Przewalski's Naked Carp (*Gymnocypris przewalskii*): An Endangered Species Taking a Metabolic Holiday in Lake Qinghai, China

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ABSTRACT

The naked carp is an endangered cyprinid that migrates annually between freshwater rivers, where it spawns, and Lake Qinghai, where it feeds and grows. Lake Qinghai is a high-altitude lake (3,200 m) in western China that currently exhibits the following composition (in mmol L^{-1}: $[Na^+]$ 200, $[Cl^-]$ 173, $[Mg^{2+}]$ 36, $[Ca^{2+}]$ 0.23, $[K^+]$ 5.3, total CO_2 21, titration alkalinity 29; osmolality 375 mOsm kg^{-1}; pH 9.3), but concentrations are increasing because of water diversion and climate change. We studied the physiology of river water to lake water transfer. When river fish are transferred to lake water, there is a transitory metabolic acidosis followed by a slight respiratory alkalosis, and hemoconcentration occurs. All plasma electrolytes rise over the initial 48 h, and final levels in lake water–acclimated fish are very close to lake water concentrations for $[Na^+]$, $[Cl^-]$, $[K^+]$, and osmolality, whereas $[Ca^{2+}]$ continues to be regulated well above ambient levels. However, $[Mg^{2+}]$ rises to a much greater extent (fourfold in 48 h); final plasma levels in lake fish may reach 12 mmol L^{-1} but are still much lower than in lake water (36 mmol L^{-1}). At the same time, urine flow rate decreases drastically to <5% of river water values; only the renal excretion of Mg^{2+} is maintained. Both gill and kidney Na^+,K^+-ATPase rapidly decline, with final levels in lake water fish only 30% and 70%, respectively, of those in river water fish. Metabolic rate also quickly decreases on exposure to lake water, with O_2 consumption and ammonia-N excretion rates eventually falling to only 60% and 30%, respectively, of those in river fish, while plasma ammonia rises fivefold. The fish appear to be benefiting from a metabolic holiday at present because of decreases in iono- and osmoregulatory costs while in lake water; elevated plasma $[Mg^{2+}]$ and ammonia may be additional factors depressing metabolic rate. If the lake continues to dehydrate, these benefits may change to pathology.

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Introduction

Przewalski's naked carp *Gymnocypris przewalskii* (Kessler), also known as the scaleless carp, is endemic to the austere environment of Lake Qinghai, the largest inland water body in China (Wu and Wu 1992; Shi and Qi 2000). Lake Qinghai is a high-altitude (3,200 m), alkaline (pH ~ 9.3), saline (9–13 ppt) lake on the Qinghai-Tibet plateau in the western highlands. Because of the altitude, O_2 content and Po_2 are only about 60% of sea level values. Lake temperature is close to 0°C for much of the year (mean annual air temperature is −0.6°C) but may rise to 15°–20°C at the surface in the summer (Qin and Huang 1998). Between March and July, fish migrate into the freshwater rivers that supply the lake in order to spawn, because the gravel substrate and swift water flow of the rivers are required for the redds in which the eggs incubate and hatch (Muir 1990). The carp then return to the lake for the rest of the year, during which most feeding and growth is thought to occur (Muir 1990; Wu and Wu 1992; Walker et al. 1996; Shi and Qi 2000). Not surprisingly, these planktivores appear to be very slow growing, with estimates of 7–10 yr to reach the typical reproductive size of 300–500 g (Guong and Hu 1975; Muir 1990; Walker et al. 1996). Although historically abundant, the population collapsed because of overfishing and destruction of spawning hab-

itat through dam building for irrigation. By the early 1990s, the species had become endangered. The commercial fishery was officially suspended in 1994, and some spawning habitat has been restored. These measures have resulted in a slow recovery, though present population numbers are only a fraction of historical levels.

However, diversion of river water for agriculture continues; this practice, compounded by climate change, has resulted in a decline of the water level in the lake by about 10–12 cm yr^{-1} over the past 50 yr. Modeling indicates that evaporative losses clearly exceed freshwater input (Qin and Huang 1998), so it is not surprising that the lake water appears to have become increasingly saline over this period. Reliable water chemistry data are scarce; the available measurements in Table 1 suggest considerable regional and seasonal variability. However, overall they indicate that an increase in salinity has occurred since 1978 and may have accelerated since our previous expedition to Lake Qinghai in 1998 (Wang et al. 2003). Total dissolved solids concentration is now about 13 ppt. Mg^{2+}, an ion usually considered to be highly toxic to fish (Marshall and Grosell 2005), is now 36 mmol L^{-1}, while Ca^{2+} has remained low and pH and titratable alkalinity constant, the latter representing the HCO_3^- and CO_3^- ions responsible for the high pH, presumably due to selective $CaCO_3$ precipitation. This suggests that ionic-osmotic challenge may pose yet another threat to the species, since cyprinids are usually considered to be rather stenohaline (e.g., De Boeck et al. 2000), although *Chalcalburnus tarichi* of Lake Van, Turkey (pH ~ 9.8, salinity ~ 22 ppt), is a notable exception (Danulat and Kempe 1992).

With this background in mind, the focus of this study from our 2004 expedition to Lake Qinghai was the physiology of ionoregulation, osmoregulation, and acid-base balance of the naked carp in river water and lake water. We were particularly interested in the possible challenges faced by the fish during their return migration from the circumneutral freshwater rivers to the alkaline, saline lake water, so an experimental river water to lake water transfer was performed. In one series, fish were fitted with indwelling catheters for measurements of blood gases, acid-base status, plasma chemistry, and urine composition and flow rate. The latter provided an index of osmotic water flux and allowed us to examine the possible role of the kidney in Mg regulation (Hickman and Trump 1969; Oikari and Rankin 1985). The plasma chemistry measurements were repeated in a second series using noncannulated animals to control for any disturbances associated with catheterization. Na^+,K^+-ATPase activities were measured in the three ionoregulatory tissues, gills, kidney, and intestine. The question of metabolic costs, together with our earlier data indicating differences in N-metabolism between fish in river water versus lake water (Wang et al. 2003), led us to measure O_2 consumption and ammonia excretion rates at rest and after exercise in the two environments. Overall, our results provide a picture of a fish undergoing profound physiological changes yet reaping surprising benefit from the current composition of the lake water.

Material and Methods

Experimental Site, Animals, and Logistics

Experiments were performed between June 13 and June 26, 2004. Our field camp was located at the site of the Lake Qinghai Fish Processing Plant (36°33′18″N, 100°38′50″E) on the southeastern shore of Lake Qinghai. The plant is currently shut down because of the moratorium on fishing, but other commercial activities continue there. The management kindly placed a disused fishing vessel at our disposal to use as an outdoor laboratory, together with electrical and water supplies. The bow of the vessel, covered with tarpaulins for protection from the elements, served as a wet laboratory, and a tent secured on the stern of the vessel provided a dry area for analytical equipment.

