Recovery from High pH Exposure in the Rainbow Trout: White Muscle Ammonia Storage, Ammonia Washout, and the Restoration of Blood Chemistry

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Abstract

The physiological responses of rainbow trout were followed during 48 h of high pH (pH 9.5) exposure and a further 48 h of recovery at pH 8.0. High pH exposure temporarily inhibited ammonia excretion (J_{Amm}) and led to a sixfold increase in plasma total ammonia (T_{Amm}) concentration. By 24 h at pH 9.5, J_{Amm} had returned to preexposure (control) rates but plasma T_{Amm} concentration remained elevated. The fish also developed a transient metabolic alkalosis (increased metabolic base) and a sustained respiratory alkalosis (decreased plasma CO_2 tension [P_{aCO_2}]). Plasma Na^+ and Cl^- concentrations were reduced by 5% after 48 h at pH 9.5. An "ammonia washout," of about 5,000 $\mu mol \cdot kg^{-1}$ occurred during the first 12 h of the recovery period, with fivefold elevations in J_{Amm} during the first few hours. This ammonia washout was accompanied by a return of plasma T_{Amm} concentration to preexposure levels after 3 h. The amount of excess T_{Amm} excreted by the fish during the washout was about 50-fold greater than extracellular fluid (ECF) T_{Amm} stores. Subsequent experiments indicated the white muscle (WM) intracellular fluid (ICF) compartment had stored at least 40% of the excess T_{Amm}. The T_{Amm} concentrations in the WM ICF were 2.5-fold greater in fish held at pH 9.5 for 48 h than in those held at pH 8.0. Estimates of the ECF and WM ICF pH, NH_3 partial pressures (P_{NH_3}), and NH_4^+ concentrations indicated the development of favorable ECF:ICF electrochemical gradients for NH_4^+ uptake, and P_{NH_3} gradients for NH_3 uptake, by the WM during the initial period of high pH exposure. There was a partial return toward steady state by 48 h. Thus, the WM serves as an "ammonia reservoir" for rainbow trout when plasma T_{Amm} increases owing to temporary reductions in branchial ammonia excretion. The rapid return of other physiological indices such as arterial pH (pH_a), P_{aCO_2}, and plasma Na^+ and Cl^- to control levels during recovery (in 3–8 h), as well as constant arterial O_2 tension (P_{aO_2}), suggested that high-pH-induced physiological disturbances are reversible and that there is no high-pH-induced gill histopathology during such short-term exposures.

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Introduction

Fish may encounter temporary elevations in environmental pH due to the photosynthetic removal of CO_2 in eutrophic waters (Jordan and Lloyd 1964; Barica 1974; Wetzel 1983). Permanently alkaline lakes, which result from high local concentrations of dissolved alkaline salts (e.g., $NaHCO_3$ and $Ca(HCO_3)_2$), are also present in many regions (see, e.g., Johansen, Maloiy, and Lykkeboe 1975; Galat et al. 1981; Danulat and Kempe 1992). Exposure of rainbow trout to highly alkaline water (pH $\geq$ 9.0) inhibits ammonia excretion and results in increased plasma total ammonia (T_{Amm}) concentration (Wright and Wood 1985; Wilkie and Wood 1991; Yesąki and Iwama 1992). It also causes respiratory and/or metabolic alkalosis (Wright and Wood 1985; Heming and Blumhagen 1988; Lin and Randall 1990; Wilkie and Wood 1991; Yesaki and Iwama 1992) and ionoregulatory disturbances characterized by decreases in plasma Na^+ and Cl^- concentration (Heming and Blumhagen 1988; Wilkie and Wood 1991, 1994; Yesaki and Iwama 1992). Wilkie and Wood (1991) demonstrated that rainbow trout are capable of counteracting these disturbances. For instance, they reported that over 72 h of high pH (pH = 9.5) exposure, ammonia excretion returns to normal, plasma Na^+ and Cl^- concentrations stabilize at slightly reduced levels, and elevated blood pH is partially compensated by the development of a simultaneous metabolic acidosis.

Surprisingly, the ability of fish to recover from short-term alkaline exposure (i.e., 8 h to 3 d), on return to water of normal pH, has received little attention. Many investigators have reported that highly alkaline water results in histological changes in the gill epithelium and other tissues (Eicher 1946; Jordan and Lloyd 1964; Daye and Garside 1976; Galat et al. 1985; Wilkie and Wood 1994; Wilkie et al. 1994). Changes in gill structure could alter branchial gas, ion, and acid-base exchange processes, not only during high pH exposure, but also following reintroduction into a circumneutral pH environment. Accordingly, we followed ammonia excretion patterns, acid-base status, plasma electrolytes, and arterial O_2 tension (Pao_2) in rainbow trout that had been reintroduced into circumneutral pH (pH 8.0) water after 48 h of exposure to pH 9.5.

Wilkie and Wood (1991) observed that, despite a full recovery of ammonia excretion rate (J_{Amm}) by 48 h of high pH, approximately 3,600 μmol N · kg^{-1} of T_{Amm} that should have been excreted by rainbow trout during the first 2 d of exposure to high pH remained missing, even after taking elevated

urea-N excretion into account. They suggested that (1) this missing ammonia was directed to other end products, such as glutamine, (2) nitrogenous waste production was reduced, and/or (3) ammonia was stored in a body compartment other than the extracellular fluid (ECF). In the present study we investigated the latter hypothesis.

To establish whether the missing ammonia was retained by the rainbow trout during high pH exposure, we followed J_{Amm} for 48 h after the fish had been returned to pH 8.0 water. We hypothesized that if the fish were storing ammonia, reintroduction into circumneutral pH would result in elevated J_{Amm}'s that would allow the fish to repay its "ammonia deficit."

The white muscle (WM) intracellular fluid (ICF) compartment constitutes approximately 45% percent of the trout's total body weight (Stevens 1968; Milligan and Wood 1986*a,* 1986*b*) and constitutes the largest potential ammonia reservoir in the fish. Therefore, we also investigated whether there were significant elevations in the WM ICF T_{Amm} content and/or changes in intracellular pH (pH_i) in rainbow trout held at high pH for 48 h. Such a strategy would appear advantageous because it would have lower metabolic cost relative to responses that might include conversion of ammonia to an alternate waste product and it would not affect the metabolic scope of the fish through reductions in overall metabolic rate.

