} over this first hour. Had we instead chosen to calculate J_{Amm} values from the T_{Amm} changes over the full 3-h periods, absolute rates would have been lower but none of the conclusions would have been qualitatively altered. The much smaller J_{Urea} values were measured over the full 3-h period, because changes in water urea concentration were too small to measure reliably over 1-h periods. We have no information on whether J_{Urea} values are influenced by the levels of water T_{Amm} that accumulated. Between flux periods, the boxes were opened to the pH-stated recirculating system and operated in

this open mode until the next determination. We ran several flux tests with known T_{Amm} but no fish present to check for ammonia loss at pH 9.50; this was negligible.

Blood samples (450–550 μL, drawn anaerobically via the catheters into ice-cold, gastight Hamilton syringes) were taken immediately prior to the start of all flux periods except the first experimental period. Sampling at this time avoided disturbance during flux determinations while providing measurements of plasma T_{Amm} and acid-base status only 30 min before the midpoint of the J_{Amm} measurements, before water T_{Amm} had increased to any great extent. This prevented overestimation of plasma T_{Amm} levels that might have been caused by progressive elevation of plasma T_{Amm} levels due to box closure during the flux period. Diffusion gradients of P_{NH_3} and NH_4^+ from plasma to bulk water (ΔP_{NH_3} and $\Delta[NH_4^+]$, respectively) were calculated from the blood measurements prior to the flux period and the water levels measured at the start of the flux period. The diffusion gradients reported for the first 1 h of exposure to pH 9.50 are based on the control blood sample, because it is unlikely that blood parameters would change during the first few minutes of exposure to high pH.

Blood samples were analyzed for hematocrit (Ht), pHa, arterial O_2 tension (Pa_{O_2}), lactate, true plasma total CO_2 (Ca_{CO_2}), plasma T_{Amm}, urea, Na^+, and Cl^- concentrations. To avoid excessive removal of blood, we did not measure all parameters in all fish; rather, different experimental series focused on different combinations of parameters. After sampling, remaining blood from the P_{O_2} electrode was returned to the fish, together with sufficient Cortland saline (Wolf 1963) to restore the blood volume.

Analytical Techniques and Calculations

Analytical methods were identical to those employed by Wright and Wood (1985), with the following exceptions. Arterial P_{O_2} was measured with a Radiometer E5046 electrode thermostated to 15°C; Ca_{CO_2} was measured with a Capnicon (Cameron Instruments); plasma and water urea were measured by the diacetyl monoxime method of Crocker (1967); lactate was measured by the L-lactate dehydrogenase method/NADH method as described by Turner, Wood, and Clark (1983); plasma Na^+ was measured by atomic absorption (Varian AA1275); and plasma Cl^- was measured by coulometric titration (Radiometer CMT10).

Values of J_{Amm} and J_{Urea} were calculated over 1-h and 3-h flux periods, respectively, from measured concentration changes in the water, the known

volume of water in the flux box, and the fish's body weight. Arterial P_{CO_2} and arterial plasma HCO_3^- were calculated from pHa and C_{aCO_2} by using appropriate solubility coefficients (αCO_2), pK_1' values from Severinghaus (1965) as tabulated by Boutilier, Heming, and Iwama (1984), and standard manipulations of the Henderson-Hasselbalch equation. Plasma and water NH_3, NH_4^+, P_{NH_3}, and diffusion gradients from blood plasma to water were similarly calculated from T_{Amm} and pH measurements in each medium, appropriate solubility coefficients (αNH_3), and pK_{Amm} values from Cameron and Heisler (1983), and the Henderson-Hasselbalch relationship. All relevant equations are given by Wright and Wood (1985). The net load of acidic equivalents ("metabolic acid load" [ΔH^+m]) in the blood at each sample time was calculated from changes in pHa, HCO_3^-, and Ht (as an index of non-HCO_3^- buffer capacity), as outlined by McDonald, Hobe, and Wood (1980).

One important difference from Wright and Wood (1985) is that all reported blood plasma values are based on the measured arterial value alone. Ventral aortic concentrations were not measured. Therefore the diffusion gradients were calculated from arterial blood plasma to bulk water and are undoubtedly lower than if a mean arterial-venous figure had been used. In view of the large changes observed in plasma T_{Amm} during the experiment, we were not confident in applying the 1.33× correction factor developed by Wright and Wood (1985) to estimate the mean arterial-venous T_{Amm} levels.