Table 1: Chemistry of Lake Qinghai and the Black Horse River

	1962[a]	1978[a]	1986[b]	1998[c]	1998[d]	2004[e]	River 2004[e]
Na^+ (mmol L^{-1})	142	71	163	88	178	200	1.0
Cl^- (mmol L^{-1})	148	71	165	99	176	173	2.1
Mg^{2+} (mmol L^{-1})	34	13	33	19	35	36	.3
Ca^{2+} (mmol L^{-1})	.25	.06	.32	.30	.13	.23	.96
K^+ (mmol L^{-1})	3.8	2.3	4.0	2.5	5.2	5.3	.03
Titration alkalinity (mmol L^{-1})	…	…	…	30	30	29	2.9
Total CO_2 (mmol L^{-1})	15.6	…	19.8	…	…	21.2	1.6
Osmolality (mOsm kg^{-1})	…	…	…	…	…	375	8
pH	…	9.4	…	9.2	9.2	9.3	7.2

[a] Academia Sinica 1979.
[b] Chen 1991.
[c] Wang et al. 2003.
[d] R. Gonzalez, personal communication.
[e] This study.

The fishing vessel was moored at a dock where water depth was about 15 m, so continuously flowing lake water (2004 values in Table 1) was pumped from a depth of about 10 m. Continuously flowing well water originating from a nearby stream was also plumbed onto the vessel. This water was essentially identical in composition (Table 1) to that of the Black Horse River, our principal fish collection site, and is subsequently referred to as river water. Temperatures fluctuated diurnally in both water sources from nighttime lows of about 11°C to late afternoon highs of 15°C.

All fish were collected under permits issued by local and national authorities, and experimental procedures were in accord with national animal care regulations. River water–acclimated carp were collected by beach seine on the Black Horse River, approximately 8 km upstream from its entry point into the southwestern corner of Lake Qinghai. Typical fish mass was about 150 g. These fish were on migration to their spawning grounds further upstream, but interestingly, many of them were immature and exhibited little or no evidence of gonadal development, suggesting that immature fish may participate in the freshwater migration without actually spawning. Lake water–acclimated carp of similar size were collected from the southeast region of Lake Qinghai by an artisanal fisherman using short sets of a gill net. Mortality from gill netting was high, and only apparently healthy fish without visible gill damage were used, so the number of lake water fish available for experiments was limited. Fish were transported back to the wet laboratory in aerated buckets and then placed in round 160-L polyethylene pails at a density of about 30 fish per pail. The pails were covered, aerated, and served with a continuous flow of river water or lake water, as appropriate. The fish were allowed to settle for 1–3 d before experimentation.

While some analyses were performed on-site, plasma, urine, and tissue samples for assay of ions, metabolites, and enzymes were frozen in liquid N_2 and transported back to Canada at −80°C in two dry shippers. One of these dry shippers failed in transit, thawing the samples, so a considerable amount of metabolite and enzyme data were lost, though it was still possible to measure ion concentrations on the sealed samples.

Experimental Series

Series 1. This series focused on changes in blood gases, acid-base status, plasma chemistry, and renal function during the river water to lake water transition in cannulated animals. River water–acclimated fish were anesthetized in neutralized MS-222 (0.1 g L^{-1}; Sigma) on an operating table and fitted with indwelling blood (PE-50 polyethylene tubing; Clay-Adams) and urinary (PE-60) catheters. The internal urinary bladder catheterization technique was performed exactly as described by Wood and Patrick (1994). This method collects urine as soon as it drains from the ureters with a siphon of 3 cm below the water surface, negating any reabsorptive actions of the urinary bladder. Dorsal aortic catheterization through the roof of the pharynx was initially attempted by the method of Soivio et al. (1972) designed for salmonids, but it proved to have a low success rate because of the far posterior and deeply buried position of the vessel in the naked carp. Furthermore, these catheters often shifted in position and became nonfunctional. Some data were collected with this technique, but most fish were instead fitted with blood-sampling catheters in the caudal vein through a lateral incision in the peduncle. The technique was essentially identical to that described for the toadfish by Wood et al. (1997). Catheters were filled with Cortland saline (Wolf 1963) heparinized with 50 IU mL^{-1} of lithium heparin (Sigma). Incisions were dusted with oxytetracycline (Sigma) and closed with silk sutures. Fish were then allowed to recover for 36 h in individual polyethylene chambers (~6 L volume) served with aeration and continuously flowing river water (~ 0.2 L min^{-1}). During this period, the patency of the catheters was assessed. Urine flow rate (UFR) was determined gravimetrically and was expressed relative to the mass of the fish, and urinary excretion rates of osmolytes were expressed as the product of concentration times mass-specific UFR.

The experimental protocol ($N = 7$) consisted of two 12-h periods of urine collection in river water (pretransfer), followed by four 12-h periods in lake water (posttransfer). The transfer was effected rapidly by vigorously flushing the fish boxes with lake water and then restoring the flow rate to ~0.2 L min^{-1}. In a parallel control series ($N = 5$), fish were transferred from river water to river water. Blood samples (500 μL) were drawn into chilled gas-tight Hamilton syringes from the catheters in between the two control periods (pretransfer) and at 12 and 36 h (posttransfer), again in between the urine-collection periods. A water sample from in front of the fish's mouth was taken for measurement of inspired Po_2, and blood samples were immediately analyzed for pH, Po_2, and plasma total CO_2, while the remainder of the plasma (obtained by centrifugation at 10,000 *g* for 30 s) was frozen in liquid N_2 for later analysis of ions, osmolality, and metabolites. Red blood cells were resuspended in Cortland saline and reinfused to maintain hematocrit and blood volume. Urine samples were similarly frozen for later analysis. At the end of the experiment, the urinary catheters were checked for leaks. Reliable urinary data were obtained from five experimental fish but only two control fish.

Series 2. This series repeated the plasma chemistry measurements of series 1, using terminal blood sampling from noncannulated animals. The goal was to ensure that changes seen in series 1 were not an artifact of disturbance as well as to provide a more detailed description of the time course of responses. This series also provided additional measures (plasma protein, blood hemoglobin) indicative of potential hemoconcentration and tissue samples for composition and Na^+,K^+-ATPase measurements, plus some terminal samples of bladder urine and gut fluids.

A group of about 40 river water–acclimated fish were placed in a single 160-L pail that was covered, aerated, and continually flushed with flowing river water. At 0 h, the water was rapidly replaced with flowing lake water. Before this transfer and at 12, 24, 36, and 48 h posttransfer, a minimum of six fish at each time point were individually removed, terminally anesthetized in MS-222 (0.5 g L^{-1}), and blood sampled by blind caudal puncture. Blood was immediately assayed for hemoglobin concentration and plasma for protein concentration; the remainder of the plasma was then frozen in liquid N_2 for later analysis. Gills, kidney, and anterior and posterior intestine, followed by a variety of other tissues, were quickly dissected, wrapped in aluminum foil, and similarly frozen. A group of lake water–acclimated fish held in flowing lake water were also sampled in this manner. In these fish as well as in pretransfer river water fish, intestinal fluids and bladder urine were collected when present.

