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Author(s): Michael P. Wilkie, Patricia A. Wright, George K. Iwama and Chris M. Wood

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The Physiological Adaptations of the Lahontan Cutthroat Trout (*Oncorhynchus clarki henshawi*) following Transfer from Well Water to the Highly Alkaline Waters of Pyramid Lake, Nevada (pH 9.4)

Michael P. Wilkie*
Patricia A. Wright†
George K. Iwama‡
Chris M. Wood*
Pyramid Lake Fisheries, Star Route, Sutcliffe, Nevada 89510

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Abstract

*Salmonids experience severe disturbances in the excretion and internal regulation of ammonia, acid-base balance, and ionoregulation when challenged with alkaline pH. We followed the responses of a high-pH-tolerant salmonid, the Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) for 72 h after transfer from pH 8.4 well water into the alkaline water (pH 9.4) of Pyramid Lake, Nevada. Fish that had been living in Pyramid Lake for 3 wk, 5 wk, and 2 yr were also examined. A combined metabolic and respiratory alkalosis (negative metabolic acid load [ΔH_m^+] and decreased arterial CO_2 tension [$Paco_2$], respectively) occurred initially. The metabolic component was corrected within 24 h, but the respiratory component persisted for up to 5 wk. Transfers also resulted in an immediate 70% reduction in the ammonia excretion rate (J_{Amm}) and a 30% increase in total plasma ammonia (T_{Amm}). The T_{Amm} was corrected within 3 d, but J_{Amm} remained depressed, which indicates reduced ammonia production rates. Because the urea excretion rate (J_{Urea}) did not change, the contribution of J_{Urea} to total N excretion increased from 10% in well water to 25% in fish acutely and chronically exposed to lake water. Liver enzyme activities indicated that the pathway for urea production was uricolysis, not the ornithine-urea cycle. Branchial chloride cell fractional surface area increased in lake water, and this may have counteracted the base load by promoting base equivalent excretion via Cl^-/HCO_3^- exchange. Plasma Na^+ and Cl^- levels were slightly higher in Pyramid Lake water. We conclude that the Lahontan cutthroat trout are able to survive in Pyramid Lake's alkaline environment because of their ability to reduce ammo-*

* Present address: Department of Biology, McMaster University, Hamilton, Ontario L8S 4K1, Canada.
† Present address: Department of Pathology, University of Guelph, Guelph, Ontario N1G 2W1, Canada.
‡ Present address: Department of Animal Science, University of British Columbia, Vancouver, British Columbia V6T 2A2, Canada.

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nia production, thereby avoiding chronic elevation of plasma T_{Amm}, *and their ability to control blood acid-base and ionic status under alkaline conditions.*

Introduction

Wilkie and Wood (1991) recently described the physiological responses of rainbow trout (*Oncorhynchus mykiss*) following transfer from circumneutral (pH 8.1) to alkaline (pH 9.5) water . Substantial disturbances occurred in nitrogenous waste (N waste) excretion, blood acid-base balance, and ionic status, none of which were fully corrected within 72 h. Other studies on rainbow trout have documented similar effects (Wright and Wood 1985; Heming and Blumhagen 1988; Lin and Randall 1990; Yesaki and Iwama 1992). Natural acute exposure to such a high pH is, however, a very unusual circumstance for this species.

We examined the physiological responses of a related salmonid, the Lahontan cutthroat trout (LCT; *Oncorhynchus clarki henshawi*) following transfer to pH 9.4. Acute alkaline exposure is part of the LCT's "natural" life cycle. The first year of life is spent in well water (pH 8.4), following which the fish are moved abruptly into the highly alkaline (pH 9.4) and moderately saline waters (4.4 ‰) of Pyramid Lake, Nevada (table 1). Attention has recently shifted to this species (Wilkie et al. 1993; Wright, Iwama, and Wood 1993) because it exhibits unusual tolerance to high pH and thrives in highly alkaline lakes throughout the northwestern United States (Trotter 1991). Attempts to stock other salmonids, such as coho salmon (*Oncorhynchus kisutch*), kokanee (*Oncorhynchus nerka*), brown trout (*Salmo trutta*), and rainbow trout into these lakes have failed (Galat et al. 1985; Kucera, Koch, and Marco 1985; Coleman and Johnson 1988).

The LCT is now designated as "threatened" because of a paucity of successfully reproducing populations (Williams et al. 1989). Historically, the Pyramid Lake LCT spawned and passed through the juvenile life stages in the freshwater environment of the Truckee River, which feeds into Pyramid Lake. Prolonged drought and water diversion have made this river inaccessible to spawning-condition LCT for many years, however (Galat et al. 1981, 1985; Kucera et al. 1985; Coleman and Johnson 1988). Indeed, by 1944 the original Pyramid Lake LCT was declared extinct. A vigorous stocking program, in which juvenile LCT are reared for 1 yr in well water prior to introduction into Pyramid Lake's alkaline waters, has revived the lake's cutthroat trout fishery (Coleman and Johnson 1988).

TABLE 1
Typical chemical composition of well water (pH 8.4) and Pyramid Lake water (pH 9.4)

	Well Water	Pyramid Lake Water
pH[a]	8.35	9.36
$[H^+]$ (μmol · L^{-1})	4.40×10^{-3}	$.43 \times 10^{-3}$
$[OH^-]$ (μmol · L^{-1})	.66	6.70
P_{CO_2} (Torr)	.78	.26
$[HCO_3^-]$ (mmol · L^{-1})	4.35	13.80
$[CO_3^=]$ (mmol · L^{-1})	.04	4.97
Titration alkalinity[b] (mmol · L^{-1})	4.45	23.08
$[Na^+]$ (mmol · L^{-1})	7.30	58.20
$[Cl^-]$ (mmol · L^{-1})	4.15	59.70
Total salinity (g · L^{-1})	.59	4.43

[a] Measured at 10.4°C.
[b] Titration alkalinity to pH = 4.0.

The minimal mortality experienced by LCT following transfer into Pyramid Lake (Coleman and Johnson 1988; D. Mosely and P. Wagner, personal communication) suggests that these fish are able to rapidly correct, or resist, the physiological disturbances observed in other salmonids at high pH. The purpose of the present investigation was to determine the physiological characteristics that allow LCT to adapt to alkaline Pyramid Lake water (pH 9.4) and to establish the time course of these adaptations. We followed the responses of naive LCT, which had never been exposed to high alkalinity, through a 72-h acute exposure to Pyramid Lake water. Analyses focused on N waste excretion, acid-base balance, and ionoregulation. Apart from the fact that this study was performed in the field using the well water and alkaline lake water available on site, methods closely duplicated those of our earlier laboratory study on *O. mykiss* (Wilkie and Wood 1991).

In addition, we investigated long-term adaptations by studying fish that had been in lake water for 3 wk, 5 wk, and 2 yr (returning spawners). In light of emerging evidence on the possible importance of urea production as an adaptation to high pH (Wood 1993), we also measured hepatic activities of uricolytic and ornithine-urea cycle (OUC) enzymes. Similarly, in view of recent findings on alterations in branchial chloride cell (CC) surface area

in response to acid-base challenge (Goss et al. 1992*b*), we looked for changes in the surface morphometry of these cells following transfer to alkaline lake water. Galat et al. (1985) reported apparent CC hyperplasia in LCT living in Pyramid Lake.

Material and Methods

All experiments were performed at the lakeside laboratory of Pyramid Lake Fisheries during May and June. We followed responses for 72 h following exposure to Pyramid Lake water in 1-yr-old LCT (*Oncorhynchus clarki henshawi; n* = 8) of both sexes. The mean weight of these fish was 243.5 ± 16.6 g (standard error of the mean [SEM]). Fish were kept indoors for 3 wk prior to the experiments in 500-L fiberglass holding tanks served with flowing hatchery well water. The chemistry of this well water (table 1; moderately high HCO_3^- and pH) reflects its origin from deep desert wells. The fish had been hatchery reared in the well water for the first year of their lives and were exposed to highly alkaline, and moderately saline, Pyramid Lake water for the first time during our experiments (table 1).

Separate groups of 1-yr-old, well-water-reared LCT were sampled 3 wk (202.3 ± 17.5 g [n = 9]) and 5 wk (242.4 ± 9.2 g [n = 13]) after exposure to lake water. Data are also reported for 3–4-yr-old cutthroat trout (500.0 ± 16.3 g [n = 6]) that had been free-living in Pyramid Lake for approximately 2 yr. These latter trout were netted as they migrated up an artificial spawning channel. All fish from these three groups were held in 500-L tanks provided with flowing lake water for at least 1 wk prior to experimentation. All fish were starved a minimum of 1 wk to minimize variation in N metabolism (Fromm 1963).