All results have been expressed as means ± 1 SEM (n) where n represents the number of animals contributing data to the mean. A paired Student's t-test (two-tailed; $P < 0.05$) was used to determine the significance of changes, with each animal serving as its own control.

Results

Survival

Approximately 40% of the fish tested died during the 72-h exposure to pH 9.50. In most, though not all, cases these mortalities could be attributed to experimental mishap—for example, overt bleeding, abnormally low Ht, blood clots, and pH-stat or aeration failure. However, it was our impression that the fish were much less resistant to experimental disturbance at pH 9.50 than at pH 8.15. As a check, 10 cannulated trout were subjected to an identical confinement and blood sampling regime in the experimental system, but water pH was kept at 8.15. None died, despite very low Ht's in several fish. To confirm that the exposure to pH 9.50 did not itself induce mortality, we performed four separate series of experiments, using a total

of 24 uncannulated fish. Only one of these fish died during 72 h of exposure to pH 9.50 in the experimental system. In a subsequent study (M. P. Wilkie and C. M. Wood, unpublished observations) conducted in the same water quality at pH 9.50 with free-swimming, routinely fed trout, there was no mortality during a 5-wk exposure. We conclude that exposure is not in itself toxic but does render the fish more susceptible to other stresses.

Ammonia Excretion

Rainbow trout exhibited a control J_{Amm} of approximately 220 μmol N · kg^{-1} · h^{-1} at pH 8.15 (fig. 1*A*). Immediately on exposure to pH 9.50, ammonia excretion was blocked and there was a short-lived net uptake of ammonia. By 8 h, a net efflux of ammonia resumed, but this remained significantly lower than the control rate at both 8 h and 24 h. The rate of ammonia N excretion continued to recover with time; control rates were reestablished at 48 h and 72 h. Arterial plasma T_{Amm} was about 100 μmol · L^{-1} under control conditions (fig. 1*B*). Plasma T_{Amm} had increased about fourfold by 8 h and almost sixfold by 24 h, in accord with the inhibition of J_{Amm}; it then remained at this level for the duration of the exposure.

At pH 8.15, ΔP_{NH_3} was not significantly different from zero (fig. 1*C*). However, in the first hour of exposure to pH 9.50, estimated ΔP_{NH_3} declined to −400 μTorr, which explained the negative J_{Amm} at this time. By 8 h, ΔP_{NH_3} had increased markedly, to almost +150 μTorr, and it remained there through 72 h. The recovery of J_{Amm} did not appear to be completely correlated with the establishment of the elevated ΔP_{NH_3}, because J_{Amm} was still significantly lower at 8 and 24 h, while ΔP_{NH_3} had rebounded by 8 h (cf. fig. 1, *A* and *C*). At pH 8.15, $\Delta[NH_4^+]$ was about +60 μmol · L^{-1} (fig. 1*D*). On initial exposure to pH 9.50, $\Delta[NH_4^+]$ was unchanged but by 8 h and 24 h had increased to about 6 and 12 times the control level, respectively, stabilizing thereafter.

Control J_{Urea} at pH 8.15 was approximately 32 μmol N · kg^{-1} · h^{-1}, accounting for only about 12% of total waste N excretion (fig. 2*A*). The rate of urea N excretion increased significantly, to 53 μmol N · kg^{-1} · h^{-1} after 8 h alkaline exposure, at which time it represented 45% of total waste N excretion; J_{Urea} remained significantly elevated through 72 h (fig. 2*A*), but its relative contribution to total waste N excretion progressively decreased toward control levels as J_{Amm} recovered (fig. 1*A*). Plasma urea N was about 5,000 μmol N · L^{-1} at pH 8.15 (fig. 2*B*), or about 50-fold higher than plasma ammonia N (fig. 1*B*). Throughout most of the exposure to pH 9.50, plasma urea N did not change; it was significantly lower than control only at 48 h (fig. 2*B*).