Series 3. The objective here was to determine metabolic and N-excretion rates at rest and after exhaustive exercise in naked carp acclimated to river water, acclimated to lake water, and during the transfer between the two. Fish were placed in the same 6-L polyethylene chambers used in series 1 and allowed to settle overnight. These could be sealed as respirometers. The same river water–acclimated fish ($N = 6$) were assayed in river water and then again at 12 and 36 h after transfer to lake water. A control group ($N = 4$) of river water–acclimated carp were put through an identical protocol and assayed in river water and at 12 and 36 h after transfer to river water. For comparison, lake water–acclimated fish ($N = 5$) were assayed at one time point only in lake water.

At each time point, the resting measurement was made first, followed by the postexercise measurement. Flow and aeration were stopped and the respirometer sealed for a 1-h period, during which the rates of O_2 depletion and ammonia-N and urea-N accumulation were monitored. The respirometer was then flushed and reaerated for 0.5–1 h. After this, the fish was transferred to a 50-L bucket, manually chased until refractory to stimulation (~5 min), and returned to its chamber for an additional 30 min of closed-system respirometry, after which flow and aeration were reestablished. Water samples were analyzed immediately for Po_2 and stored briefly at 4°C before the assay for ammonia-N and urea-N.

Analytical Methods and Calculations

On-site measurements included the following. Plasma protein was measured by refractometry (American Optical TS-meter) and whole blood hemoglobin by the cyamethemoglobin method using Drabkin's reagent (Sigma-Aldrich). Oxygen tensions (Po_2) in water and blood samples as well as blood pH were measured using Radiometer microelectrodes kept at the same temperature as the fish with water jackets. Electrode outputs were displayed on Cameron OM-200 oxygen meters (Port Aransas, Texas) and Radiometer pHM 71 and pHM 72 acid-base analyzers (Copenhagen). True plasma CO_2 was measured by the method of Cameron (1971), again using a Radiometer microelectrode, on plasma obtained from blood samples centrifuged in sealed tubes. The same method was used to measure water total CO_2 levels. Titratable alkalinity was measured by titrating 10-mL water samples, with continual aeration, to pH 4.0 with standardized 0.2 N HCl (Sigma-Aldrich) using a Gilmont microburette (McDonald and Wood 1981). Blood plasma carbon dioxide tensions (Pco_2) and bicarbonate concentrations ($[HCO_3^-]$) were calculated using the solubility of carbon dioxide (α_{CO_2}), the apparent pK (pK_{app}) for teleost blood at the experimental temperature, and rearrangements of the Henderson-Hasselbalch equation according to Boutilier et al. (1984). Po_2 measurements in water were converted to O_2 concentrations using the solubility coefficient (α_{O_2}) for O_2 at the appropriate temperature and salinity, again tabulated by Boutilier et al. (1984). Ammonia-N and urea-N concentrations in water were determined by the salicylate hypochlorite (Verdouw et al. 1978) and diacetyl monoxime (Rahmatullah and Boyde 1980) methods, respectively; for the former, we found it important to make up the standards in the appropriate water quality (river water or lake water). O_2 consumption rates (Mo_2) were calculated from the rates of decline of O_2 concentration in the closed respirometers, factored by fish mass and water volume. Ammonia-N and urea-N excretion rates were similarly calculated from rates of increase of these compounds in the respirometers. Ammonia quotients (AQ), an indicator of fuel utilization (Lauff and Wood 1996), were calculated as the molar ratios of ammonia-N excretion to O_2 consumption.

The rest of the analyses were performed at McMaster University. Na^+, Ca^{2+}, Mg^{2+}, and K^{2+} levels in water, plasma, intestinal fluid, and urine were measured by flame atomic absorption spectroscopy (Varian SpectrAA-220FS, Mulgrave, Australia) and Cl^- by coulometric titration (Radiometer CMT 10 chloridometer). Note that these are measurements of total ion concentrations, not free ion concentrations. Osmolality was determined by vapor pressure osmometry (Wescor 5100C). Ammonia levels in plasma and urine were determined using the glutamate dehydrogenase method (Raichem, ammonia reagent, product 85446). For determination of Na^+,K^+-ATPase activities, gills, kidney, and intestinal tissues were homogenized in SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) containing 0.1% Na^+-deoxycholate and centrifuged at 5,000 *g* for 30 s at 4°C. Supernatants were immediately frozen in liquid nitrogen and stored at −80°C until analyzed. ATPase activity (McCormick 1993) was determined in the presence or absence of 0.5 mM ouabain and normalized to total protein content (measured using the bicinchoninic acid method; Sigma-Aldrich). Ouabain-sensitive ATPase activity was expressed as μmol ADP mg protein^{-1} h^{-1}. For other measurements, tissues were initially ground into a fine powder under

Table 2: Arterial and venous blood gases of naked carp in river water in series 1

	Arterial	Venous
N	6	9
Inspired Po_2 (torr)	78.5 ± 2.4	79.2 ± 3.0
Po_2 (torr)	49.9 ± 6.2	12.2 ± 1.6
Pco_2 (torr)	1.45 ± .04	1.98 ± .21
pH	8.10 ± .02	8.07 ± .04
$[HCO_3^-]$ (mmol L^{-1})	9.23 ± .49	11.79 ± 1.47

Note. Data are means ± 1 SEM. Po_2 at air saturation = 102 torr. Measured total CO_2 levels (not shown) in plasma were slightly higher than $[HCO_3^-]$ levels, which were calculated by the Henderson-Hasselbalch equation.

liquid N_2 in a mortar and pestle. Protein content was determined via the Lowry method as modified by Miller (1959) using bovine serum albumin (Sigma) as a standard. Glucose, glycogen, and lactate were determined as by Bergmeyer (1985). The sum, in glucosyl unit equivalents, was converted to mass assuming a molecular weight of 180 to provide a measure of carbohydrate. Lipids were determined gravimetrically after extraction in chloroform-methanol (2 : 1) exactly as described by Lauff and Wood (1996).

Statistics

Unless otherwise stated, data have been expressed as means ± 1 SEM (N), where N = number of fish. In series 1 and 3, Dunnett's paired multiple comparison test was employed to detect specific differences (indicated by asterisks) within a treatment group (control or experimental) at times posttransfer relative to the pretransfer value. Student's unpaired t-tests were applied to detect specific differences (indicated by crosses) between experimental and control groups at the same sampling time in these same series as well as between river water– and lake water–exposed carp in series 2 and 3. The Bonferroni correction for multiple comparisons was applied. Student's paired t-tests were employed to detect differences between resting and postexercise values at the same time posttransfer in series 3, reflecting the paired design. In a few cases, data were log transformed before analysis to equalize variances. A significance level of $P \leq 0.05$ was used throughout.