Material and Methods

Experimental Animals and Setup

Rainbow trout (*Oncorhynchus mykiss;* mean weight = 290.5 ± 12.9 g, n = 22) were obtained from Spring Valley Trout Farm (Petersburg, Ontario) and held in moderately hard (140 mg · L^{-1} as $CaCO_3$), dechlorinated, 15°C Hamilton tap water (composition: $[Na^+]$ = 0.6, $[Cl^-]$ = 0.8, $[Ca^{++}]$ = 0.9, $[Mg^{++}]$ = 0.15, titration alkalinity = 2.0 mmol · L^{-1}) for no more than 3 mo. The fish were starved 1 wk prior to surgery, at which time they were anesthetized with MS222 (1:10,000 dilution; Sigma), fitted with chronic indwelling dorsal aortic catheters (Soivio, Westman, and Nyholm 1972), and then allowed to recover 48 h in individual darkened Plexiglas flux boxes (volume = 3.0 L; see McDonald and Rogano [1986] for details), supplied with the same tap water at approximately 0.5 L · min^{-1} and 15°C. The boxes received their water from a mixing head tank served by an inflow of tap water (5.0 L · min^{-1}) and fitted with a pH regulation setup.

A flow-through system was employed to minimize changes in water Ca^{++} concentration that occur when hard Hamilton tap water is raised to pH 9.5.

Wilkie and Wood (1991) reported that water Ca^{++} concentrations declined by 80% during a 72-h high pH exposure regime in a recirculating system that had partial water replacement. In contrast, in the present study, water Ca^{++} concentrations were maintained above 0.5 mmol · L^{-1}. The pH regulation setup consisted of a Radiometer GK2401C pH electrode and PHM 84 pH meter connected to a TTT80 autotitrator; when pH dropped below 9.65, the autotitrator signaled an electromagnetic control valve (Nacon Industries) which regulated the dropwise addition of 1 N KOH into the head tank from a 15-L KOH reservoir. Resultant water K^+ concentrations did not exceed 0.7 mmol · L^{-1}. Previous experiments have shown that water K^+ concentrations in this range cause no apparent physiological effects in rainbow trout (Wilkie et al. 1993; Wilkie and Wood 1994).

Experimental Protocol

Part 1: Recovery of Physiological Status Following High pH Exposure. Ammonia excretion rates and changes in blood chemistry were determined in seven rainbow trout held at pH 8.0 (preexposure period), then exposed to pH 9.5 for 48 h, and returned to pH 8.0 for 48 h. Ammonia excretion rate was determined at pH 8.0, and at 0–1 h, 8–9 h, 24–25 h, and 48–49 h of pH 9.5 exposure, and at continuous 30-min intervals over the first 3 h after return to pH 8.0 (postexposure period), as well as at 8–9 h, 12–13 h, 24–25 h, and 48–49 h of the postexposure period. Ammonia excretion rate measurements required that water flow to the boxes be cut off for 30–60 min. Vigorous aeration during this period ensured thorough mixing and oxygenation. Water ammonia levels usually approached 50 μmol · L^{-1} after 1 h. During the 0–3-h postexposure period, greatly elevated J_{Amm} necessitated flushing the boxes every 60 min to prevent water ammonia concentrations from reaching levels that might inhibit ammonia excretion (Cameron and Heisler 1983; Wright and Wood 1985; Wilkie and Wood 1991). Water pH control during J_{Amm} determination at high pH was complicated by CO_2 addition to the box via aeration and CO_2 excretion by the fish, which drove water pH down. Accordingly, pH was maintained via manual monitoring of box pH with an independent pH meter (Radiometer PHM 72) and electrode (GK2401C) and successive 0.5-mL additions of 1 N KOH to the box every 30 min or when pH dropped below pH 9.5.

Blood samples were taken during the preexposure period, after 8 h and 48 h of pH 9.5 exposure, and at 3 h, 8 h, and 24 h of the postexposure period. To minimize effects that box closure might have had on blood parameters, such as ammonia, we took blood samples 30–60 min prior to J_{Amm}

measurement (see Wilkie and Wood 1991; Wilkie et al. 1993). Blood samples (700 μL) were drawn through the dorsal aortic catheter into an ice-cold, heparinized, gastight Hamilton syringe and replaced with an equal volume of Cortland saline (Wolf 1963). Whole blood was immediately analyzed for arterial pH (pH_a), hematocrit, lactate, and arterial O_2 tension. Blood used in Pa_{O_2} determinations (approximately 150 μL) was reinfused into the fish. Remaining unused blood was centrifuged at 10,000 *g* and the plasma used for immediate determination of plasma total CO_2 and protein concentration; the remainder was frozen for later determination of plasma T_{Amm} ($T_{Amm} = NH_3 + NH_4^+$), Na^+, and Cl^- concentrations. Simultaneous water samples were taken to measure inspired P_{O_2} and pH.

Part 2: Ammonia Storage in the WM Compartment. To elucidate the potential role that WM might play as a reservoir for excess internal T_{Amm}, 12 rainbow trout of similar size and age were cannulated as previously described and exposed to pH 9.5 ($N = 6$, experimental group) or pH 8.0 ($N = 6$, controls) for 48 h. Blood samples (700 μL) were taken immediately prior to high pH exposure (control) and after 8, 24, and 48 h at pH 9.5. Samples were analyzed for pH_a and T_{Amm} as previously described. After 48 h at pH 9.5, fish were killed with an overdose of MS 222 (1.5 g · L^{-1}). Death occurred in less than 1 min; the fish were then removed from the box and a "filet" of WM excised from the trunk above the lateral line and between the adipose and dorsal fins. The tissue was immediately freeze-clamped with aluminum tongs cooled with liquid N_2 and stored initially in liquid N_2 and subsequently at −70°C until processed for determination of pH_i and T_{Amm} concentration.

Analytical Techniques and Calculations

Analytical techniques for water T_{Amm} concentrations ($T_{Amm} = NH_4^+ + NH_3$), plasma T_{Amm}, hematocrit, whole-blood lactate, pH_a, Pa_{O_2}, and plasma Na^+ and Cl^- were identical to those described by Wilkie and Wood (1991). Plasma total CO_2 was measured on a Corning total CO_2 analyzer, and plasma protein was measured by refractometry (Alexander and Ingram 1980).

Ammonia excretion rates were calculated from the change in water T_{Amm} during the flux period, the box volume, and fish weight (see Wright and Wood 1985). The NH_3 partial pressure (P_{NH_3}) and NH_4^+ concentration ($[NH_4^+]$) were calculated by using the Henderson-Hasselbalch equation, and employing the appropriate solubility coefficients and ammonia pK values provided by Cameron and Heisler (1983). The P_{CO_2} and HCO_3^- concentrations ($[HCO_3^-]$) in arterial blood plasma were determined in a similar manner

using pK′ values and solubility coefficients provided by Boutilier, Heming, and Iwama (1984). The net load of acidic equivalents ("metabolic acid load," ΔH_m^+) was calculated from changes in pH_a, $[HCO_3^-]$, and hematocrit (as an index of blood buffer capacity) as outlined in McDonald, Hobe, and Wood (1980).

Measurements of WM T_{Amm} concentration were made on tissue that was ground to a fine powder under liquid N_2 and deproteinized in 10 volumes of 8% perchloric acid. The T_{Amm} concentration of the extract was determined, after neutralization with tris(hydroxymethyl)aminomethane (Tris) buffer, by the methods of Kun and Kearney (1971).