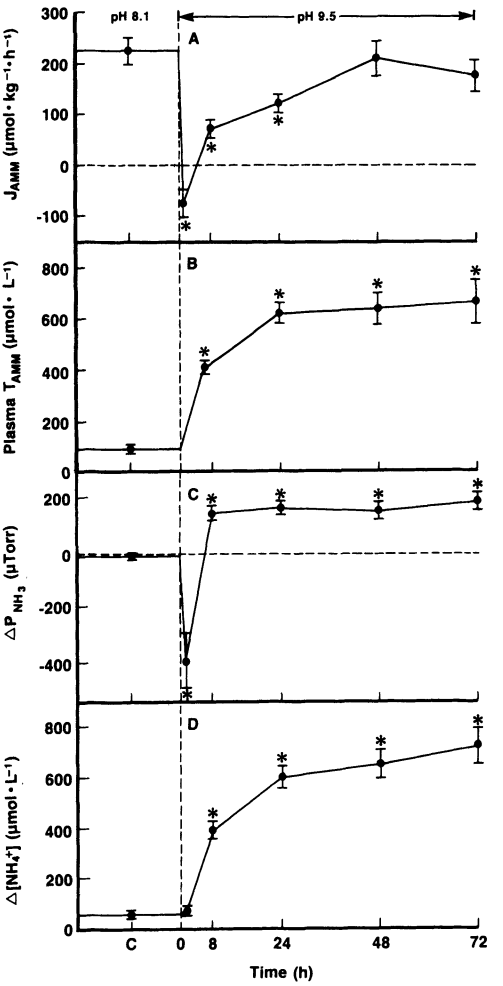

Fig. 1. Influence of severe alkaline exposure (pH = 9.50) on (A) J_{Amm}, (B) *plasma* T_{Amm} *in rainbow trout,* (C) ΔP_{NH_3} *between arterial blood and bulk water, and* (D) $\Delta[NH_4^+]$ *from arterial blood to water across the gills of rainbow trout. Values are means* ± *1 SEM;* n > *20 for the control, 1-h, 8-h, and 24-h periods;* n = *9–11 at 48 h;* n = *7–9 at 72 h. Asterisks indicate significant differences from control values* ($P < 0.05$).

Blood Acid-Base Status

Blood acid-base disturbances are displayed on a pH-HCO_3^- diagram (fig. 3). A significant respiratory alkalosis was seen at 8 h exposure to pH 9.50, reflected in a rise in pHa from 7.83 to 7.97. Arterial P_{CO_2} declined from 3.1 to 1.4 Torr, and plasma HCO_3^- fell from 7.5 to 5.1 mmol · L^{-1}. Arterial pH stabilized after this initial increase, despite a further progressive decline in

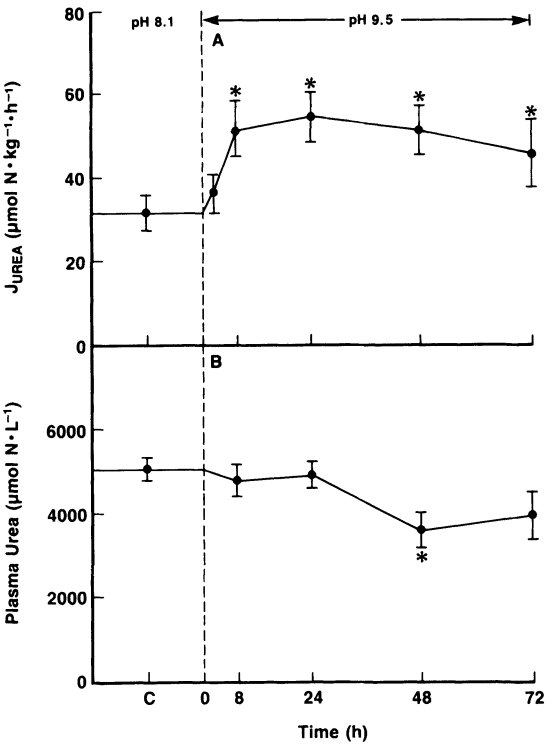

Fig. 2. Influence of severe alkaline (pH = 9.50) exposure on (A) J_{Urea} and (B) plasma urea concentration in rainbow trout. Values are means ± 1 SEM; n > *20 for the control, 3-h, 8-h, and 24-h periods for both J_{Urea} and plasma urea;* n = *26 at 48 h and* n = *19 at 72 h for J_{Urea};* n = *12 at 48 h and* n = *7 at 72 h for plasma urea. Asterisks indicate significant differences from control values (*P < *0.05).*