Results

Series 1: Cannulation Studies

Inspired Po_2 in the fish boxes was about 79 torr, approximately 80% relative to air saturation (102 torr) at this altitude. Under these conditions, river water–acclimated fish maintained PaO_2 and PvO_2 values around 50 and 12 torr, respectively (Table 2). Both pHa and pHv were around 8.1, while $PaCO_2$ and $PvCO_2$ values were about 1.5 and 2 torr, respectively, with small corresponding differences in plasma $[HCO_3^-]$ (9–12 mmol L^{-1}). Thus, despite the high altitude, blood gases were fairly typical for fish at this temperature.

Transfer from circumneutral river water to alkaline lake water induced a slight fall in $PvCO_2$ at 36 h (Fig. 1*A*). PvO_2 was unaffected (Fig. 1*B*). There was an indication of metabolic acidosis at 12 h posttransfer, with significant falls in pHv (Fig. 2*A*) and $[HCO_3^-]$v (Fig. 2*B*) at this time, but both values had recovered by 36 h, with a slight overshoot in pHv. The control series demonstrated that there were no effects of the transfer procedure or blood sampling itself on venous blood gases (Figs. 1, 2). Only one fish completed the experimental protocol with a functioning arterial catheter, but its data reinforced the trends seen in the venous samples, with no changes in PaO_2 but pHa rising to 8.26 (vs. 8.08 pretransfer) and $PaCO_2$ falling to 0.87 torr (vs. 1.59 torr pretransfer) by 36 h in lake water. These followed transitory falls in pHa and $[HCO_3^-]$a at 12 h. Unfortunately, plasma samples could not be analyzed for lactate or ammonia because of thawing in transit.

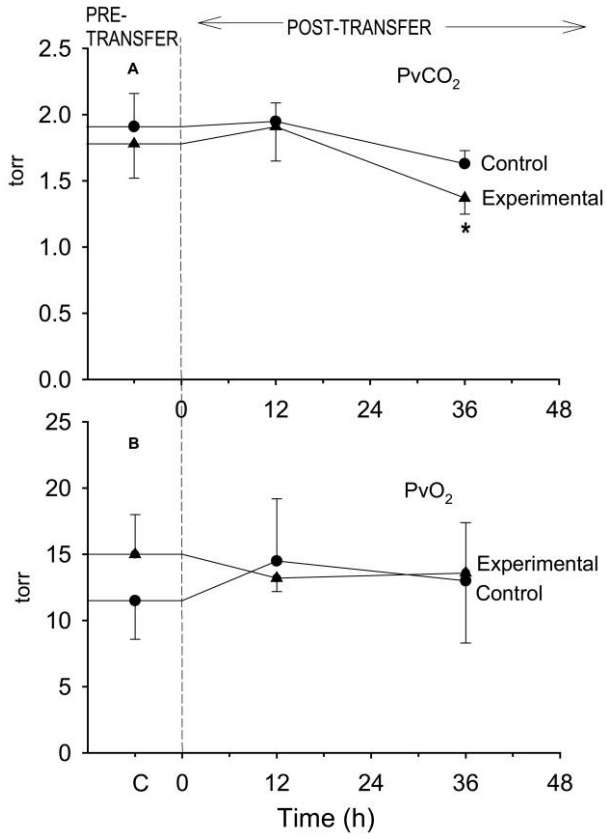

Figure 1. Partial pressures of carbon dioxide ($PvCO_2$; *A*) and oxygen (PvO_2; *B*) in venous blood of river water–acclimated naked carp before and after transfer to lake water (N = 7) in series 1. The control group (N = 5) was transferred from river water to river water. Means ± 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from respective pretransfer value in the same fish.

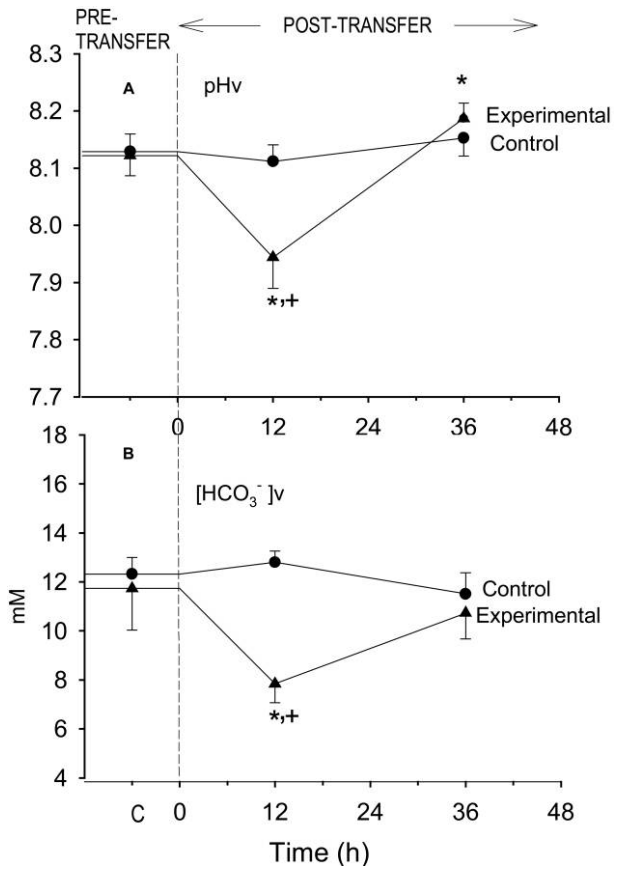

Figure 2. pH (pHv; *A*) and plasma bicarbonate concentration ($[HCO_3^-]v$; *B*) in venous blood of river water–acclimated naked carp before and after transfer to lake water ($N = 7$) in series 1. The control group ($N = 5$) was transferred from river water to river water. Means ± 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from respective pretransfer value in the same fish. Cross indicates significantly different ($P \leq 0.05$) from simultaneous value in the control group.

Plasma ion concentrations and osmolality in these cannulated carp in river water were all very close to the values tabulated in Table 3 for noncannulated, terminally sampled fish in series 2, apart from $[K^+]$, which was slightly elevated in the cannulated animals. Transfer to lake water caused marked increases in plasma osmolality (Fig. 3*A*), $[Na^+]$ (Fig. 3*B*), $[Cl^-]$ (Fig. 3*C*), $[Mg^{2+}]$ (Fig. 4*A*), $[Ca^{2+}]$ (Fig. 4*B*), and $[K^+]$ (Fig. 4*C*) over the following 36 h. For all except $[K^+]$, the increases were already significant by 12 h posttransfer. The most dramatic response was seen in plasma $[Mg^{2+}]$, which increased fourfold by 36 h. For the other ions and osmolalities, increases were in the range of 15%–40%. As with blood gases, the control series demonstrated that there were no effects of the experimental protocol itself on the parameters measured (Figs. 3, 4).