White muscle pH_i was determined according to the method of Pörtner et al. (1990). In brief, WM was ground to a fine powder under liquid N_2; 100 mg of the powder was then added to approximately 400 μL of ice-cold metabolic inhibitor consisting of 6 mmol · L^{-1} nitrilotriacetic acid and 150 mmol · L^{-1} potassium fluoride. The sample was subsequently stirred with a needle cooled in liquid N_2, vortexed, and subsequently centrifuged at 10,000 *g* for 30 s. The supernatant was then immediately analyzed on the same pH microelectrode setup that was used for the determination of pH_a (same as extracellular pH [pH_e]).

White muscle T_{Amm} concentration was converted to μmol · L^{-1} ICF from μmol · kg^{-1} wet weight, according to the following expression:

$$\text{ICF } T_{Amm} = (\text{wet } [T_{Amm}] - \text{ECFV} \cdot \text{plasma } [T_{Amm}])/\text{ICFV},$$

where ICFV and ECFV are the respective WM intracellular and extracellular fluid volumes for rainbow trout WM at 15°C reported by Milligan and Wood (1986*a*); wet $[T_{Amm}]$ and plasma $[T_{Amm}]$ are the wet WM and plasma ammonia concentrations, respectively. White muscle P_{NH_3} and $[NH_4^+]$ were estimated in a manner identical to calculations used for extracellular (plasma) determinations of P_{NH_3} and $[NH_4^+]$.

Statistics

All results are expressed as means ± 1 standard error of the mean (SEM) ($N = 6$–7). Statistically significant differences in part 1 (among data collected at pH 8.0, during exposure to pH 9.5, and during the postexposure period) were determined with repeated-measures ANOVA followed by a Bonferroni posttest at $P \leq 0.05$. An unpaired two-tailed Student's *t*-test was used to test for statistical significance ($P \leq 0.05$) in part 2 (ammonia storage in the WM) after first establishing homogeneity of variance using an *F*-test.

Results

Part 1: Recovery of Physiological Status following pH 9.5 Exposure

Ammonia Metabolism. Ammonia excretion rates of rainbow trout held in pH 8.0 water (preexposure) were approximately 240 μmol · kg^{-1} · h^{-1}. Over the first hour of pH 9.5, J_{Amm} was reduced 80% to 44 μmol · kg^{-1} · h^{-1} (fig. 1). This was followed by a gradual recovery of J_{Amm}, which by 8 h at pH 9.5 returned to levels that were 50% of the preexposure values; by 24–48 h at pH 9.5 J_{Amm} had returned to values not significantly higher than preexposure rates (fig. 1). A dramatic increase in J_{Amm} was observed after the trout were returned to pH 8.0 (fig. 1). Relative to J_{Amm} at 48 h, J_{Amm} was approximately threefold higher during the first hour of the postexposure period and five times greater than preexposure rates. Ammonia excretion rates gradually declined, from approximately 1,200 μmol · kg^{-1} · h^{-1} over the first hour of the postexposure period to 760 μmol · kg^{-1} · h^{-1} after 3.0

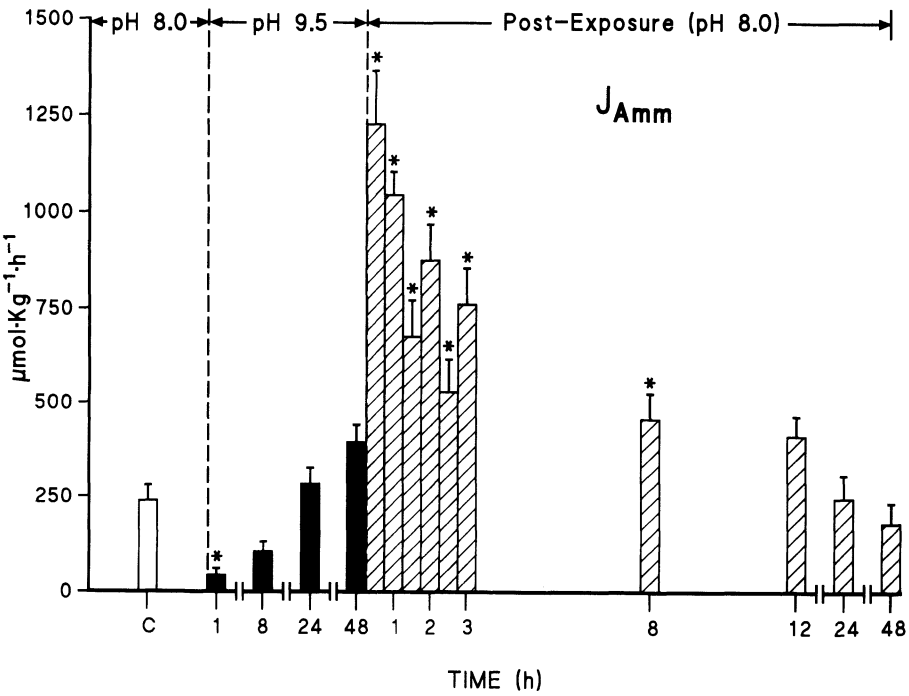

Fig. 1. Rates of ammonia excretion in rainbow trout prior to pH 9.5 exposure (preexposure; pH 8.0), during 48 h of pH 9.5 exposure, and during 48 h following pH 9.5 exposure (postexposure; pH 8.0). Values are means ± 1 SEM; N = 7. *Asterisks indicate significant differences from preexposure (control) values* ($P < 0.05$).

h (fig. 1). At 8 h J_{Amm} was still significantly elevated, but by 12–24 h it was reduced to levels comparable to preexposure rates (fig. 1).

In accordance with reduced J_{Amm} at pH 9.5, plasma T_{Amm} increased from a preexposure concentration of 60 μmol · L^{-1} to 330 μmol · L^{-1} by 8 h at pH 9.5, with a further increase to approximately 400 μmol · L^{-1} after 48 h (fig. 2*A*). At pH 9.5, the levels of circulating un-ionized ammonia (NH_3) increased to a greater relative extent than plasma T_{Amm} concentration because of the development of a simultaneous blood alkalosis (see below). Prior to pH 9.5 exposure P_{NH_3} was 20 μTorr. By 8 h at pH 9.5, it had increased to approximately 180 μTorr, and further increased to about 270 μTorr at 48 h. Return to pH 8.0 led to a rapid recovery of plasma T_{Amm} concentration. Three hours after return to pH 8.0 plasma T_{Amm} had returned to preexposure concentrations of approximately 50 μmol · L^{-1} (fig. 2*A*) and stabilized at about 30 μmol · L^{-1} by 8 h. Plasma P_{NH_3} levels were also rapidly restored to preexposure levels by 3 h (fig. 2*B*). At all times during the experiment, NH_4^+ (not shown) constituted more than 97% of the plasma T_{Amm} concentration and therefore followed trends virtually identical to those in T_{Amm} concentration (fig. 2*A*).