Two days prior to sampling, fish were fitted with chronic indwelling dorsal aortic catheters (Soivio, Westman, and Nyholm 1972) under MS 222 anesthesia (1:10,000 dilution; Sigma) and immediately placed in darkened, well-aerated (gas partial pressure of O_2 [P_{O_2}] = 125–130 Torr), acrylic flux boxes (McDonald 1983). Water ammonia levels were less than 5 μmol N · L^{-1} when the boxes were operated as open systems. Mean water temperature during the study of the acute responses was 10.4° ± 0.4°C. The mean well water pH for this study was 8.35 ± 0.010, and the mean lake water pH was 9.36 ± 0.006. For the longer-term comparisons, the water temperature, at the time of sampling, was 7.5°C (pH = 9.41 ± 0.005) for the fish sampled after 3 wk of exposure to lake water (3-wk fish), 9.5°C (pH = 9.39 ± 0.003) for those sampled after 5 wk (5-wk fish), and 10.0°C (pH = 9.38 ± 0.008) for those sampled after 2 yr (2-yr fish).

Well-aerated water was distributed to each flux box at 0.5 L · min^{-1} via a flow-splitter. Two 500-L central reservoirs continuously served the flux boxes with an excess of either well water (pH 8.4) or lake water (pH 9.4). Water pH was monitored with a Radiometer GK 2401C combination electrode and PHM 72 pH meter.

Experimental Protocol for Acute Response Experiments

Experimental methods followed those of Wilkie and Wood (1991). Water samples (15 mL) were taken at 0 h, 1 h, and 3 h of each period. The ammonia excretion rate (J_{Amm}) and urea excretion rate (J_{Urea}) were determined first in well water, then at 0–3 h, 8–11 h, 24–27 h, 48–51 h, and 72–75 h of exposure to Pyramid Lake water. With the exception of the 0–3-h period of lake water exposure, blood samples were taken 30 min prior to box closure. This was done to minimize disturbance to the fish and to ensure that truly representative J_{Amm} and J_{Urea} were measured (see Wilkie and Wood 1991; Wilkie et al. 1993). Blood samples (1 mL) were drawn into two heparinized 500-μL gastight Hamilton syringes via the arterial catheter. Arterial pH (pH_a) and O_2 tension (Pa_{O_2}) were measured immediately; blood used for the latter (approximately 200 μL) was reinfused into the fish. Cortland's saline was then infused to replace blood lost on account of sampling and to maintain the internal ionic and osmotic status of the fish (Wolf 1963). Aliquots of whole blood were saved for later analysis of hemoglobin (20 μL) and lactate content (100 μL). The remainder was centrifuged, and a small amount of plasma (50 μL) decanted for immediate determination of plasma total CO_2 and protein concentration. The remaining plasma (400–500 μL) was frozen for later analysis of total ammonia (T_{Amm}), urea, Na^+ and Cl^-, glucose, and cortisol. Water P_{O_2}, pH, and total CO_2 concentration in each box were also measured at the time of blood sampling. To establish how ammonia excretion was achieved in Pyramid Lake's highly alkaline environment, the blood-to-water partial pressure gradients for NH_3 (ΔP_{NH_3}) and concentration gradients for NH_4^+ ($\Delta[NH_4^+]$) were estimated from the measured pH values and T_{Amm} values in plasma and water (Wright and Wood 1985; Wilkie and Wood 1991). In a few fish, the transepithelial potential (TEP) across the gills between the arterial blood and the environment was also measured with methods identical to those described by Perry and Wood (1985). Determination of TEP allowed us to estimate the transbranchial electrochemical gradients for OH^- (H^+), HCO_3^-, and $CO_3^=$.

Fish were sacrificed after 72 h with an overdose of MS 222 (1.5 g · L^{-1}), and the second left gill arch excised for morphometric analysis of branchial CC surface area. In addition, the livers were quickly extracted, freeze-

clamped, and stored in liquid N_2 for later determination of ureagenic enzyme activity. A control group, kept in well water, was similarly sacrificed and sampled.

Our analytical techniques and the calculations used to estimate N waste excretion rates, water and blood chemical parameters, and electrochemical gradients are described by Wilkie and Wood (1991), Wilkie et al. (1993), and Wright et al. (1993).

Experimental Protocol for Long-Term Comparisons

Water and blood samples were taken essentially as described above. Only single samples were taken from the 3-wk fish and 2-yr fish. These fish were then sacrificed for gill and liver excision. Tissue samples were not extracted from the 5-wk fish.

Gill Sampling Techniques and Analysis

The methods used in this study are based on those of Laurent and Perry (1990) and Goss, Laurent, and Perry (1992*a*). The gill filaments were trimmed from the excised second left gill arch in small pieces (approximately 10 filaments per piece), rinsed in ice-cold, 0.15 mol $\cdot$ L^{-1} Na^+ cacodylate buffer, and then fixed in 5% glutaraldehyde for 60–70 min. After fixation, pairs of filaments, joined at the septum, were dissected away from one another and washed three times with ice-cold buffer and refrigerated at 4°C for several hours. The paired filaments were then taken through a partial ethanol dehydration series (30%, 50%, and 70% ethanol) and shipped back to McMaster University in 70% ethanol. Subsequently, the gills were completely dehydrated in 95% ethanol and then absolute ethanol, and then taken through two successive baths (2 min each) of 1,1,1,3,3,3-hexamethyldisilazane (Aldrich) and air-dried. The paired filaments were then mounted on aluminum stubs, sputter-coated, and viewed on an ISI-DS130 dual-stage scanning electron microscope at 2,000 times magnification. At least eight noncontiguous fields (approximately 2,500 μm^2 per field), along the trailing edge of a filament, were randomly photographed for each fish. The individual surface areas of CC in each field were subsequently determined with a Graphic Master digitizing tablet (Numonics) and an accompanying software program (Sigma Scan; Jandel Scientific). Chloride cell fractional surface area (CC FSA) and CC density were calculated from the estimates of individual CC surface areas and the total filamental surface areas measured per fish. The filamental epithelium was used for morphometry, rather than the lamellar epithelium, because the paired filaments could be mounted parallel

to the face of the aluminum stub. This made the flat, relatively uniform surface of the trailing filamental epithelium accessible for examination with the scanning electron microscope. Furthermore, the gill lamellae are less appropriate for such analysis because the undulating nature of their topography makes viewing more difficult and measurements prone to error (Goss et al. 1992*a*).

Statistics

All data are expressed as means ± 1 SEM (n). For the 72-h acute lake water exposure experiment, each animal served as its own control, and paired, two-tailed t-tests were used to determine statistical significance ($P < 0.05$). For long-term exposures, data for fish held in lake water were compared with data generated for fish held in well water. Therefore, an F ratio was calculated to test for homogeneity of variance, followed by an unpaired, two-tailed t-test to determine significant differences ($P < 0.05$). Ureagenic enzymes and gill morphometric data were evaluated by ANOVA, and subsequent paired contrasts ($P < 0.05$) were made by a Tukey-Kramer Honestly Significant Difference test using a commercially available statistics package (SAS JMP; SAS Institute 1989).

Results

Nitrogenous Waste Excretion

Lahontan cutthroat trout in well water exhibited a J_{Amm} of approximately 330 μmol N · kg^{-1} · h^{-1}. This was reduced nearly 70% during the first hour of exposure to alkaline lake water; depression of J_{Amm} persisted throughout the 3-d exposure (fig. 1*A*). The associated initial 30% rise in plasma T_{Amm}, to about 300 μmol N · L^{-1}, seen at 8–24 h (fig. 1*B*) was no longer evident by 72 h (fig. 1*B*). Urea excretion rates remained stable around 40 μmol N · kg^{-1} · h^{-1} for the duration of the 3-d exposure (fig. 1*A*). As a result, the percentage contribution of J_{Urea} to total N excretion ($J_{Total\ N}$) increased from 10% in well water to approximately 25% in lake water. Plasma urea levels were between 5,500 and 6,500 μmol N · L^{-1} for 24 h following exposure to lake water but then declined to approximately 4,000 μmol N · L^{-1} after 48 h.