The two control fish with patent urinary catheters showed no substantive changes in UFR, urine composition, or urinary osmolyte excretion rates (data not shown) as a result of the experimental protocol. Urinary ion concentrations and osmolality in urine collected by bladder catheterization in river water–acclimated carp (data not shown) were all similar to but slightly higher than measurements made by terminal spot sampling in series 2, which are tabulated in Table 3. The only significant difference was for urinary $[Cl^-]$ (18.4 ± 3.1 vs. 9.4 ± 2.3 mmol L^{-1} in spot-sampled fish). Therefore, we have elected to illustrate urinary osmolyte excretion rates rather than concentrations in Figures 6 and 7.

UFR was about 4 mL kg h^{-1} in river water carp (Fig. 5). UFR fell precipitously by 80% within the first 12 h after transfer to lake water and reached <5% of the lake water rate by 36–48 h (Fig. 5). This was accompanied by decreases of 70%–90% in the excretion rates of total osmolytes (Fig. 6*A*), Na^+ (Fig. 6*B*), Ca^{2+} (Fig. 7*B*), and K^+ (Fig. 7*C*) and about 50% for Cl^- (Fig. 6*B*). The urinary excretion rate of Mg^{2+} behaved very differently (Fig. 7*A*). It fell initially but thereafter was restored (and indeed was slightly elevated at 24–36 h) because of substantial increases in Mg^{2+} concentration (30.32 ± 5.89 mmol L^{-1} at 36–48 h posttransfer vs. 0.82 ± 0.11 mmol L^{-1} pretransfer). This 37-fold increase in urinary $[Mg^{2+}]$ may be contrasted with fourfold to 12-fold increases in the concentrations of other urinary osmolytes.

Series 2: Terminal Sampling Studies

Terminal sampling demonstrated substantial differences in plasma composition between river water–acclimated and lake water–acclimated carp (Table 3). Plasma osmolality, $[Na^+]$, $[Cl^-]$, and $[K^+]$ were all about 60% higher in lake water fish, while $[Ca^{2+}]$ was 23% higher. However, the most marked differences were in plasma $[Mg^{2+}]$ (13-fold higher in lake water fish) and [ammonia] (fivefold higher).

Terminal sampling of noncannulated fish after transfer from river water to lake water confirmed the plasma ion patterns seen in the cannulated fish of series 1. All ions and osmolality rose significantly after transfer (Figs. 8, 9). Over 48 h, the increases were in the range of 20%–30% for osmolality (Fig. 8*A*), $[Na^+]$ (Fig. 8*B*), $[Cl^-]$ (Fig. 8*C*), and $[Ca^{2+}]$ (Fig. 9*B*) but were 4.3-fold for $[Mg^{2+}]$ (Fig. 9*A*), all very similar to the responses of series 1. Only the plasma $[K^+]$ response (2.4-fold increase) was larger than in series 1 (Fig. 9*C* vs. Fig. 4*C*), but these noncannulated fish started with lower $[K^+]$ levels in river water and exhibited an apparent overshoot at 48 h. The additional measurement of plasma [protein] (Fig. 10*A*) and blood [hemoglobin] (Fig. 10*B*) provided indexes of the extent of hemoconcentration (20%–25%) due to fluid shifts (see "Discussion"), indicating that observed changes in osmolality (Fig. 8*A*), $[Na^+]$ (Fig. 8*B*), $[Cl^-]$ (Fig. 8*C*), and $[Ca^{2+}]$ (Fig. 9*B*) were all largely attributable to this phenomenon, whereas the larger increases in plasma $[Mg^{2+}]$ (Fig. 9*A*) and $[K^+]$ (Fig. 9*C*) had a different origin.

Table 3: Measurements of total ion concentrations and osmolalities in plasma, urine, and intestinal fluid obtained by terminal sampling in naked carp collected from river water and lake water in series 2

	Plasma		Urine		Gut Fluid
	River	Lake	River	Lake	Lake
N	6	14	8	8	5–9
Na^+ (mmol L^{-1})	138.3 ± 4.2	222.0 ± 8.1*	20.7 ± 2.4	68.9 ± 15.1*	139.4 ± 9.6
Cl^- (mmol L^{-1})	130.0 ± 5.9	208.9 ± 8.8*	9.4 ± 2.3	54.9 ± 14.2*	176.3 ± 14.7
Mg^{2+} (mmol L^{-1})	.91 ± .03	11.96 ± 1.36*	.66 ± .06	14.6 ± 3.3*	40.4 ± 6.7
Ca^{2+} (mmol L^{-1})	2.87 ± .13	3.53 ± .26*	1.33 ± .14	4.91 ± 1.02*	31.1 ± 6.5
K^+ (mmol L^{-1})	2.74 ± .19	4.35 ± .17*	1.50 ± .14	16.73 ± 6.88*	…
Ammonia (mmol L^{-1})	.57 ± .04	2.43 ± .24*	1.23 ± .16	…	…
Osmolality (mOsm kg^{-1})	282.7 ± 5.1	447.5 ± 8.9*	68.6 ± 5.4	…	707.5 ± 64.3

Note. Data are means ± 1 SEM. Ellipsis indicates that the measurement was lost because of dry shipper failure or insufficient volume remaining.
* $P \leq 0.05$ relative to corresponding value in river water fish.

The plasma values from terminal samples of lake water–acclimated fish are also included for reference in Figures 8 and 9, as well as the relevant concentrations in lake water (from Table 1). These data suggest that plasma osmolality (Fig. 8*A*), $[Na^+]$ (Fig. 8*B*), and $[Cl^-]$ (Fig. 8*C*) would continue to rise over time after transfer, eventually stabilizing at values slightly higher than in lake water (but see "Discussion"). Plasma $[K^+]$, after an initial overshoot, also would appear to equilibrate with the lake water $[K^+]$ (Fig. 9*C*). However, plasma $[Ca^{2+}]$ (Fig. 9*B*) appears to be regulated at much higher levels than the low concentrations (Table 1) in either river water or lake water. Finally, while the fish allow plasma $[Mg^{2+}]$ (Fig. 9*A*) to rise considerably in the face of the Mg^{2+} challenge from lake water (Table 1), they are successful in keeping it at less than 35% of the levels in lake water.

Bladder urine obtained by terminal sampling (Table 3) of river water–acclimated versus lake water–acclimated carp confirmed that all ions were in much higher concentrations in the latter. Most notable were an 11-fold difference in urinary $[K^+]$ and a 22-fold difference in urinary $[Mg^{2+}]$.

Intestinal fluid could be obtained only from the anterior region and only in lake water–acclimated fish (Table 3) and was mixed with partially digested plankton. Notably, osmolality, $[Mg^{2+}]$, and $[Ca^{2+}]$ were all markedly higher than plasma levels, in contrast to $[Na^+]$ and $[Cl^-]$.