Plasma Ions. By 48 h of high pH exposure plasma Na^+ and Cl^- were 5% lower than the preexposure concentrations of 143 and 136 mmol · L^{-1}, respectively (fig. 3). The trout reestablished plasma Cl^- concentrations after only 3 h following return to pH 8.0, while plasma Na^+ did not return to preexposure values until 8 h. Plasma protein concentrations fluctuated around 2 g · 100 mL^{-1} and did not change significantly, despite repetitive blood sampling (data not shown).

Acid-Base Status. Upon exposure to pH 9.5, the rainbow trout underwent a combined metabolic and respiratory alkalosis. At 8 h, this alkalosis was characterized by a 0.15-unit elevation in pH_a, a ΔH_m^+ of −2.68 mmol · L^{-1}, and a 40% lower arterial CO_2 tension (P_{aCO_2}) of approximately 1.6 Torr (fig. 4*A, B,* and *D*). By 48 h, the metabolic component of the alkalosis was corrected, in association with a threefold elevation in blood lactate concentration, but pH_a was further elevated to about 8.17 owing to a further decline in P_{aCO_2} to 0.45 Torr. No changes in P_{aO_2} were observed (fig. 4*C;* note the P_{aO_2} data at 8 h were lost owing to P_{O_2} electrode failure).

Acid-base status was rapidly restored during the postexposure period. Arterial pH had returned to preexposure values after only 3 h at pH 8.0, although P_{aCO_2} remained slightly depressed and ΔH_m^+ remained close to zero (fig. 4*A, B,* and *D*). It is interesting that blood lactate was still signifi-

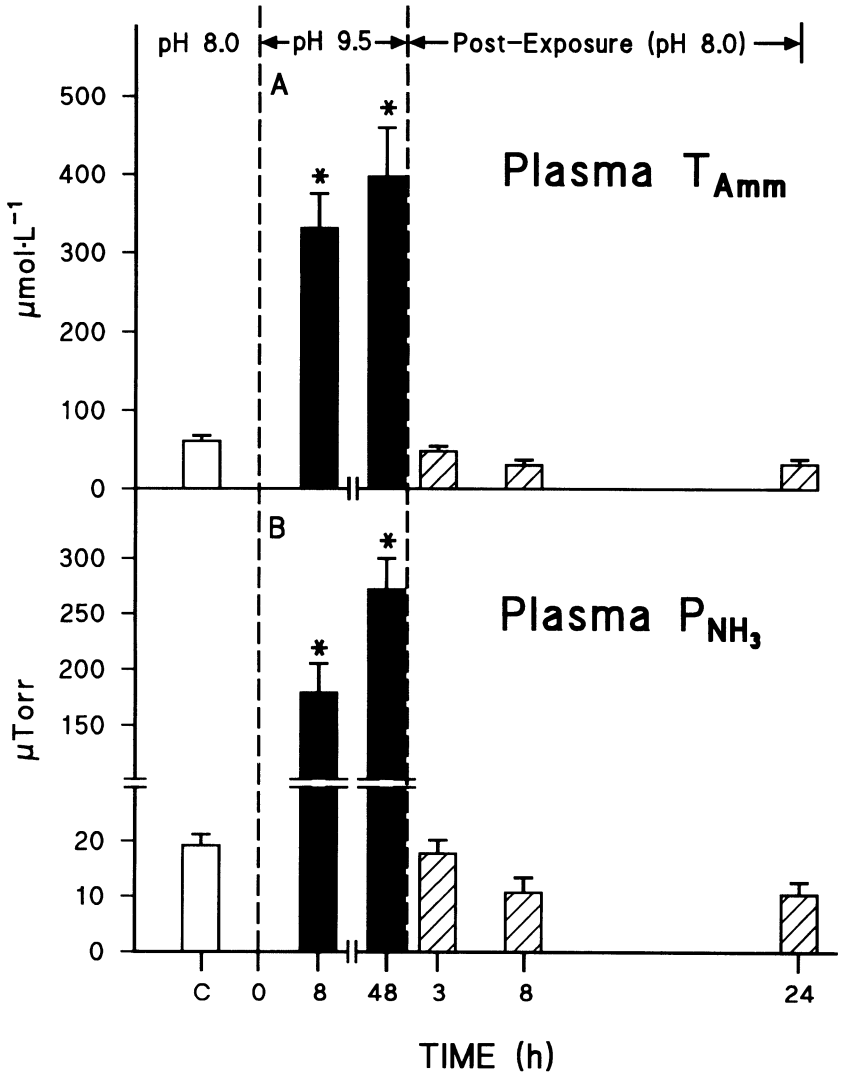

Fig. 2. Changes in plasma T_{Amm} concentration (A) and plasma P_{NH_3} (B) of rainbow trout prior to pH 9.5 exposure (preexposure; pH 8.0), during 48 h of pH 9.5 exposure, and during 48 h following pH 9.5 exposure (postexposure; pH 8.0). Values are means ± 1 SEM; N = 7. *Asterisks indicate significant differences from preexposure (control) values* ($P < 0.05$).

cantly elevated at this time (fig. 4*D*). By 8 h, however, $Paco_2$ and blood lactate concentration had returned to levels not significantly different from preexposure values. Again, no changes in blood Pao_2 were observed (fig. 4*C*).

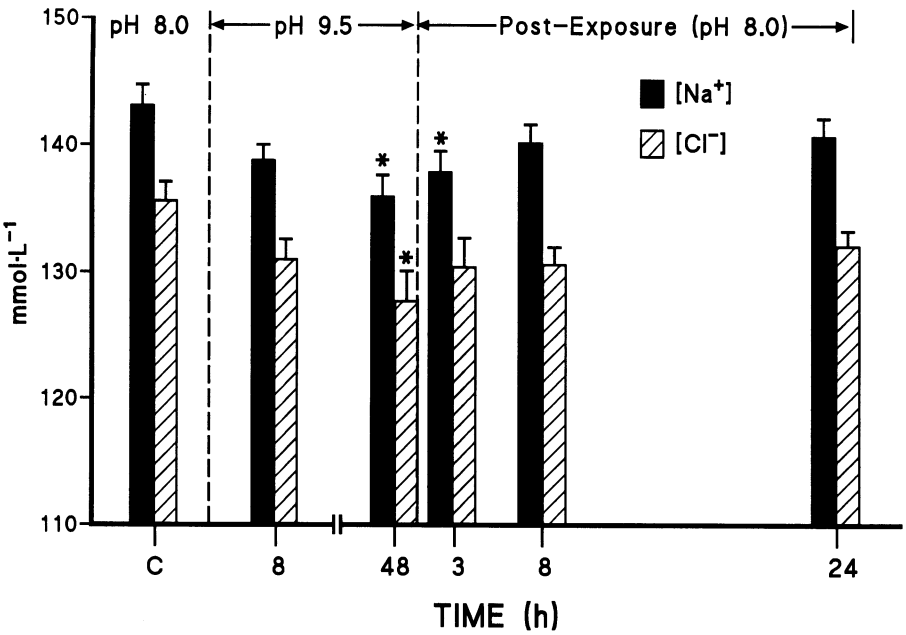

Fig. 3. Changes in the plasma Na^+ (solid bars) and Cl^- (hatched bars) concentrations of rainbow trout prior to pH 9.5 exposure (preexposure; pH 8.0), during 48 h of pH 9.5 exposure, and during 48 h following pH 9.5 exposure (postexposure; pH 8.0). Values are means ± 1 SEM; N = 7. *Asterisks indicate significant differences from preexposure (control) values (*$P < 0.05$*).*