In well water, both the ΔP_{NH_3} and $\Delta[NH_4^+]$ gradients were outwardly directed (approximately 25 μTorr and 165 μmol N · L^{-1}, respectively; fig. 1*C* and *D*) and were in accordance with the increase in plasma T_{Amm} (fig. 1*B*). After 8 h of exposure to lake water both ΔP_{NH_3} and $\Delta[NH_4^+]$ increased sig-

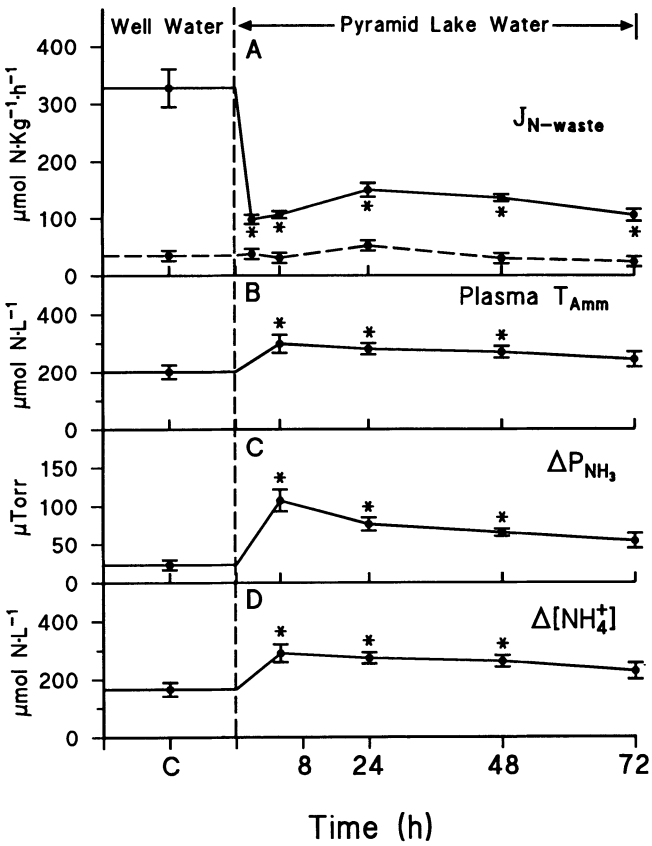

Fig. 1. Changes in (A) J_{Amm} *(solid line) and* J_{Urea} *(dashed line);* (B) T_{Amm}; (C) *the calculated* ΔP_{NH_3}; *and* (D) *the calculated* $\Delta[NH_4^+]$ *of Lahontan cutthroat trout, following transfer into alkaline Pyramid Lake water (pH 9.4) from well water (pH 8.4). Values are means ± 1 SEM;* n = 7. *Asterisks indicate significant differences from well water values* ($P < 0.05$).

nificantly to approximately 100 μTorr and 290 μmol N · L^{-1}, respectively (fig. 1*C* and *D*). The greater relative increase in ΔP_{NH_3} reflected a marked elevation in blood pH. Thereafter, ΔP_{NH_3} and $\Delta[NH_4^+]$ gradually declined in parallel with the fall in plasma T_{Amm}, and, by 72 h, neither was significantly different from control values.

Values of ΔP_{NH_3} in fish sampled at 3 and 5 wk were not significantly different from well water values. Similarly, $\Delta[NH_4^+]$, approximately 180 μmol N · L^{-1} in these fish, was also similar to that seen in well water. Fish sampled after 2 yr in lake water had significantly elevated ΔP_{NH_3} and $\Delta[NH_4^+]$ (approximately 70 μTorr and 280 μmol N · L^{-1}, respectively).

The reduction in J_{Amm} seen upon initial exposure to lake water persisted for an extended period. Fish sampled after 3- and 5-wk exposure to lake

water exhibited J_{Amm}'s of only about 100 μmol N · kg^{-1} · h^{-1}. These values are the same as those measured over the first hour at pH 9.4. The J_{Amm} of the 2-yr trout was not significantly different from the rates observed in well water fish, however (fig. 2*A*).

Despite the persistent depression of J_{Amm} in lake-water-adapted fish, plasma T_{Amm} was not persistently elevated. Rather, T_{Amm} remained relatively stable, from 72 h onward in all groups. The 2-yr fish, however, showed elevated plasma T_{Amm} (fig. 2*B*). The partial pressure of NH_3 (P_{NH_3}) in plasma was

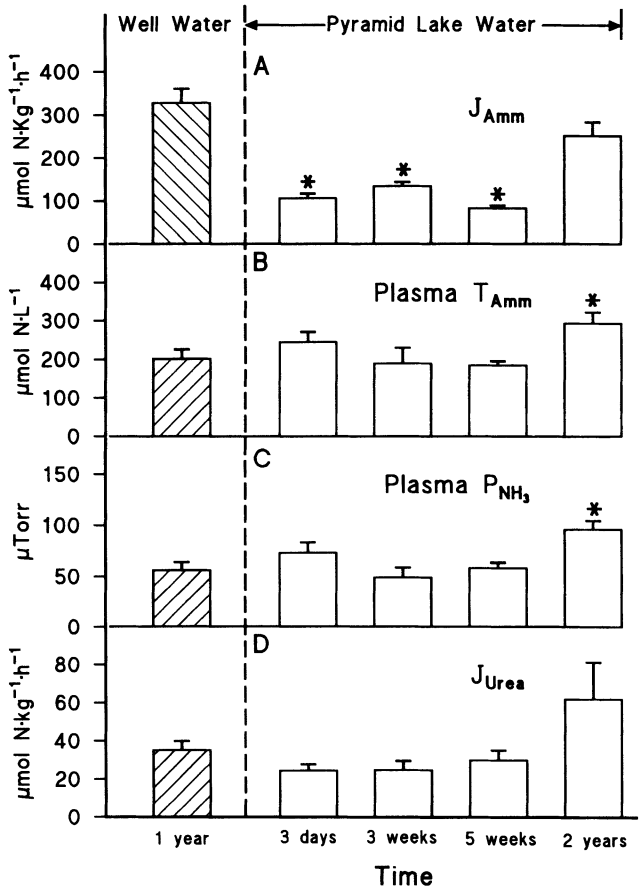

Fig. 2. The influence of long-term exposure to alkaline Pyramid Lake water (pH 9.4) upon (A) J_{Amm}, (B) T_{Amm}, (C) P_{NH_3} *in arterial blood, and* (D) J_{Urea} *of Lahontan cutthroat trout previously reared in well water (pH 8.4). Values are means ± 1 SEM;* n = *7 in well water and after 3 d at pH 9.4;* n ≥ *8 after 3 wk;* n = *13 after 5 wk; and* n ≥ *6 after 2 yr of exposure to lake water. Asterisks indicate significant differences from well water values* ($P < 0.05$).

about 50 μTorr in the well water fish and not significantly different from values measured in fish exposed to lake water for 72 h, 3 wk, or 5 wk. Blood P_{NH_3} was, however, significantly higher in the 2-yr fish (fig. 2*C*).

The J_{Urea} was not significantly different from preexposure values in any of the groups (fig. 2*D*). The percentage contribution of J_{Urea} to $J_{Total\ N}$ was, however, two- to threefold higher in all groups of lake-water-adapted fish.

Ureagenic Enzyme Activities

The activity of the key regulatory enzyme in the OUC pathway, carbamoyl phosphate synthetase III (CPS III), was just above the level of detection (table 2). Activities of glutamine synthetase, ornithine carbamoyl transferase, and argininosuccinate synthetase were relatively low compared with teleosts with a functional OUC pathway (Mommsen and Walsh 1989; Randall et al. 1989). The activity of arginase, which hydrolyzes dietary arginine to ornithine and urea (see Wood [1993] for review) was typical for a teleost (Mommsen and Walsh 1991; table 2). None of these enzymes were altered by exposure to lake water.

Significant uricolytic enzyme activity was present in LCT (table 2). The activities of uricase and allantoicase were within the range of values reported for fish by Cvancara (1969) and Goldstein and Forster (1965), respectively. Quantitative differences in activity were also observed between the groups of trout examined; uricase activity was twofold greater in the 3-wk fish but was significantly lower in the 2-yr fish. Relative to that in well-water-reared trout, allantoicase activity was significantly lower in the 2-yr fish (table 2).

Blood Parameters

No significant changes in P_{aO_2}, plasma glucose, or cortisol were observed after 72 h of exposure to lake water (table 3). Plasma protein levels remained stable over the first few hours of exposure to lake water but declined by 20% after 48–72 h of exposure (table 3). Blood hemoglobin had declined by almost 50% after 72 h. These latter effects are largely explicable as the consequences of repetitive blood sampling.