$Paco_2$ to 0.7 Torr by 72 h. This stabilization was attributable to a concurrent metabolic compensation (see Discussion) reflected in a marked, continual decline in plasma HCO_3^- to 2.5 mmol · L^{-1} by 72 h. The calculated ΔH^+m rose progressively during alkaline exposure, reaching 3.3 mmol · L^{-1} by 72 h (fig. 4*A*).

Control blood lactate was approximately 0.5 mmol · L^{-1} at pH 8.15 (fig. 4*B*). After 8 h in alkaline water, lactate increased about threefold, and after 24 h about sixfold. Up to 24 h, lactate and ΔH^+m were similar, but thereafter lactate declined while ΔH^+m continued to rise (fig. 4*A, B*). However, lactate remained significantly elevated above the control level through 72 h of alkaline exposure. This increase in blood lactate was not correlated with any marked disturbance of arterial blood oxygenation. Arterial Po_2, approximately 105 Torr at pH 8.15, exhibited a slight but significant decline after

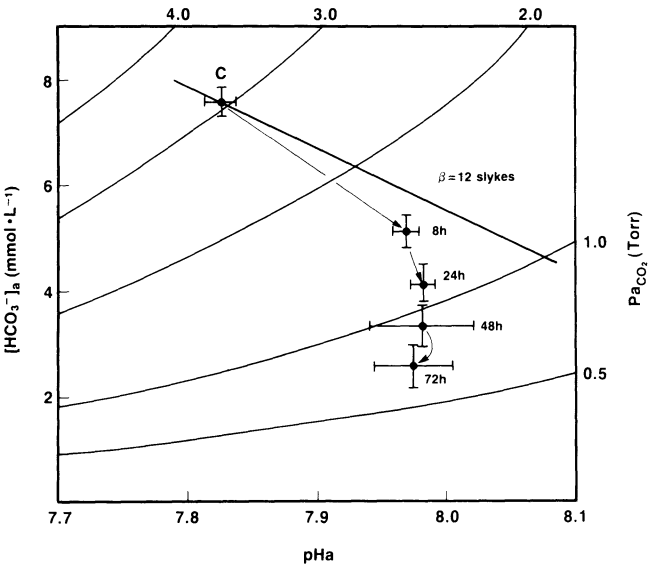

Fig. 3. A pH-HCO_3^- diagram showing changes in arterial blood acid-base parameters (pHa, Pa_{CO_2}, and plasma $[HCO_3^-]$) over time during exposure of rainbow trout to extremely alkaline water (pH = 9.50). Values are means ± 1 SEM. For all three parameters, n > *20 during the control, 8-h, and 24-h periods,* n = *13 at 48 h, and* n = *9 at 72 h. All parameters were significantly different from control values at 8, 24, 48, and 72 h.*

8 h at high pH (fig. 4*C*). However, Pa_{O_2} did not deviate significantly from the control value for the remainder of the exposure period.

Ionoregulation

Control Na^+ and Cl^- levels in blood plasma were approximately 143 and 138 mmol · L^{-1} (fig. 5). By 24 h exposure to pH 9.50, these declined by about 6% and 8%, respectively, but there was little further change during the remainder of the experiment.

Discussion

Survival

Rainbow trout can survive at pH 9.50, but the exposure clearly disturbs internal homeostasis of ammonia, acid-base status, and ions and renders the fish more susceptible to mortality from other causes. It is likely that experimental stresses (catheterization, blood sampling, confinement, etc.) were