Part 2: Ammonia Storage in the WM Compartment

The plasma T_{Amm} concentrations, P_{NH_3}, and acid-base status of fish exposed to pH 9.5 in part 2 of the study (fig. 5; table 1) exhibited changes similar to those observed in part 1, though ammonia levels peaked at 8 h rather than 48 h and absolute elevations were smaller (fig. 5*A, B*). There were no significant changes in control fish held at pH 8.0. The buildup of plasma T_{Amm} concentration, during pH 9.5 exposure, coincided with a 2.2-fold elevation in WM T_{Amm} concentrations that approached 3,000 μmol · L^{-1} ICF (fig. 5*A*). Fish held at pH 8.0 had WM T_{Amm} concentrations of approximately 1,250 μmol · L^{-1} ICF (fig. 5*A*). The development of a significant intracellular alkalosis (pH_i elevation of 0.09 units; table 1) contributed to the 2.8-fold elevation in WM P_{NH_3} in the fish exposed to pH 9.5 (fig. 5*B*).

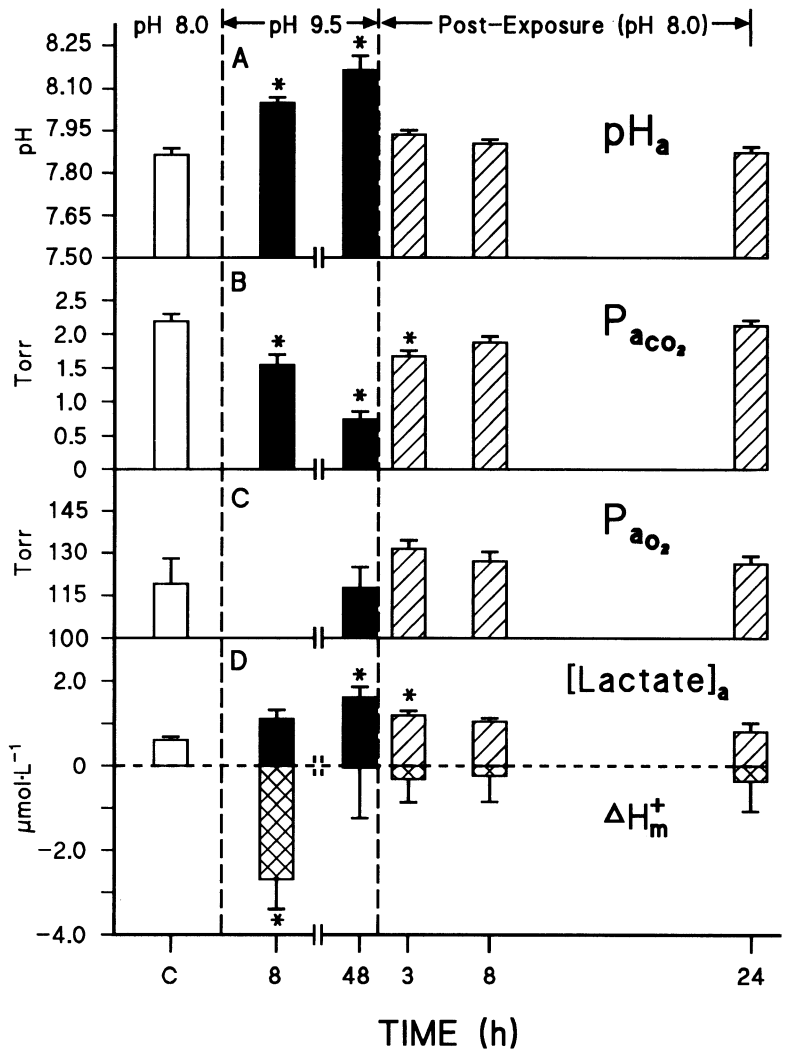

Fig. 4. Changes in the (A) pH_a, (B) $Paco_2$, (C) Pao_2, and (D) metabolic acid load (ΔH^+_m; downward-directed crosshatched bars) and whole blood lactate concentration (upward-directed bars) of rainbow trout prior to pH 9.5 exposure (preexposure; pH 8.0), during 48 h of pH 9.5 exposure, and during 48 h following pH 9.5 exposure (postexposure; pH 8.0). Values are means ± 1 SEM; N = 7. *Asterisks indicate significant differences from preexposure (control) values* ($P < 0.05$).