Fish sampled after 3 wk and 5 wk in lake water had plasma protein, hemoglobin, and P_{aO_2} levels that were comparable to those of fish in well water. Glucose concentrations were depressed after 5 wk in lake water but were elevated in fish that had been residing in the lake for 2 yr. Plasma protein levels were depressed in the latter group. Plasma cortisol concentrations were variable (135–280 ng · mL^{-1}) in all groups (table 3).

TABLE 2

Activities of OUC enzymes and uricolytic enzymes in Lahontan cutthroat trout living in well water or Pyramid Lake water

		Lake Water	
	Well Water	3 wk	2 yr
OUC enzymes:[a]			
Glutamine synthetase	.43 ± .08 (6)	.35 ± .07 (8)	.57 ± .08 (6)
Carbamoyl phosphate synthetase III	.01 ± .01 (4)	.02 ± .01 (4)	.03 ± .01 (6)
Ornithine carbamoyl transferase	.03 ± .01 (6)	.03 ± .00 (8)	.03 ± .00 (6)
Argininosuccinate synthetase	.05 ± .01 (5)	.04 ± .00 (5)	.05 ± .01 (5)
Arginase	38.51 ± 2.99 (6)	40.96 ± 3.92 (8)	40.76 ± 6.32 (6)
Uricolytic enzymes:			
Uricase	.92 ± .19 (6)	1.83 ± .23[b] (7)	.51 ± .11[c] (6)
Allantoinase	1.70 ± .27 (6)	1.61 ± .22 (8)	.78 ± .27 (6)
Allantoicase	.66 ± .09 (6)	.48 ± .08 (8)	.28 ± .10[b] (6)

Note. Data are means ± 1 SEM, with *n* in parentheses below.

[a] Activities are expressed as μmol · g^{-1} wet liver tissue · min^{-1}, except CPS III, which is expressed as μmol · g^{-1} mitochondria · h^{-1}.

[b] Significantly different from fish held in well water ($P < 0.05$).

[c] Significantly different from fish held in lake water for 3 wk ($P < 0.05$).

Transepithelial Potential

Transepithelial potentials were −6.2 ± 1.7 mV in well water fish and significantly lower than values measured in trout exposed to lake water for 5 wk (−3.2 mV;

TABLE 3

Arterial blood and plasma measurements taken from Lahontan cutthroat in well water (pH 8.4) and over the initial 72-h exposure to lake water and after 3 wk, 5 wk, and 2 yr of exposure to lake water

	Plasma Protein ($g \cdot 100\ mL^{-1}$)	Hemoglobin ($mg \cdot 100\ mL^{-1}$)	Glucose ($mmol \cdot L^{-1}$)	Cortisol ($ng \cdot mL^{-1}$)	Pa_{O_2} (Torr)
Well water:					
1 yr	4.1 ± .2	12.0 ± .9	6.3 ± .7	254.5 ± 26.7	97.6 ± 7.5
Pyramid Lake water:					
8 h	4.0 ± .2	10.0 ± .6	5.1 ± .4	281.3 ± 6.3	99.1 ± 2.7
24 h	3.6 ± .2	8.8 ± .8*	5.2 ± .4	146.3 ± 28.4	104.9 ± 5.4
48 h	3.2 ± .2*	7.9 ± .7*	5.0 ± .4	146.3 ± 28.6	98.8 ± 8.8
72 h	3.2 ± .2*	6.1 ± .7*	4.8 ± .3	157.6 ± 22.4	99.8 ± 4.0
3 wk	3.9 ± .2	8.9 ± .9*	6.7 ± .8	135.8 ± 39.3	90.9 ± 6.9
5 wk	3.9 ± .2	8.0 ± .5*	3.3 ± .6*	248.8 ± 25.5	88.7 ± 4.0
2 yr	2.5 ± .1*	...	9.4 ± .8*	277.1 ± 43.3	69.3 ± 14.3

* Significantly different from well water values ($P < 0.05$).

TABLE 4

Electrochemical gradients for acid-base-relevant ions between the blood and water of Lahontan cutthroat trout in well water (pH 8.4; n = *3) or adapted to lake water (pH 9.4;* n = *13) for 3 wk*

	TEP		Nernst Potential		F_{ION}[a]	
Ion	Well Water	Lake Water	Well Water	Lake Water	Well Water	Lake Water
OH^- (H^+) . . .	−6.2 ± 1.7	−3.2 ± .4*	−19.8 ± 1.5	−70.6 ± 1.4*	+13.6 ± 1.7	+67.4 ± 1.3*
$CO_3^=$	−6.2 ± 1.7	−3.2 ± .4*	+33.2 ± 2.6	−38.6 ± 1.5*	−39.4 ± 4.3	+35.5 ± 1.3*
HCO_3^-	−6.2 ± 1.7	−3.2 ± .4*	+12.4 ± 1.5	−16.7 ± 2.2*	−18.6 ± 3.1	+13.6 ± 2.0*

Note. All data expressed in mV.

[a] F_{ION} = TEP − Nernst potential. A positive (+) F_{ION} indicates an inwardly directed electrochemical gradient for anions.

* Significantly different from well water values ($P < 0.05$).

table 4). Estimates of the electrochemical gradients for OH^- indicated that the inwardly directed gradient for OH^- (= outwardly directed electrochemical gradient for H^+) increased fivefold in lake water (table 4). Furthermore, the outwardly directed electrochemical gradients for HCO_3^- and $CO_3^=$ in well water were reversed following transfer into lake water and resulted in large inwardly directed gradients for these basic anions (table 4).

Acid-Base Balance and Ionoregulation

The LCT in well water had a pH_a of 7.9 (fig. 3*A*), an arterial plasma CO_2 tension ($Paco_2$) of 1.8 Torr (fig. 3*B*), and an arterial plasma concentration of HCO_3^- ($[HCO_3^-]_a$) of approximately 6.7 mmol · L^{-1}. By 8 h, following

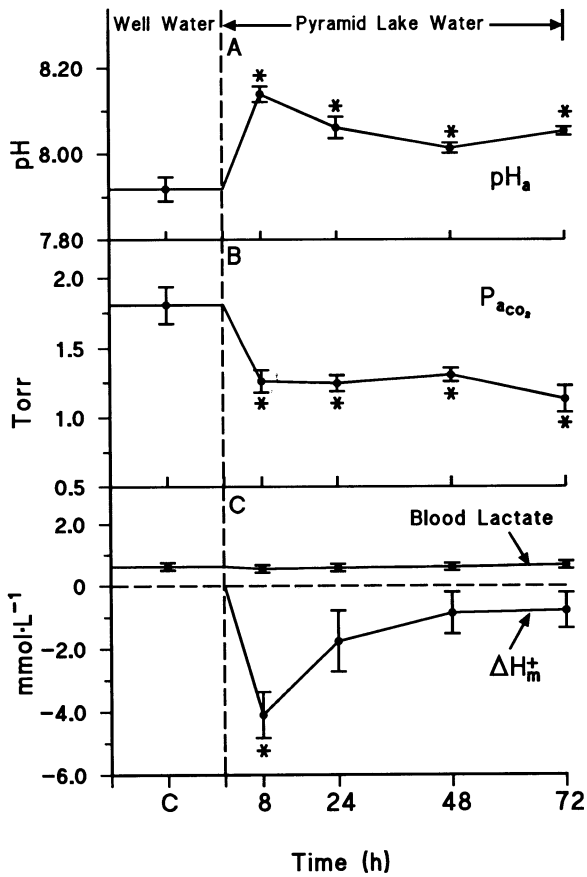

Fig. 3. Changes in (A) pH_a, (B) $Paco_2$, and (C) blood lactate and ΔH^+_m of Lahontan cutthroat trout following transfer into alkaline Pyramid Lake water (pH 9.4) from well water (pH 8.4). Values are means ± 1 SEM; n = 7. *Asterisks indicate significant differences from well water values* ($P < 0.05$).

transfer into lake water, the fish rapidly underwent a combined respiratory and metabolic alkalosis, which was characterized by a 0.2-unit increase in pH_a, a 30% reduction in $Paco_2$, no change in $[HCO_3^-]_a$, and a metabolic acid load (ΔH_m^+) of approximately -4 mmol $\cdot$ L^{-1} (i.e., a metabolic base load; fig. 3*A, B,* and *C*). The partial correction and subsequent stabilization of pH_a, at approximately pH 8.05, after 24 h, was the result of a stabilization of $Paco_2$ and the elimination of the metabolic base load (fig. 3*A, B,* and *C*). These alterations in blood acid-base status occurred without alterations in blood lactate concentration (fig. 3*C*). In the long-term exposure groups, blood acid-base status remained very similar to that attained by 24–72 h and was characterized by a chronic respiratory alkalosis (fig. 4).