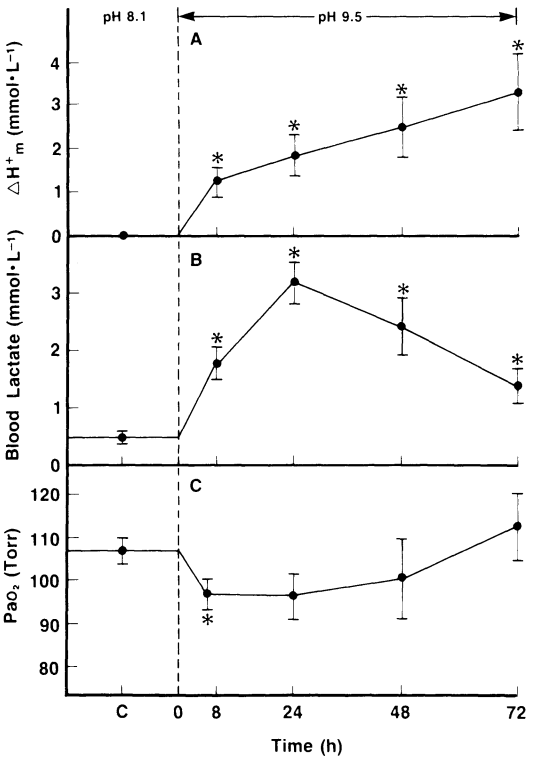

Fig. 4. Influence of severe alkaline exposure (pH = 9.50) on (A) $\Delta H^+ m$, (B) blood lactate concentration, and (C) Pa_{O_2}. Values are means ± 1 SEM; n > *15 for the control, 8-h, and 24-h periods for all three parameters;* n = *13 at 48 h and* n = *9 at 72 h for $\Delta H^+ m$;* n = *11 at 48 h and* n = *7 at 72 h for blood lactate;* n = *8 at 48 h and* n = *5 at 72 h for Pa_{O_2}. Asterisks indicate significant differences from control values* (P < *0.05).*

responsible for unexplained fatalities and contributed to the higher rates of mortality at comparable pH reported by Jordan and Lloyd (1964), Heming and Blumhagen (1988), and Randall and Wright (1989). In the latter two studies, mortality was attributed to ionoregulatory disturbance and ammonia toxicity, respectively. The trout of Heming and Blumhagen (1988) exhibited much larger decreases in plasma electrolytes despite a less severe alkaline exposure (pH 8.7), presumably due to differences in the experimental conditions or fish stocks used, because water chemistry was comparable in the two studies. Randall and Wright (1989), based on the work of Lin and Randall (1990), reported that trout died when plasma T_{Amm} built up to only about 20% of the levels observed in surviving trout of the present study. However, firm conclusions are difficult because the alkaline exposure was more severe (pH 10.0), the water chemistry was very different (extremely soft water with

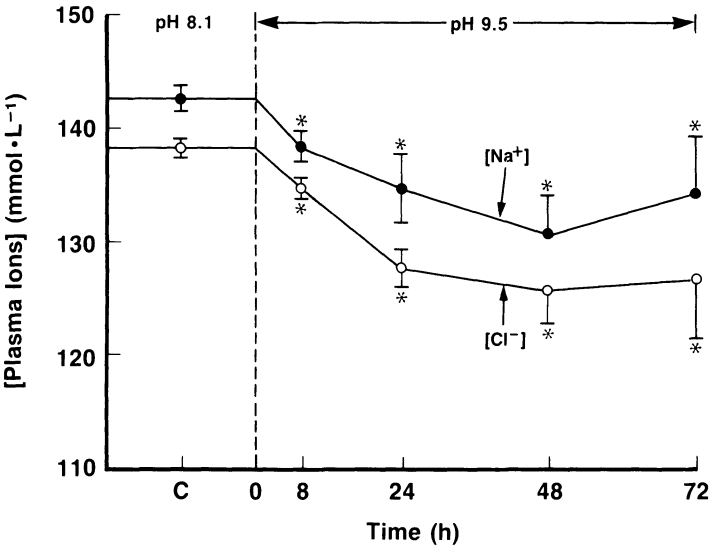

Fig. 5. Influence of severe alkaline exposure (pH = 9.50) on plasma Na^+ and Cl^- concentrations in rainbow trout. Values are means ± 1 SEM. For both Na^+ and Cl^-, n > 20 during control, 8-h, and 24-h periods; n *= 14 at 48 h and* n *= 9 at 72 h for plasma Cl^-;* n *= 13 at 48 h and* n *= 9 at 72 h for plasma Na^+. Asterisks indicate significant differences from control values (*P *< 0.05).*

elevated NaCl and $CaCl_2$), and the trout were loaded with the additional stress of ventilation masks. Whatever the exact cause of death, it is clear from all three investigations that water pH's around 9.50 are marginal for the survival of *Oncorhynchus mykiss*. This probably explains the failure of stocking efforts for rainbow trout in alkaline lakes where the Lahontan cutthroat trout thrives (Galat et al. 1985).