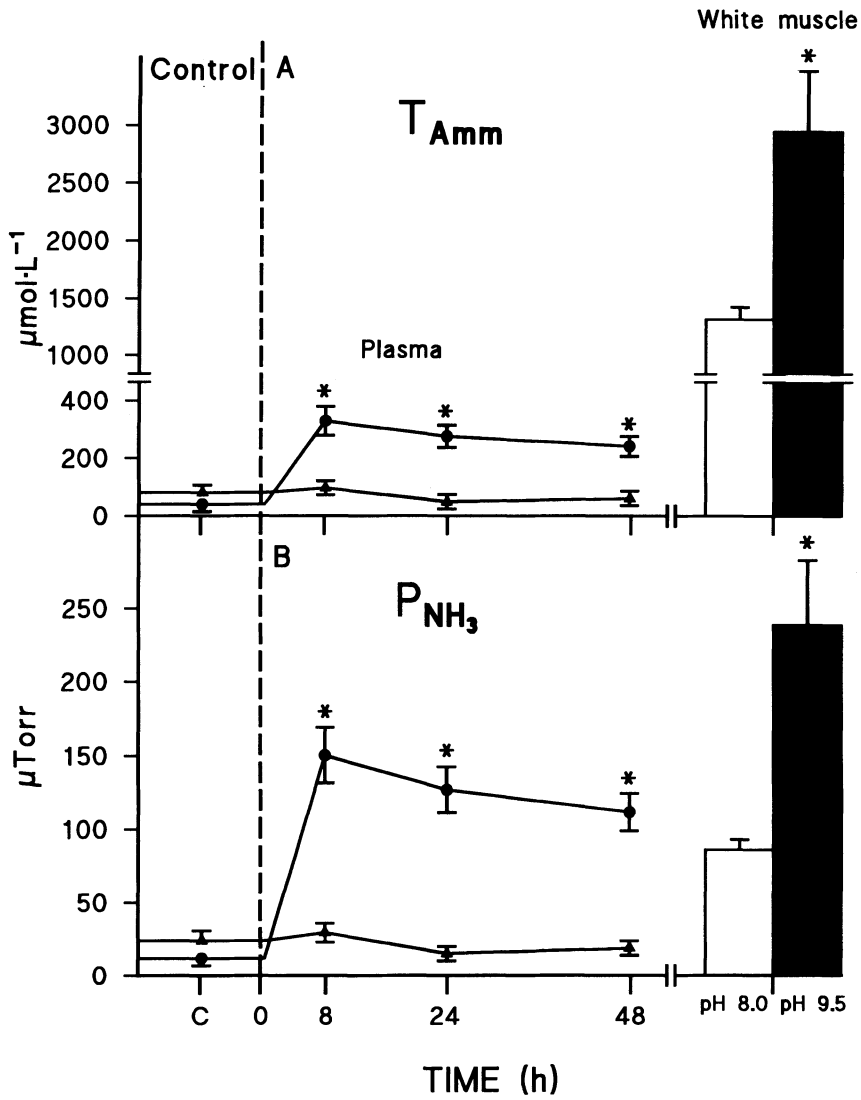

Fig. 5. Plasma (same as ECF; line graphs) and terminal WM ICF (bar graphs) (A) T_{Amm} concentrations, and (B) P_{NH_3} of fish held at pH 8.0 (triangle and open bar, respectively) or exposed to pH 9.5 (solid circle and solid bar, respectively) for 48 h. Values are means ± 1 SEM; N = *7 for plasma values and* N = *6 for WM intracellular measurements. Asterisks indicate significant differences between fish exposed to pH 9.5 and those held at pH 8.0 (controls;* $P < 0.05$*).*

TABLE 1

Estimates of the WM intracellular:extracellular P_{NH_3}, NH_4^+ electrochemical gradients (F_{NH_4}) and ICF:ECF ammonia distribution ratios in rainbow trout held at pH 9.5 or pH 8.0

		pH 9.5	
	pH 8.0	8 h	48 h
WM ICF:			
pH_i	7.164 ± .029	. . .	7.255 ± .018*
T_{Amm} (μmol · L^{-1})	1,316.1 ± 107.7	. . .	2,944.8 ± 524.8*
$[NH_4^+]$ (μmol · L^{-1})	1,311.8 ± 107.5	. . .	2,933.0 ± 522.7*
P_{NH_3} (μTorr)	86.7 ± 6.5	. . .	238.4 ± 43.4*
ECF (plasma):			
pH_a	7.840 ± .016	8.018 ± .011*	8.026 ± .024*
T_{Amm} (μmol · L^{-1})	59.5 ± 6.38	330.5 ± 49.9*	241.1 ± 34.1*
$[NH_4^+]$ (μmol · L^{-1})	58.6 ± 6.3	323.0 ± 49.0*	235.6 ± 33.5*
P_{NH_3} (μTorr)	18.4 ± 1.9	151.0 ± 18.8*	111.7 ± 12.7*
E_{NH_4} (mV)	−77.2 ± 4.4	−35.3 ± 3.6[a,*]	−61.1 ± 7.3*
F_{NH_4} (mV)[b]	−5.8 ± 4.4	−47.7 ± 3.6[a,*]	−21.9 ± 7.3*
ICF P_{NH_3} − ECF P_{NH_3} (μTorr)	68.3 ± 7.8	−62.1 ± 23.8[a,*]	126.8 ± 48.8
$[T_{Amm}]_i/[T_{Amm}]_e$	24.0 ± 3.9	4.3 ± .6[a,*]	13.4 ± 2.2*

[a] For these calculations WM pH_i and T_{Amm} values at 8 h were assumed to be the same as values in the control (pH 8.0) muscle samples.

[b] F_{NH_4} = transepithelial potential − E_{NH_4}, where the transepithelial potential is assumed to be −83 mV (see Wright, Randall, and Wood 1988). The E_{NH_4} is the calculated Nernst equilibrium potential for NH_4^+. A negative F_{NH_4} indicates an inwardly directed gradient for NH_4^+.

* Significantly different from control (pH 8.0) values ($P < 0.05$; $N = 6$).

Discussion

Ammonia Metabolism and Storage in the WM

Previously, Wilkie and Wood (1991) observed that, despite full recovery of ammonia excretion after 48 h at pH 9.5, approximately 3,600 μmol · kg^{-1} of ammonia that should have been excreted by rainbow trout were unaccounted for. They suggested this "ammonia deficit" resulted from reduced ammonia production (i.e., lower metabolic rate), conversion of the ammonia to other waste products, or ammonia storage in other body compartments. In the present study, recovery of J_{Amm} by 24 h of pH 9.5 exposure and a

slight elevation at 48 h suggest that there was no reduction in ammonia production. Moreover, the "ammonia washout" during recovery, which approximated 5,000 μmol · kg^{-1} (fig. 6), suggests that there may have been *increased* ammoniogenesis during exposure to pH 9.5. The rapidity of the washout also implies ammonia was not converted to an alternate end product to any great extent, although there may have been a small stimulation of urea (Olson and Fromm 1971; Wilkie and Wood 1991; Wilkie et al. 1993; Wright 1993) or glutamine production (Levi et al. 1974; Arillo et al. 1981).

The postexposure washout of ammonia is similar to that seen in fish recovering from exhaustive exercise (Milligan and Wood 1986*a*, 1986*b*). The ammonia washout following exercise results from increased WM NH_3 production via increased adenylate breakdown (Driedzic and Hochachka 1978). In the present study, the ammonia washout was likely related to the initial high-pH-induced blockade of ammonia excretion at the gill and possibly elevated hepatic ammoniogenesis during and after high pH exposure. Ac-