A progressive 6%–8% increase in plasma Na^+ and Cl^- was observed during the first 72 h of exposure to lake water (fig. 5*A*). This trend toward greater plasma Na^+ and Cl^- was still seen at 3 wk. At 5 wk and 2 yr, levels had decreased slightly, but, even at the latter time, the elevation in Cl^- above the levels in well-water-adapted fish remained significant (fig. 5*B*).

Gill Structure and CC Morphometry

The filamental surface of the LCT gill was predominantly composed of microridged pavement (or respiratory) cells and contained varying numbers of villous CCs, which appeared in openings of the respiratory epithelium (fig. 6*A, B,* and *C*). Mucous cells were rarely seen. Qualitatively, the relative absence and small size of filamental epithelial CCs in well water fish is apparent in figure 6*A*. Following transfer into lake water, the exposure of CCs on the filamental surface became much more pronounced through changes in both individual cell surface areas and cell densities (fig. 6*B* and *C*). Similarly, the lamellar gill surface of well-water-adapted fish had very few CCs. Exposure to lake water led to changes in lamellar CC exposure and density that paralleled those observed on the filamental epithelium (fig. 6*D, E,* and *F*).

The CC FSA was fourfold greater after 3 d of exposure to lake water and 10-fold and 20-fold higher after 3 wk and 2 yr in lake water, respectively (fig. 7*A*). Differences in CC FSA between the naive trout and those exposed to lake water for 3 d were due to the twofold greater individual CC surface area, combined with a twofold greater CC density (fig. 7*B* and *C*). The higher CC FSAs observed in fish exposed to lake water for 3 wk and 2 yr were solely due to greater individual CC surface areas (fig. 7*B*).

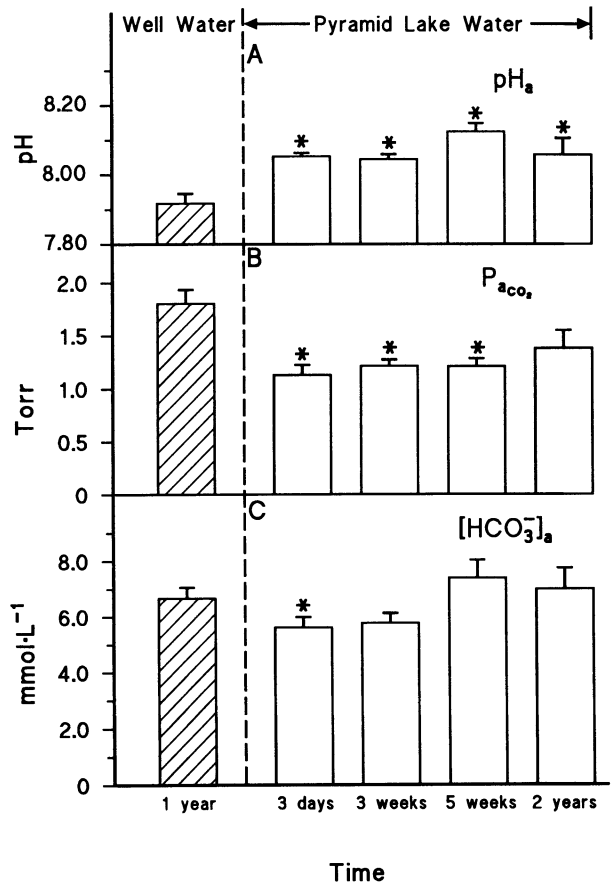

Fig. 4. The influence of long-term exposure to alkaline Pyramid Lake water (pH 9.4) upon (A) pH_a, (B) $Paco_2$, and (C) $[HCO_3^-]_a$ in Lahontan cutthroat trout previously reared in well water (pH 8.4). Values are means ± 1 SEM; n = *7 in well water and after 3 d in lake water;* n = *8 after 3 wk;* n = *13 after 5 wk; and* n = *6 after 2 yr of exposure to Pyramid Lake water. Asterisks indicate significant differences from well water values (*P < *0.05).*

Discussion

Survival and Stress

The absence of mortality following acute transfer of LCT into Pyramid Lake water illustrates how readily these fish adapt to the highly alkaline environment. This is underscored by the rapid adjustments that we observed in a variety of physiological parameters, including N waste excretion, ionoregulation, and acid-base regulation. Background cortisol levels were, however, relatively high in all exposure groups (table 3). This may be attributed to

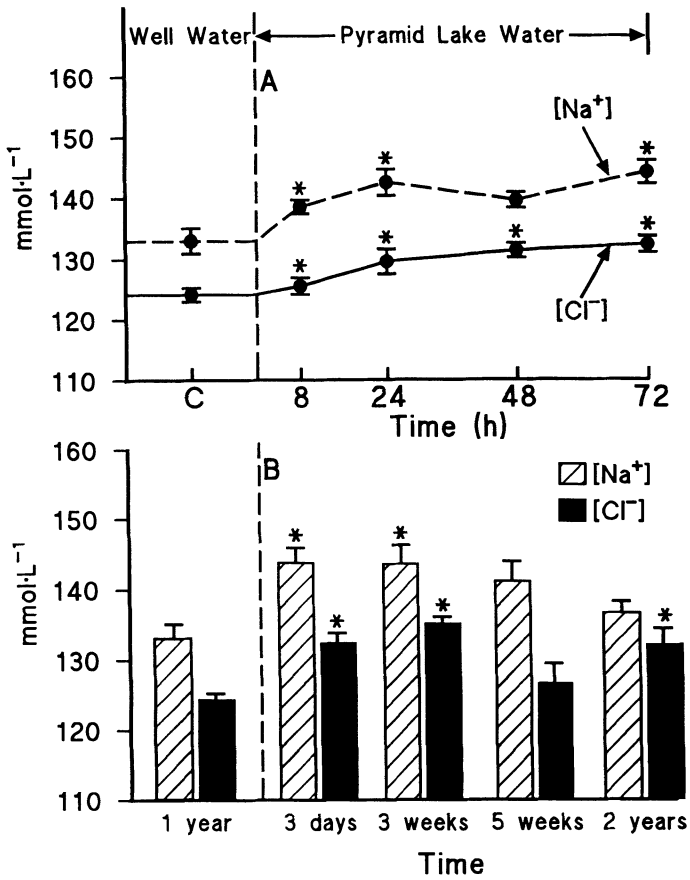

Fig. 5. (A) Changes in plasma Na^+ and Cl^- concentrations in Lahontan cutthroat trout over the first 72 h following exposure to alkaline Pyramid Lake water (pH 9.4) and (B) after 3 d, 3 wk, 5 wk, and 2 yr in lake water. Values are means ± 1 SEM; n = *7 in well water (pH 8.4) and after 3 d in lake water;* n = *8 after 3 wk;* n = *13 after 5 wk; and* n = *6 after 2 yr of exposure. Asterisks indicate significant differences from well water values (*$P < 0.05$*) for Na^+ and Cl^-.*

the handling, confinement, and catheterization of a wild trout (Woodward and Strange 1987; McDonald and Milligan 1992). Accordingly, we believe that transfer into lake water was not unduly stressful. The absence of any increase in plasma glucose, following acute lake water exposure (table 3), supports our conclusion.