Na^+,K^+-ATPase samples survived transit for only some of the terminal sampling times. Nevertheless, the data indicate much lower activity levels in both the gills (by 70%; Fig. 11*A*) and the kidney (by 30%; Fig. 11*B*) of lake water fish relative to river water fish. Furthermore, these decreases were initiated rapidly after river water to lake water transfer, with gill activities falling by 50%–60% (significant by 12 h; Fig. 11*A*) and kidney activities by 20% (Fig. 11*B*; significant by 48 h). In marked contrast, there were no changes in Na^+,K^+-ATPase activity levels in either the anterior (Fig. 12*A*) or posterior intestine (Fig. 12*B*) after transfer of river water fish to lake water.

Tissue proximate composition measurements (Table 4) on fish collected from river water versus lake water revealed significantly higher lipid stores (by 1.6–2.2-fold) in the red muscle, white muscle, and liver of the latter. Notably, in both populations, red muscle lipid concentrations were eightfold to 10-fold higher than in white muscle, while liver concentrations were intermediate. In contrast to lipid, carbohydrate concentrations were fourfold higher in the livers of river water–acclimated carp than in lake water fish, whereas there were no differences in white and red muscle concentrations between the two populations. Notably, in both river and lake fish, carbohydrate concentrations were much higher in liver than in either red or white muscle, which had similar levels. Protein concentrations were about 30% higher in the livers and red muscle of river fish than lake fish. Protein levels were fairly similar among different tissues.

Series 3: Respirometry Studies

Resting metabolic rates (Mo_2) of lake water–acclimated fish were only 60% of those in river water–acclimated fish (Fig. 13*A*). After transfer to lake water, river water carp exhibited a progressive decline in Mo_2 that was significant by 12 h (35% fall) and that reached about 40% by 36 h (Fig. 13*A*). The control group, put through an identical experimental procedure in river water only, exhibited no changes (Fig. 13*B*), so this was not an artifact of handling but rather a true effect of lake water exposure.

Effects on N-excretion were equally pronounced. Urea-N excretion rates were below detection in most fish (certainly below 25 μmol N $kg\ h^{-1}$ overall) and thus have been omitted. Within 12 h following transfer to lake water, resting ammonia-N excretion rates in river water fish had declined dramatically, and rates of lake water–acclimated carp were only 26% of those in river water–acclimated fish (Fig. 14*A*). Again, there were no changes in the control group (Fig. 14*B*), so the depression of

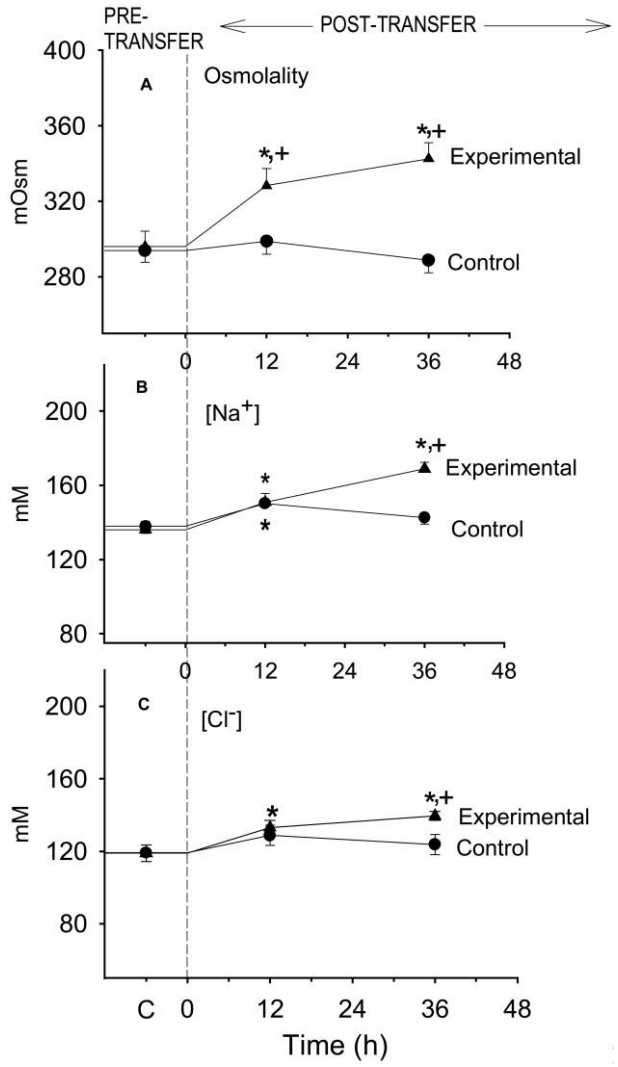

Figure 3. Plasma concentrations of osmolality (*A*), total sodium ([Na^+]; *B*), and total chloride ([Cl^-]; *C*) in the blood of river water–acclimated naked carp ($N = 8$) before and after transfer to lake water in series 1. The control group ($N = 5$) was transferred from river water to river water. Means ± 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from respective pretransfer value in the same fish. Cross indicates significantly different ($P \leq 0.05$) from simultaneous value in the control group.

ammonia-N excretion was directly due to exposure to lake water.

Interestingly, the AQ in lake water–acclimated fish (0.081 ± 0.018) was substantially lower than in river water–acclimated fish (0.202 ± 0.026). However, despite the declines in both Mo_2 (Fig. 13*A*) and ammonia-N excretion (Fig. 14*A*) after transfer to lake water, the AQ stayed high in these river water–acclimated fish (0.187 ± 0.043 at 36 h). Again, there were no changes in the control group. These AQ data indicate a pronounced difference in fuel metabolism between river water–acclimated and lake water–acclimated fish but that 36-h exposure to lake water was not sufficient to change the river water pattern (see "Discussion").

The metabolic and ammonia-N excretion rates recorded immediately after exhaustive exercise were also 50%–65% lower in lake water–acclimated fish than in river water–acclimated animals (Table 5). After the latter were transferred to lake water, postexercise Mo_2 progressively declined, as did postexercise am-