Nitrogenous Waste Excretion

Acute exposure to pH 9.50 caused an immediate blockade of J_{Amm}, which is in agreement with the earlier work of Wright and Wood (1985) conducted in the same water quality. However, the present study demonstrated that, with continued high pH exposure, J_{Amm} gradually returned to control levels and plasma T_{Amm} stabilized at about six times the control concentration. Wright and Wood (1985) attributed the immediate blockade of J_{Amm} partially to an inhibition of branchial Na^+/NH_4^+ exchange, and partially to a reversal of ΔP_{NH_3} from blood plasma to bulk water. In the present study, this gradient became highly negative during the first hour of high pH exposure, which

explains the transitory net uptake of ammonia by the fish. The subsequent rise in ΔP_{NH_3} to highly positive values (due to the build-up of plasma T_{Amm} and rise in pHa) would favor NH_3 diffusion from blood plasma to water and may have played a role in the recovery of J_{Amm}. However, this does not appear to be the complete explanation, because the recovery of J_{Amm} was slower than the elevation of ΔP_{NH_3}. At present, we can only speculate as to additional mechanisms involved in the recovery of J_{Amm}. Possibilities include (1) activation of Na^+/NH_4^+ exchange (Cameron and Heisler 1983; note the stabilization of plasma Na^+ after the first 24 h of alkaline exposure), (2) activation of H^+/NH_4^+ exchange (Cameron 1986), (3) reacidification of the gill boundary layer through increased CO_2 or H^+ production (Wright, Randall, and Perry 1989), and (4) increased simple diffusive efflux of NH_4^+ favored by the marked rise in $\Delta[NH_4^+]$ (McDonald, Tang, and Boutilier 1989). All should be amenable to experimental test in future investigations.

During high pH exposure, trout were able to tolerate a sustained increase in plasma T_{Amm} to more than 600 $\mu mol \cdot L^{-1}$, yielding a P_{NH_3} of more than 250 μTorr, both well above the normal physiological range. However, these levels are much lower than those measured in the plasma of trout dying from acute injections of ammonium salts (Hillaby and Randall 1979). They are also lower than levels in the plasma of seawater-adapted trout surviving for 24 h in elevated ambient ammonia (R. W. Wilson, personal communication). Thus rainbow trout can tolerate abrupt short-term increases in plasma T_{Amm}, but it remains an open question whether they can tolerate such levels indefinitely.

The inhibition of J_{Amm} by alkaline exposure resulted in an "ammonia deficit" of about 4,500 $\mu mol\ N \cdot kg^{-1}$ before normal rates were resumed at 48 h. On the basis of measured changes in plasma T_{Amm}, storage in the extracellular fluid volume ($\sim$0.25 $L \cdot kg^{-1}$; Milligan and Wood 1986) amounted to only 3% of this figure, which suggests that ammonia N production was inhibited, redirected to other end products, or that ammonia was stored elsewhere. While we have no information on ammonia production rates, the measured increase in urea N excretion certainly accounted for about 20% of the ammonia deficit. Conversion of ammonia to relatively nontoxic glutamine, as observed in the brain of goldfish exposed to high ambient ammonia (Levi et al. 1974), is another possibility. Intracellular white muscle ammonia levels increase by up to 7,000 $\mu mol \cdot kg^{-1}$ after exercise in trout (Mommsen and Hochachka 1988; Wright and Wood 1988), so this is one likely storage site.