Nitrogenous Waste Excretion

The initial reduction of J_{Amm}, immediately observed following transfer into lake water, parallels that seen in *Oncorhynchus mykiss* exposed to pH 9.5

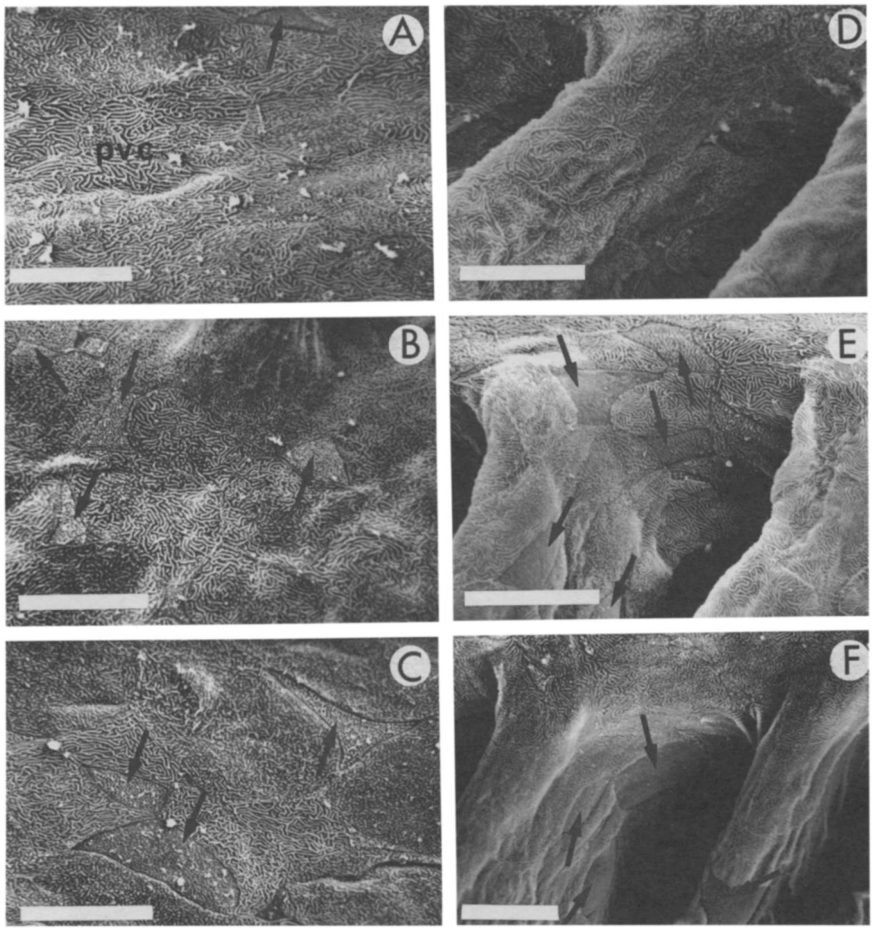

Fig. 6. Representative scanning electron micrographs of the filamental epithelium (A, B, C) *and lamellar epithelium* (D, E, F) *of Lahontan cutthroat trout reared in well water (pH 8.4;* A, D) *or alkaline Pyramid Lake water (pH 9.4) for 3 d* (B, E) *or 3 wk* (C, F). *Note the increased density of CC (indicated by arrows) on the filamental and lamellar epithelium of fish exposed to lake water for 3 d and the larger CCs after 3 wk vs. 3 d of exposure to lake water.* pvc, *Gill pavement cell; bar = 20 μm.*

water (Wilkie and Wood 1991). Unlike the rainbow trout, however, there was no tendency for J_{Amm} to return to preexposure levels in the LCT (figs. 1*A*, 2*A*). We estimate that approximately 16,000 μmol N · kg^{-1} of waste ammonia was unaccounted for during the first 72 h at pH 9.4. Plasma T_{Amm} levels (fig. 1*B*) suggest that virtually none (<1%) of the "missing" ammonia was stored in the extracellular space. The fact that J_{Amm} remained depressed

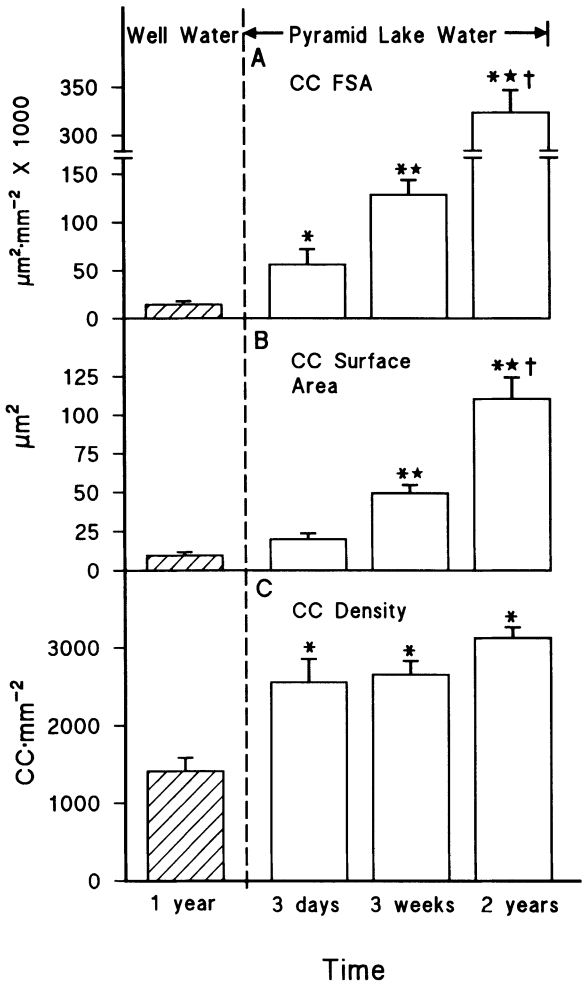

Fig. 7. Differences in branchial (A) CC FSA, (B) mean individual CC surface area, and (C) CC density of Lahontan cutthroat trout exposed to well water (pH 8.4) or Pyramid Lake water (pH 9.4) for 3 d, 3 wk, or 2 yr. Asterisks indicate significant differences from well water values; stars indicate significant differences from 3 d of exposure to Pyramid Lake water; daggers indicate significant differences from 3 wk of exposure to lake water ($P < 0.05$).

indefinitely while plasma T_{Amm} eventually returned to, and remained at, preexposure levels (figs. 1*B*, 2*B*) suggests that LCT were either excreting another N waste product(s) and/or amino acid deamination rates were permanently reduced.

The possibility that LCT were excreting another N waste product is intriguing and deserves further investigation. Absolute rates of J_{Urea} did not

change following transfer to lake water (figs. 1*A*, 2*D*), so urea did not fulfill this role. Potential alternate waste products include trimethylamine oxide (TMAO), glutamine, creatine, creatinine, or purines such as uric acid (see Forster and Goldstein 1969; Mommsen and Walsh 1992). In a related study (Wright et al. 1993), however, we were unable to detect any uric acid excretion in LCT that had been exposed to lake water for about 4 wk and that exhibited J_{Amm} and J_{Urea} very similar to those of the present study.

The alternate possibility, that of reduced use of amino acids as an energy source and reduced endogenous ammonia production, also deserves further investigation (Walton and Cowey 1982; Van Waarde 1983). The persistent respiratory alkalosis (figs. 3, 4) may have altered metabolic enzyme activities and led to a greater reliance on other fuels such as glycogen and/or fatty acids.

Pyramid Lake water's greater salinity (approximately 60 mmol · L^{-1} NaCl; table 1) may also have accounted for the some of the persistent reduction in J_{Amm}. Ammonia production may have declined in accord with an overall reduction in metabolic rate as a result of lower ionoregulatory and osmoregulatory costs in the more isotonic environment (Rao 1968). Brett (1979) noted that growth rates generally increase with salinity in euryhaline fish, such as the cutthroat trout. A greater proportion of amino acids may have been incorporated and retained in structural protein, rather than deaminated. Indeed, the rapid growth of the LCT in Pyramid Lake's alkaline/saline waters is legendary (Coleman and Johnson 1988).

The initial inhibition of J_{Amm} upon exposure of LCT to lake water was similar to that observed in rainbow trout exposed to comparable pH, and it is likely that similar explanations apply (Wright and Wood 1985; Lin and Randall 1990; Wilkie and Wood 1991; Yesaki and Iwama 1992). These include a decrease in the ΔP_{NH_3} between the blood and gill boundary layers that drives the diffusive efflux of NH_3, and/or an inhibition of Na^+/NH_4^+ exchange. Although the blood-bulk water ΔP_{NH_3} increased rather than decreased upon exposure to alkaline pH (fig. 1*C*), this value will be differentially affected by the presence or absence of boundary layer acidification and associated diffusion trapping of NH_3 as NH_4^+ in the gill water (Randall and Wright 1989). Acidification of gill water by CO_2 and possibly H^+ efflux across the gills is well established in rainbow trout in fresh water (Playle and Wood 1989; Lin and Randall 1990), and this phenomenon clearly augments J_{Amm} (Wright, Randall, and Perry 1989). It is likely that the same phenomenon occurs in the LCT in well water. Blockade of CO_2 hydration and boundary layer acidification in gill water by acetazolamide clearly reduces J_{Amm} in rainbow trout (Wright et al. 1989). In other words, boundary layer acidification increases the blood-boundary layer ΔP_{NH_3} above the

blood-bulk water ΔP_{NH_3} and effectively augments J_{Amm}. In the highly buffered waters of Pyramid Lake, however, boundary acidification is probably reduced or nonexistent. As a result, gill boundary layer pH will approach the pH of the bulk water, and therefore approximately 50% of the excreted ammonia will exist as NH_3 in the gill water. This would effectively reduce the blood-boundary layer ΔP_{NH_3} below the blood-bulk water ΔP_{NH_3} and result in reduced J_{Amm}. Indeed, Wright et al. (1993) found no effect of acetazolamide treatment on J_{Amm} in LCT adapted to Pyramid Lake water. They also found that Na^+-free water and amiloride treatment did not effect J_{Amm}, suggesting that Na^+/NH_4^+ exchange does not occur once the animals are adapted to Pyramid Lake water. In contrast, amiloride treatment consistently reduces J_{Amm} in rainbow trout in fresh water (Kirschner, Greenwald, and Kerstetter 1973; Wright and Wood 1985).