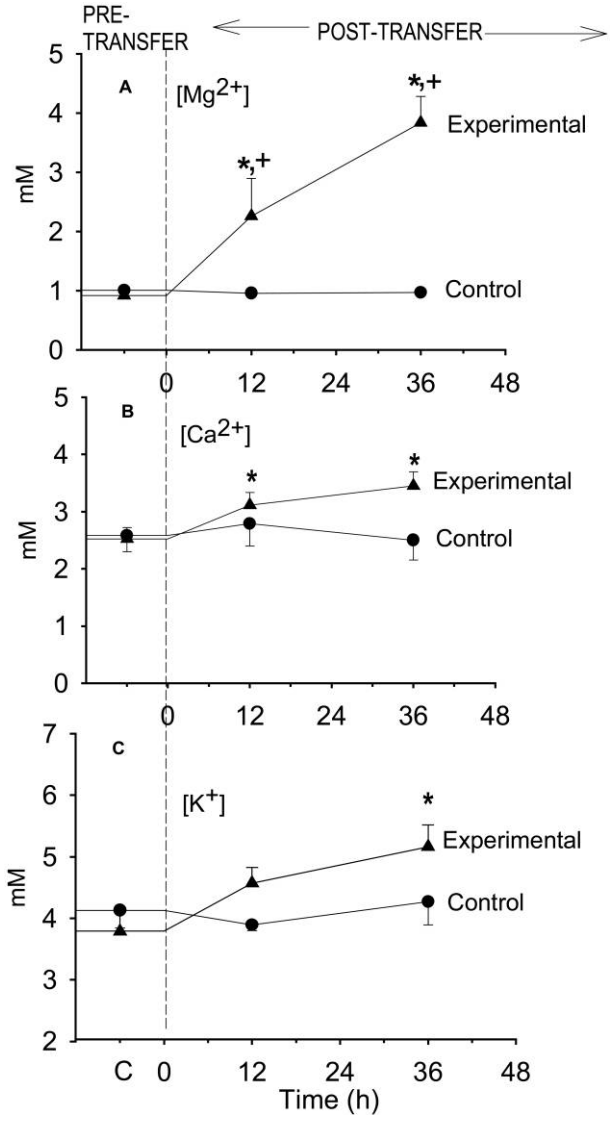

Figure 4. Plasma concentrations of total magnesium ([Mg^{2+}]; *A*), total calcium ([Ca^{2+}]; *B*), and total potassium ([K^+]; *C*) in the blood of river water–acclimated naked carp ($N = 8$) before and after transfer to lake water in series 1. The control group ($N = 5$) was transferred from river water to river water. Means ± 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from respective pretransfer value in the same fish. Cross indicates significantly different ($P \leq 0.05$) from simultaneous value in the control group.

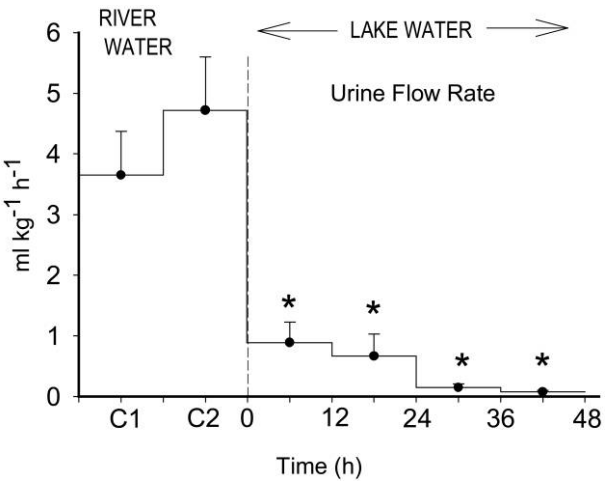

Figure 5. Urine flow rate of river water–acclimated naked carp ($N = 5$) before and after transfer to lake water in series 1. Means + 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from respective pretransfer mean value in the same fish.

monia-N excretion (Table 6). Less pronounced trends occurred in the control group, so these declines in the experimental group may have been partially due to lake water exposure and partially due to the experimental protocol itself.

Discussion

In river water, the physiology of Przewalski's naked carp appears very typical of most freshwater teleosts with respect to plasma ions (e.g., Holmes and Donaldson 1969), renal function (e.g., Hickman and Trump 1969; Beyenbach 2000), N-waste excretion (e.g., Wood 1993; Wilkie 2002), and acid-base status (e.g., Albers 1970). Despite the low ambient Po_2 at high altitude, blood gases and resting metabolic rates are not unusual for teleosts at comparable size and temperature (e.g., Perry and McDonald 1993; Clarke and Johnston 1999). However, transition into lake water causes profound changes in this physiology over the ensuing 36–48 h. These include an acid-base disturbance, hemoconcentration, a rapid rise in osmolality and in the concentrations of all measured plasma ions (most particularly Mg^{2+}), dramatic changes in renal function, decreases in the activity level of Na^+,K^+-ATPase in gills and kidney, and large falls in the rates of O_2 consumption and ammonia-N excretion. Most of these changes appear to be "permanent" because the differences are still seen, often to a greater extent, in these same parameters in fish collected from lake water. The overall picture is that of a fish taking a metabolic holiday, at least in part by allowing its plasma ionic status to largely equilibrate with the saline lake water, and thereby greatly saving on its ionoregulatory expenditures. However, the physiological picture is complicated by two potential stressors in the lake water that may attenuate the benefits of this reduction in osmoregulatory costs, namely high $[Mg^{2+}]$ and high pH/alkalinity.

Acid-Base Regulation

A rise in blood pH as seen at 36 h posttransfer (Fig. 2*A*) is not unusual for fish moving into alkaline environments (e.g., Wilkie et al. 1993, 1994; McGeer and Eddy 1998). The high pH water serves as a "Pco_2 vacuum" (an infinite trap where water Pco_2 is essentially 0; Johansen et al. 1975), thereby drawing down blood Pco_2 levels (Fig. 1*A*) and creating a persistent

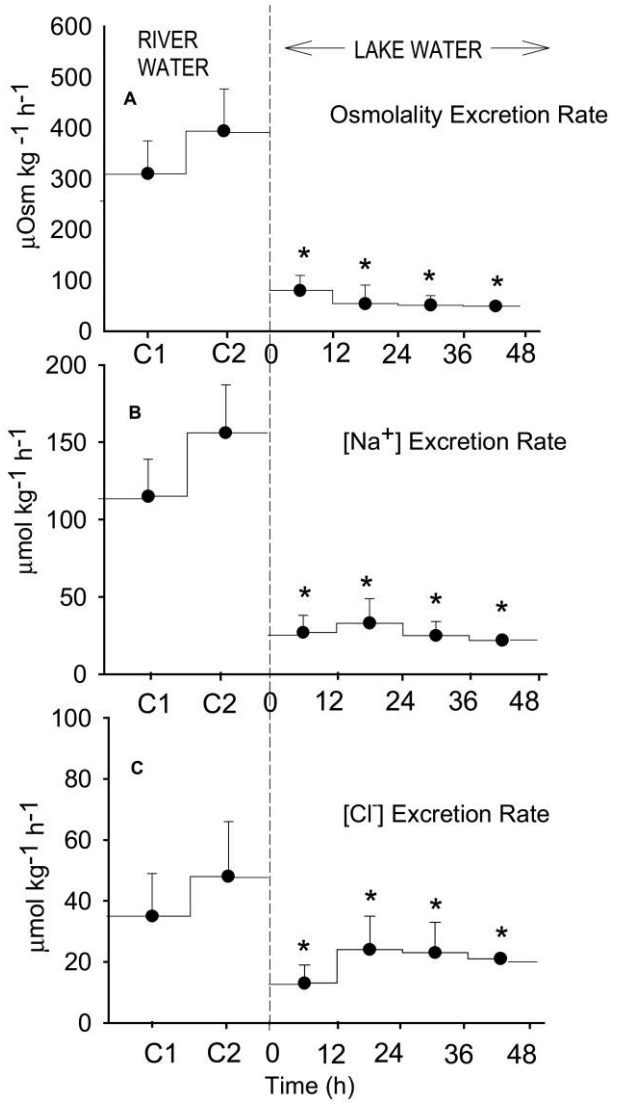

Figure 6. Urinary excretion rates of total osmolytes (*A*), total sodium ($[Na^+]$; *B*), and total chloride ($[Cl^-]$; *C*) by river water–acclimated naked carp ($N = 5$) before and after transfer to lake water in series 1. Means + 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from respective pretransfer mean value in the same fish.