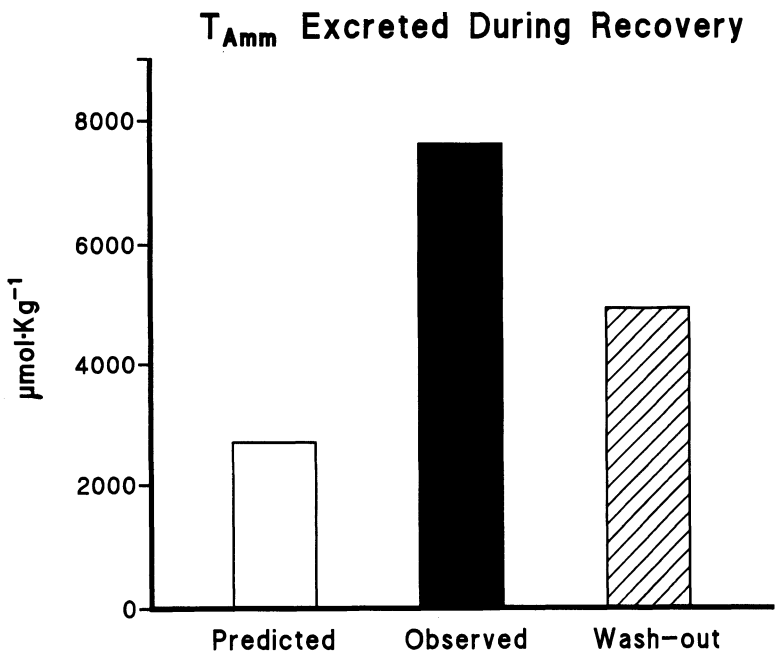

Fig. 6. Predicted (open bar) and observed (solid bar) amounts of T_{Amm} excreted by rainbow trout during the first 12 h of recovery from high pH (pH = 9.5) exposure. The predicted T_{Amm} assumes the preexposure J_{Amm} continued during the 12-h recovery period. The ammonia washout (hatched bar) is the observed T_{Amm} excreted minus the predicted T_{Amm} excreted. The estimate is based on mean J_{Amm}'s of seven fish.

cordingly, internal (plasma) T_{Amm} levels likely increased until an outward P_{NH_3} gradient between the plasma and the water, most likely the unstirred boundary layers of the gills (Wright, Randall, and Perry 1989; Wright, Iwama, and Wood 1993), was achieved, enabling J_{Amm} to return to preexposure rates. The Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) of alkaline Pyramid Lake, Nevada, similarly tolerates higher internal levels of T_{Amm} and P_{NH_3} to facilitate J_{Amm} in alkaline environments (Wright et al. 1993; Wilkie et al. 1994).

Wilkie and Wood (1991) suggested other potential mechanisms of ammonia excretion by rainbow trout at pH 9.5. These included increased branchial Na^+/NH_4^+ exchange (Maetz 1972, 1973; Cameron and Heisler 1983) or H^+/NH_4^+ exchange (Cameron 1986). The former possibility now appears unlikely since Wilkie and Wood (1994) recently found, using amiloride and radiotracers ($^{22}Na^+$), that Na^+ uptake and NH_4^+ excretion were not correlated in rainbow trout at high pH. The possibility of H^+/NH_4^+ exchange seems unlikely because alkaline water is, by definition, deficient in available protons for such an antiporter. It is possible, however, that increased CO_2 excretion and/or enhanced activity of putative proton pumps on the branchial epithelium (Avella and Bornancin 1989; Lin and Randall 1993) might supply H^+ to an unstirred boundary layer for such a process, or for increased "diffusion trapping" of NH_3. A final possibility is that NH_4^+ diffusion across the gills due to elevated plasma NH_4^+ concentration leads to recovery of J_{Amm} (Goldstein, Claiborne, and Evans 1982; McDonald and Prior 1988; Heisler 1990), though the bulk of evidence at present weighs against an important role for NH_4^+ diffusion in freshwater fish (Heisler 1990; Wood 1993).

The theory governing ammonia distribution across fish WM cell membranes has been clearly established (Wright and Wood 1988; Wright et al. 1988; Tang, Lin, and Randall 1992). In brief, the membranes appear to be freely permeable to both NH_3 and NH_4^+. Both the transmembrane pH gradient ($pH_i < pH_e$; e.g., table 1) acting on NH_3 diffusion and the electrical gradient (membrane potential negative inside by 70–90 mV; Hagiwara and Takahashi 1967; Hidaka and Toida 1969) acting on NH_4^+ diffusion favor higher intracellular T_{Amm} concentrations than extracellular concentrations. The electrical gradient appears to have the larger influence, such that under steady state conditions, the intracellular/extracellular T_{Amm} concentration ratio (i.e., $[T_{Amm}]_i/[T_{Amm}]_e$) is closer to that predicted by the electrical gradient (25–35) than to that dictated purely by the pH gradient (3–6). For example, under control conditions in part 2 of the present study, the distribution ratio was about 24 (table 1). As a result, there is a standing P_{NH_3} gradient from

intracellular to extracellular compartment of about 70 μTorr (see fig. 5*B;* table 1) and a small NH_4^+ electrochemical gradient of about −6 mV in the opposite direction (table 1). As a result there is a continuous steady state shuttle whereby NH_3 diffuses from ICF to ECF, and NH_4^+ from ECF to ICF along partial pressure and electrochemical gradients, respectively.

Given this situation, as T_{Amm} concentration built up in the ECF during the first 24 h at pH 9.5 (figs. 2, 5), owing to inhibited branchial ammonia excretion but continuing production by the liver, it would also accumulate in the WM ICF until a new steady state developed. Table 1 illustrates that early in the exposure (e.g., 8 h), the P_{NH_3} gradient would likely be reversed and favor NH_3 entry into the ICF (see fig. 5*B*), while the electrochemical gradient for NH_4^+ entry would be greatly increased. Later on (e.g., 48 h), the gradients return toward control values as a new steady state is approached. The gradients and distribution ratio did not fully return to control values in the fish in part 2, suggesting a new steady state had not been fully achieved by 48 h (table 1). Because the new steady state distribution ratio will be largely determined by the membrane potential, the increase in $[T_{Amm}]_i$ is far greater than that in $[T_{Amm}]_e$. Assuming that the total WM ICF volume constitutes 45% of the fish's body weight (Stevens 1968; Milligan and Wood 1986*a*), and the same $[T_{Amm}]_i/[T_{Amm}]_e$ relationship held in the fish of part 1 as in part 2, then T_{Amm} retention in the WM would explain about 40% of the observed postexposure ammonia washout of approximately 5,000 μmol · kg^{-1}. The remainder was likely due to similar retention of T_{Amm} in other tissues and/or elevated production. The amount stored in the total ECFV (0.25 L · kg^{-1}; Milligan and Wood 1986*a*) was no more than 100 μmol · kg^{-1}, or about 2% of the observed washout.