Wright et al. (1993) did not examine the LCT in well water, but they concluded that maintenance of a large blood-to-water ΔP_{NH_3}, via chronically elevated pH_a and plasma T_{Amm}, was probably the most important factor sustaining the (limited) J_{Amm} in lake water. The fact that the arterial blood P_{NH_3} in LCT in well water was similar to values observed after 3 wk and 5 wk in lake water (approximately 56 μTorr: fig. 2*C*) suggests that this species was "preadapted" to living in a high-pH lake. Such a preadaptation would likely be selected for, as would an ability to chronically reduce ammonia production rates, in a salmonid that has a long evolutionary history in alkaline lakes (Trotter 1991).

Ureagenic Enzyme Activities

Urea excretion did not account for the missing waste N. Its persistence at high pH and its greater percentage contribution to N excretion appears to maintain minimal rates of $J_{waste\ N}$. The presence of significant activities of enzymes involved in uricolysis in both well water fish and those adapted to Pyramid Lake for 3 wk or 2 yr suggests that the majority of urea production resulted from uricolysis (table 2). Danulat and Kempe (1992) observed very high rates of urea excretion in the cyprinid *Chalcalburnus tarichi,* endemic to highly alkaline Lake Van, Turkey (pH 9.8), and suggested that much of this excretion was due to hydrolysis of arginine, catalyzed by hepatic arginase. We found hepatic arginase activity was about 16-fold higher than that reported by Danulat and Kempe (1992) but equal to values reported by Chiu, Austic, and Rumsey (1986) for fingerling rainbow trout. Arginine is an essential amino acid for teleosts (Forster and Goldstein 1969), and, since the fish in the present study had been starved prior to experimentation, it

seems unlikely that there would have been sufficient arginine flux through arginase to sustain urea production.

The OUC pathway contribution to urea synthesis was insignificant in cutthroat trout, as CPS III and other enzyme activities in the cycle were negligible or very low. This observation is consistent with work performed on other salmonids (Huggins, Skutch, and Baldwin 1969; Chiu et al. 1986) and the high-pH-tolerant cyprinid *Chalcalburnus tarichi* (Danulat and Kempe 1992).

Acid-Base Balance and Ionoregulation

The respiratory alkalosis accompanying high pH exposure persisted indefinitely (figs. 3*B*, 4*B*), but the metabolic alkalosis was corrected within 24 h (figs. 3*C*, 4*C*). The long-term control of metabolic acid-base status is interesting in view of the fact that large electrochemical gradients, favoring losses of metabolic acid (protons) or gains of metabolic base, developed following transfer to high pH (table 4). The presence of significant inwardly directed electrochemical gradients for HCO_3^- and $CO_3^=$ can be attributed to the unusually high concentrations of these ions in Pyramid Lake water (Galat et al. 1985; table 1). Despite these exogenous factors, the similar levels of plasma HCO_3^- in well water LCT and those residing in Pyramid Lake for up to 2 yr suggests that these fish are in a steady state with respect to long-term acid-base status. One possible mechanism of acid-base control is the increased production of metabolic acid, via increased lactic acid production (Eichenholz et al. 1962). Such a response has been observed in rainbow trout at pH 9.5 and in LCT in pH 10 water (Wilkie and Wood 1991; Wilkie et al. 1993). We observed no such response in this study, however. Another possibility is that the persistent external base load was counteracted by a stimulation of gill Cl^-/HCO_3^- exchange. Increased Cl^-/HCO_3^- exchange might have been augmented by branchial CC proliferation, as has been demonstrated in other teleosts subjected to alkalotic disturbances in systemic acid-base status (see Goss et al. 1992*a*, 1992*b*).

We suggest that the greater CC FSA found in lake-water-adapted LCT (fig. 7) is linked to long-term acid-base regulation in an environment that exerts a continual base load on the fish. The rapidity (24 h) of the correction of the metabolic alkalosis, following transfer into Pyramid Lake water, does not argue against a branchial mechanism of metabolic acid-base control. Indeed, Goss et al. (1992*a*) demonstrated that changes in branchial morphology occur 6 h after the initiation of acid-base disturbances. Therefore, it is quite possible that increased CC FSA, and associated Cl^-/HCO_3^- exchange, accounted for the correction of the alkalosis after only 24 h of lake

water exposure. Further evidence in support of branchial acid-base regulation by the LCT was the presence of a significant correlation between pH_a and CC FSA (CC FSA = $[1.15 \times 10^6][pH_a] - [9.16 \times 10^6]$; $r = 0.585$, $P < 0.05$). Galat et al. (1985) reported that LCT, living in a variety of alkaline lakes, had considerable CC hyperplasia and suggested that it was correlated to the sum of external HCO_3, $CO_3^=$, and Cl^-. Our results corroborate these findings.

Ionoregulatory failure, characterized by 15%–20% decreases in plasma Na^+ and/or Cl^-, has been cited as a potential contributing factor in the deaths of rainbow trout exposed to high pH (Heming and Blumhagen 1988; Wilkie and Wood 1991; Yesaki and Iwama 1992). In contrast, the LCT exhibited slight increases in plasma Na^+ and Cl^- following transfer to lake water (fig. 5). The high salinity of Pyramid Lake water may account for this difference. The increase in branchial CC FSA accompanying lake water adaptation (figs. 6, 7), however, may have also have prevented ionoregulatory disturbance in these fish (Laurent, Hobe, and Dunel-Erb 1985; Laurent and Perry 1990; Perry, Goss, and Laurent 1992).

Conclusions

Unlike other salmonids, LCT readily adapt to the extreme pH of Pyramid Lake by making a number of unique physiological adjustments. The apparent persistence of reduced J_{Amm} in lake-water-adapted fish suggests that this species adapts to alkaline lake water by decreasing endogenous ammonia production. This allows the fish to rapidly correct internal ammonia levels and prevents NH_3 from reaching toxic levels. Furthermore, the LCT also rapidly corrects, and continues to regulate, its metabolic acid-base status, despite the presence of large inwardly directed electrochemical gradients for basic equivalents. Increases in branchial CC FSA may actually augment this acid-base regulation, and also prevent plasma ion dilution, through the modulation of Na^+ and Cl^- uptake at the gill.

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Literature Cited

BRETT, J. R. 1979. Environmental factors and growth. Pages 599–675 in W. S. HOAR, D. J. RANDALL, and J. R. BRETT, eds. Fish physiology. Vol. 8. Academic Press, New York.

CHIU, Y. N., R. E. AUSTIC, and G. L. RUMSEY. 1986. Urea cycle activity and arginine formation in rainbow trout (*Salmo gairdneri*). J. Nutr. 116:1640–1650.

COLEMAN, M. E., and V. K. JOHNSON. 1988. Summary of management at Pyramid Lake, Nevada, with emphasis on Lahontan cutthroat trout, 1954–1987. Am. Fisheries Soc. Symp. 4:107–115.

CVANCARA, V. A. 1969. Comparative study of liver uricase activity in freshwater teleosts. Comp. Biochem. Physiol. 28:725–732.

DANULAT, E., and S. KEMPE. 1992. Nitrogenous waste excretion and accumulation of urea and ammonia in *Chalcalburnus tarichi* (Cyprinidae), endemic to the extremely alkaline Lake Van (eastern Turkey). Fish Physiol. Biochem. 9:377–386.

EICHENHOLZ, A., R. O. MULHAUSEN, W. E. ANDERSON, and F. M. MACDONALD. 1962. Primary hypocapnia: a cause of metabolic acidosis. J. Appl. Physiol. 17:283–288.