The elevated J_{Urea} during high pH exposure ensures that some nitrogenous waste excretion occurs while J_{Amm} is inhibited. This may prevent acute ammonia toxicity. The response is similar to that exhibited by trout exposed

to high ambient ammonia, which similarly inhibits J_{Amm} (Olson and Fromm 1971). This small increase in J_{Urea} is reminiscent of the strategy (complete ureotelism) employed by the tilapia endemic to the pH 10 water of Lake Magadi, Kenya (Randall et al. 1989; Wood et al. 1989). In mammals, urea synthesis is favored by internal alkalosis (Bean and Atkinson 1984). However, in contrast to mammals and the Lake Magadi tilapia (Randall et al. 1989) both of which have an active ornithine urea cycle (OUC), the OUC appears to be nonfunctional (absent, repressed, or incomplete) in most teleost fish (Mommsen and Walsh 1989). The toadfish (*Opsanus* sp.; Read 1971) is the only other exception. In the rainbow trout, some or all of the enzymes may be present, though in very low levels (Huggins, Skutsch, and Baldwin 1969; Rice and Stokes 1974; Chiu, Austic, and Rumsey 1986). Therefore, the increased J_{Urea} at high pH in the trout was probably due to the standard urea production pathways of uricolysis or arginase activity (Mommsen and Walsh 1989), rather than to an induction of OUC activity.

Blood Acid-Base and Ion Regulation

The blood alkalosis on initial exposure to high-pH water ("a P_{CO_2} vacuum" [Johansen et al. 1975]) was due solely to the decrease in P_{aCO_2}, a decrease consistent with the results of Wright and Wood (1985) and Lin and Randall (1990). Over 72 h, there appeared to be no ability to restore P_{aCO_2} to normal levels. In contrast to the findings of Heming and Blumhagen (1988), there was no rise in plasma HCO_3^- at 24 h. Analysis of the acid-base data on a pH-HCO_3^- diagram (fig. 3; see Wood, McMahon, and McDonald [1977] for details) reveals two clear components of the response. The initial phase of respiratory alkalosis (titration along the non-HCO_3^- buffer line by decreased P_{aCO_2}) was followed after 8 h by a progressive "metabolic" compensation (loss of HCO_3^- base = gain of acidic equivalents), which prevented any further rise in pHa. Had this compensation not occurred, pHa would have exceeded 8.20 by 72 h, in contrast to the observed stabilization at pH 7.97.

The rise in blood lactate was similar in magnitude to ΔH^+m, over the first 24 h of alkaline exposure. This suggests that activation of glycolysis might have contributed to the metabolic compensation of acid-base balance. Either the rise in systemic pHa or the rise in internal T_{Amm} levels may have played a role in this activation. In mammalian red blood cells, for example, glycolysis is stimulated by alkalosis alone (de Loecker 1964), while NH_4^+ is reported to specifically activate phosphofructokinase, a key regulatory enzyme in glycolysis (Kuhn et al. 1974). It is notable that the rise in ΔH^+m and lactate occurred in the absence of external or internal hypoxia. The measured levels of P_{aO_2} remained well above those needed to saturate the

arterial blood (Eddy 1971). Measurements of lactate production and turnover rates are required in future studies to confirm that glycolysis contributes to acid-base control in trout exposed to alkaline environments.

Plasma Na^+ and Cl^- stabilized after the first 24 h of decline at levels well above those associated with mortality in trout exposed to acid water (McDonald 1983*a*; Wood 1989). While ion flux rates were not measured, this suggests that the initial inhibition of branchial Na^+ and Cl^- uptake, seen on exposure to pH 9.50 in previous studies (Wright and Wood 1985; Wood 1989), does not continue for long. The recovery of Na^+ balance may have played a role in the recovery of ammonia excretion via Na^+/NH_4^+ exchange. The reason for the much larger electrolyte declines in the trout of Heming and Blumhagen (1988) exposed to a more moderate alkaline pH (8.7) is unknown.

In conclusion, rainbow trout can survive at pH 9.50 for at least 72 h, although marked disturbances occur in ammonia excretion, acid-base balance, and ionoregulation that may render the fish more susceptible to death from other causes. Several adaptations occur, including a rapid increase in urea excretion, a subsequent reestablishment of ammonia excretion, a metabolic compensation opposing respiratory alkalosis, and a limitation of plasma ion losses. Recently, Yesaki (1990) has shown generally similar effects in rainbow trout exposed to pH 10, in very hard water. Further studies are needed to characterize the longer-term response and to determine the physiological and biochemical mechanisms that permit these adaptations to take place.

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