Although plasma P_{NH_3} and WM intracellular P_{NH_3} were dramatically elevated during pH 9.5 exposure, they did not appear to be toxic. The rapid return of plasma P_{NH_3}, and presumably WM P_{NH_3}, to preexposure levels following reintroduction into pH 8.0 water (fig. 2) supports our earlier arguments that fish modulate internal T_{Amm} levels in accordance with external environmental conditions. The plasma P_{NH_3}'s of part 1, though elevated, are still likely below the toxic thresholds for rainbow trout and are comparable to those observed by Wilkie and Wood (1991) (approx. 300 μTorr) in trout that experienced negligible mortality at pH 9.5 and about 25% lower than levels observed in salmonids suffering mortality in alkaline or hyperammonemic environments (see, e.g., Yesaki and Iwama 1992; Wilkie et al. 1993; R. W. Wilson and C. M. Wood, unpublished observations). In conclusion, the WM is capable of storing much of the ammonia that was retained by the fish during exposure to pH 9.5. Some of this ammonia retention was

due to initial high-pH-induced inhibition of ammonia excretion and the remainder was likely generated through increased rates of ammoniogenesis. In terms of environmental relevance, the ability of fish to store ammonia in the WM ICF would benefit the animal not only at high pH, but also when it was subjected to high environmental ammonia concentrations, such as would occur following the turnover of eutrophic water bodies or as a result of anthropogenic ammonia deposition (Barica 1974; Wetzel 1983).

Restoration of Acid-Base and Ion Balance

The respiratory alkalosis observed during the high pH exposure has been observed in a number of previous studies on trout (Wright and Wood 1985; Lin and Randall 1990; Wilkie and Wood 1991; Yesaki and Iwama 1992), and the metabolic alkalosis in several others (Heming and Blumhagen 1988; Yesaki and Iwama 1992; Wilkie et al. 1993, 1994). Wilkie and Wood (1994) and Wilkie et al. (1994) postulated that correction of the alkalosis resulted in part from increased branchial Cl^-/HCO_3^- exchange brought about by increases in the fractional surface area of branchial chloride cells. Significant metabolic production of lactic acid also likely contributed to the stabilization of blood pH as observed in the present and previous studies (Wilkie and Wood 1991; Wilkie et al. 1993). Most likely a combination of increased lactic acid production and modulation of branchial ion fluxes corrected the metabolic alkalosis and partially offset the respiratory alkalosis experienced by rainbow trout at high pH.

Reintroduction of the fish into pH 8.0 water led to rapid reestablishment of blood acid-base status. Only $Paco_2$ was still significantly reduced 3 h after return to circumneutral pH; all other acid-base parameters had returned to preexposure values. Surprisingly, despite the continued significant elevation of blood lactate, there was no postexposure acidosis (i.e., "overshoot") as $Paco_2$ moved toward preexposure levels. The continued significant depression of $Paco_2$ at 3 h contributed to the normalization of blood pH at this time. Given the known rapidity with which branchial chloride cell fractional surface area can change during acid-base disturbances (Goss, Laurent, and Perry 1992), it is likely that branchial ion fluxes were already returning toward normal by this time.

As in previous studies (Heming and Blumhagen 1988; Wilkie and Wood 1991; Yesaki and Iwama 1992; Wilkie et al. 1993), the trout in the present study experienced significant reductions in plasma Na^+ and Cl^- by 48 h of high pH exposure. Trout suffering mortality at high pH experience severe ionoregulatory disturbances (Yesaki and Iwama 1992; Wilkie et al. 1993),

and such a response may be symptomatic of direct high-pH-induced damage to the gill epithelium. Indeed, Daye and Garside (1976), studying brook trout (*Salvelinus fontinalis*), reported severe histological changes in the gills, including mucus cell hypertrophy and epithelial lifting. Galat et al. (1985) and Wilkie et al. (1994) also observed chloride cell hyperplasia in Lahontan cutthroat trout inhabiting alkaline lakes. Wilkie and Wood (1994) have reported similar responses in chloride cell morphometry in rainbow trout following high pH exposure. The fact that no changes in Pa_{O_2} were observed in the present or previous high pH exposure studies (Wilkie and Wood 1991; Wilkie et al. 1993, 1994) suggests high-pH-induced alterations in gill morphology do not impede gas exchange and are likely adaptive, rather than pathological. The rapid postexposure restoration of the rainbow trouts' plasma ion, acid-base, and plasma ammonia balance to preexposure levels supports this theory. It should be kept in mind, however, that factors such as water hardness, temperature, and ionic composition play an important role in determining a fish's ability to tolerate environmental stressors such as elevated environmental pH (Yesaki and Iwama 1992).

The Costs of Survival at Alkaline pH

Although rainbow trout are capable of adapting to high pH, adjustments that facilitate survival in a laboratory setting might impose additional costs on animals that are stocked into some of the alkaline, saline lakes of western North America (see, e.g., Coleman and Johnson 1988; Yesaki 1990). For instance, chronically elevated internal T_{Amm} concentrations could stimulate glycolytic flux in the WM and red blood cells (Kuhn et al. 1974) and the corresponding lactacidosis might help the animal to regulate systemic pH (Wilkie and Wood 1991). These energetically expensive responses to high pH would, however, ultimately reduce the rainbow trout's "metabolic scope" and might explain why rainbow trout populations have not taken hold in alkaline lakes, such as Pyramid Lake (pH = 9.4), Nevada. In contrast, Lahontan cutthroat trout endemic to Pyramid Lake rapidly correct the slight, transient elevations in internal ammonia that occur immediately following transfer into Pyramid Lake water, presumably via reductions in overall basal metabolic ammonia production (Wilkie et al. 1994). Furthermore, energetically expensive adaptations, such as increased lactic acid production, are noticeably absent in the Lahontan cutthroat trout following transfer into Pyramid Lake water. Thus, the ability of the Lahontan cutthroat trout to make rapid, energetically inexpensive adaptations to alkaline water might explain

why this animal thrives in an environment that is unsuitable for other salmonids such as the rainbow trout.

In conclusion, despite numerous previous investigations detailing the toxicity of alkaline environments to teleosts (e.g., Eicher 1946; Jordan and Lloyd 1964; Daye and Garside 1975, 1976; Alabaster and Lloyd 1980; Murray and Ziebell 1984; Heming and Blumhagen 1988; Yesaki 1990; Yesaki and Iwama 1992; Wilkie et al. 1993), the physiological disturbances initiated by exposure to pH 9.5 are readily reversible in rainbow trout. Although physiological disturbances occur across the gill epithelium, Pa_{O_2} remains constant prior to, during, and after alkaline exposure. This suggests there was no impairment of oxygen uptake and, hence, no high-pH-induced gill histopathology. The rapid restoration of acid-base, ion, and ammonia balance in the plasma during the postexposure period supports our conclusion. During the period of initial exposure to high environmental pH, increased plasma P_{NH_3} enables the fish to reestablish a positive P_{NH_3} diffusion gradient across the gill epithelium and ultimately results in reestablishment of J_{Amm} despite continued high pH exposure. Greater plasma T_{Amm} also leads to the development of ECF:ICF gradients that favor NH_3 and NH_4^+ uptake into the WM compartment such that far more T_{Amm} accumulates in the muscle ICF than in the ECF after 48 h at pH 9.5. Storage of ammonia in the tissues probably accounts for much of the prolonged ammonia washout observed during the postexposure period.

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