FORSTER, R. P., and L. GOLDSTEIN. 1969. Formation of excretory products. Pages 313–350 in W. S. HOAR and D. J. RANDALL, eds. Fish physiology. Vol. 1. Academic Press, New York.

FROMM, P. O. 1963. Studies on renal and extra-renal excretion in a freshwater teleost, *Salmo gairdneri*. Comp. Biochem. Physiol. 10:121–128.

GALAT, D. L., E. L. LIDER, S. VIGG, and S. R. ROBERTSON. 1981. Limnology of a large deep North American terminal lake, Pyramid Lake, Nevada, U.S.A. Hydrobiologia 82:281–317.

GALAT, D. L., G. POST, T. J. KEEFE, and G. R. BOUCKS. 1985. Histological changes in the gill, kidney and liver of Lahontan cutthroat trout, *Salmo clarki henshawi*, living in lakes of different salinity-alkalinity. J. Fish Biol. 27:533–552.

GOLDSTEIN, L., and R. P. FORSTER. 1965. The role of uricolysis in the production of urea by fishes and other aquatic vertebrates. Comp. Biochem. Physiol. 14:567–576.

GOSS, G. G., P. LAURENT, and S. F. PERRY. 1992*a*. Evidence for a morphological component in acid-base regulation during environmental hypercapnia in the brown bullhead (*Ictalurus nebulosus*). Cell Tissue Res. 268:539–552.

GOSS, G. G., S. F. PERRY, C. M. WOOD, and P. LAURENT. 1992*b*. Mechanisms of ion and acid-base regulation at the gills of freshwater fish. J. Exp. Zool. 263:143–159.

HEMING, T. A., and K. A. BLUMHAGEN. 1988. Plasma acid-base and electrolyte status of rainbow trout exposed to alum (aluminum sulphate) in acidic and alkaline environments. Aquatic Toxicol. 12:125–140.

HUGGINS, A. K., G. SKUTCH, and E. BALDWIN. 1969. Ornithine-urea cycle enzymes in teleostean fish. Comp. Biochem. Physiol. 28:587–602.

KIRSCHNER, L. B., L. GREENWALD, and T. H. KERSTETTER. 1973. Effect of amiloride on sodium transport across body surfaces of freshwater animals. Am. J. Physiol. 224: 832–837.

KUCERA, P. A., D. L. KOCH, and G. F. MARCO. 1985. Introductions of Lahontan cutthroat trout in Omak Lake, Washington. North Am. J. Fisheries Manage. 5:296–301.

LAURENT, P., H. HOBE, and S. DUNEL-ERB. 1985. The role of environmental sodium chloride relative to calcium in gill morphology of freshwater salmonid fish. Cell Tissue Res. 240:675–692.

LAURENT, P., and S. F. PERRY. 1990. Effects of on gill chloride cell morphology and ionic uptake in freshwater trout, *Salmo gairdneri.* Cell Tissue Res. 259:429–442.

LIN, H. L., and D. J. RANDALL. 1990. The effect of varying water pH on the acidification of expired water in rainbow trout. J. Exp. Biol. 149:149–160.

MCDONALD, D. G., and C. L. MILLIGAN. 1992. Chemical properties of the blood in fish. Pages 55–133 in W. S. HOAR and D. J. RANDALL, eds. Fish physiology. Vol. 12. Academic Press, New York.

MCDONALD, D. G., and C. L. MILLIGAN. 1992. Chemical properties of the blood in Fish. Pages 55–133 in W. S. HOAR and D. J. RANDALL, eds. Fish physiology. Vol. 12. Academic Press, New York.

MOMMSEN, T. P., and P. J. WALSH. 1989. Evolution of urea synthesis in vertebrates: the piscine connection. Science 243:72–75.

———. 1991. Urea synthesis in fishes: evolutionary and biochemical perspectives. Pages 137–163 in P. HOCHACHKA and T. P. MOMMSEN, eds. The biochemistry and molecular biology of fishes. Elsevier, New York.

———. 1992. Biochemical and environmental perspectives on nitrogen metabolism in fishes. Experientia 48:583–593.

PERRY, S. F., G. G. GOSS, and P. LAURENT. 1992. The interrelationships between gill chloride cell morphology and ionic uptake in four freshwater teleosts. Can. J. Zool. 70:1775–1786.

PERRY, S. F., and C. M. WOOD. 1985. Kinetics of branchial calcium uptake in the rainbow trout: effects of acclimation to various external calcium levels. J. Exp. Biol. 116:411–433.

PLAYLE, R. C., and C. M. WOOD. 1989. Water chemistry changes in the gill microenvironment of rainbow trout: experimental observations and theory. J. Comp. Physiol. 159B:527–537.

RANDALL, D. J., C. M. WOOD, S. F. PERRY, H. BERGMAN, G. M. O. MALOIY, T. P. MOMMSEN, and P. A. WRIGHT. 1989. Urea excretion as a strategy for survival in a fish living in a very alkaline environment. Nature 337:165–166.

RANDALL, D. J., and P. A. WRIGHT. 1989. The interaction between carbon dioxide and ammonia excretion and water pH in fish. Can. J. Zool. 67:2936–2942.

RAO, G. M. M. 1968. Oxygen consumption of rainbow trout (*Salmo gairdneri*) in relation to activity and salinity. Can. J. Zool. 46:781–786.

SAS Institute. 1989. SAS JMP user's guide. SAS Institute, Cary, N.C.

SOIVIO, A. K., K. WESTMAN, and K. NYHOLM. 1972. Improved method of dorsal aortal catheterization: haematological effects followed for three weeks in rainbow trout. Finn. Fisheries Res. 1:11–21.

TROTTER, P. 1991. Cutthroat trout. Pages 236–265 in J. STOLZ and J. SCHNELL, eds. Trout. Stackpole, Harrisburg, Pa.

VAN WAARDE, A. 1983. Aerobic and anaerobic ammonia production by fish. Comp. Biochem. Physiol. 74B:675–684.

WALTON, M. J., and C. B. COWEY. 1982. Aspects of intermediary metabolism in salmonid fish. Comp. Biochem. Physiol. 73B:59–79.

WILKIE, M. P., and C. M. WOOD. 1991. Nitrogenous waste excretion, acid-base regulation, and ionoregulation in rainbow trout (*Oncorhynchus mykiss*) exposed to extremely alkaline water. Physiol. Zool. 64:1069–1086.

WILKIE, M. P., P. A. WRIGHT, G. K. IWAMA, and C. M. WOOD. 1993. The physiological responses of the Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*), a resident of highly alkaline Pyramid Lake (pH 9. 4), to challenge at pH 10. J. Exp. Biol. 175:173–194.

WILLIAMS, J. E., J. E. JOHNSON, D. A. HENDRICKSON, S. CONTRERAS-BALDERAS, J. D. WILLIAMS, J. NAVARRO-MENDOZA, D. E. MCALLISTER, and J. E. DEACON. 1989. Fishes of North America endangered, threatened, or of special concern: 1989. Fisheries 14: 2–20.

WOLF, K. 1963. Physiological salines for freshwater teleosts. Progressive Fish Culturalist 25:615–625.

WOOD, C. M. 1993. Ammonia and urea metabolism and excretion. Pages 379–425 in D. H. EVANS, ed. The physiology of fishes. CRC, Boca Raton, Fla.

WOODWARD, J. J., and R. J. STRANGE. 1987. Physiological stress responses in wild and hatchery-reared rainbow trout. Trans. Am. Fisheries Soc. 116:574–579.

WRIGHT, P. A., G. K. IWAMA, and C. M. WOOD. 1993. Ammonia and urea excretion in Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) adapted to highly alkaline Pyramid Lake (pH 9.4). J. Exp. Biol. 175:153–172.

WRIGHT, P. A., D. J. RANDALL, and S. F. PERRY. 1989. Fish gill boundary layer: a site of linkage between carbon dioxide and ammonia excretion. J. Comp. Physiol. 158B:627–635.

WRIGHT, P. A., and C. M. WOOD. 1985. An analysis of branchial ammonia excretion in the freshwater rainbow trout: effects of environmental pH change and sodium uptake blockade. J. Exp. Biol. 114:329–353.

YESAKI, T. Y., and G. K. IWAMA. 1992. Some effects of water hardness on survival, acid-base regulation, ion regulation and ammonia excretion in rainbow trout in highly alkaline water. Physiol. Zool. 65:763–787.