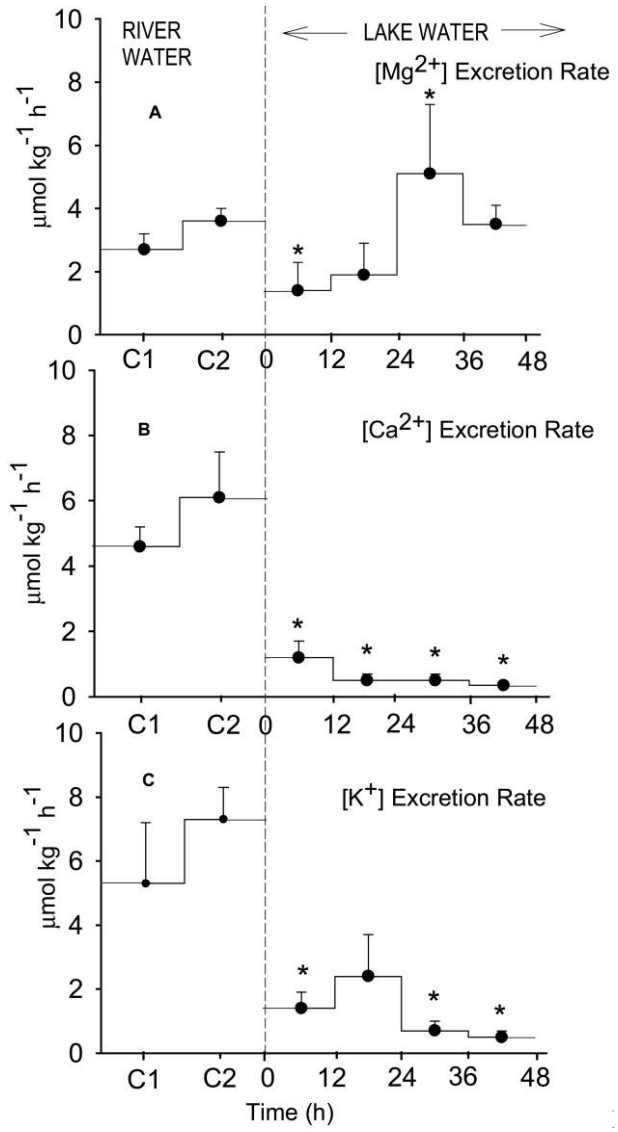

Figure 7. Urinary excretion rates of total magnesium ([Mg^{2+}]; *A*), total calcium ([Ca^{2+}]; *B*), and total potassium ([K^+]; *C*) by river water–acclimated naked carp ($N = 5$) before and after transfer to lake water in series 1. Means + 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from respective pretransfer mean value in the same fish.

respiratory alkalosis. The preceding metabolic acidosis (Fig. 2) was probably due to activation of lactate and associated proton production as a short-term compensation against respiratory alkalosis, as has been seen in several studies on salmonids transferred to alkaline water (Wilkie and Wood 1991; Wilkie et al. 1993). Unfortunately, because of sample deterioration in transit, it was not possible to measure plasma lactate. Regardless, the extent of the acid-base disturbance, at least over the first 36 h, was fairly modest and unlikely to have a serious impact on the animal's health in lake water.

Iono- and Osmoregulation

Clearly, transfer from river water to lake water creates a significant ionic and osmotic challenge to the naked carp (Table 1). Data were similar regardless of whether obtained by cannulation (Figs. 3, 4) or terminal sampling (Figs. 8, 9). Within

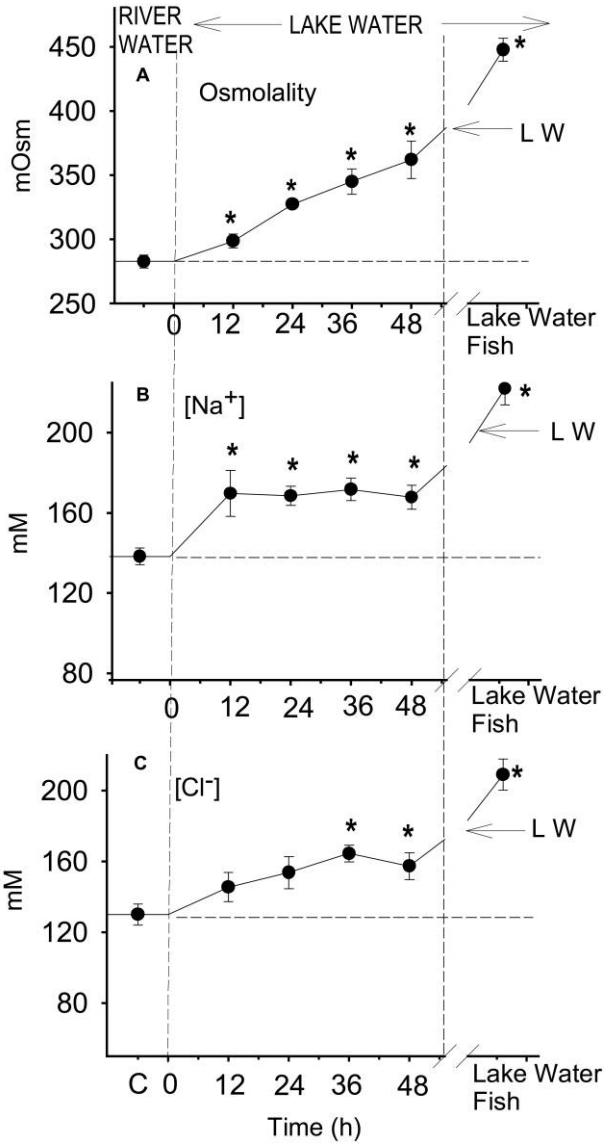

Figure 8. Plasma concentrations of osmolality (*A*), total sodium ([Na^+]; *B*), and total chloride ([Cl^-]; *C*) in the blood of naked carp obtained by terminal sampling in series 2. Data are shown for fish acclimated to river water (*far left*), fish acclimated to lake water (*far right*), and fish at various times after transfer from river water to lake water ($N = 6$–7 for all groups). The concentration of the moiety in lake water is indicated by an arrow (*LW*) to illustrate the extent of equilibration between plasma and the external environment. Means ± 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from the value in the river water–acclimated fish.

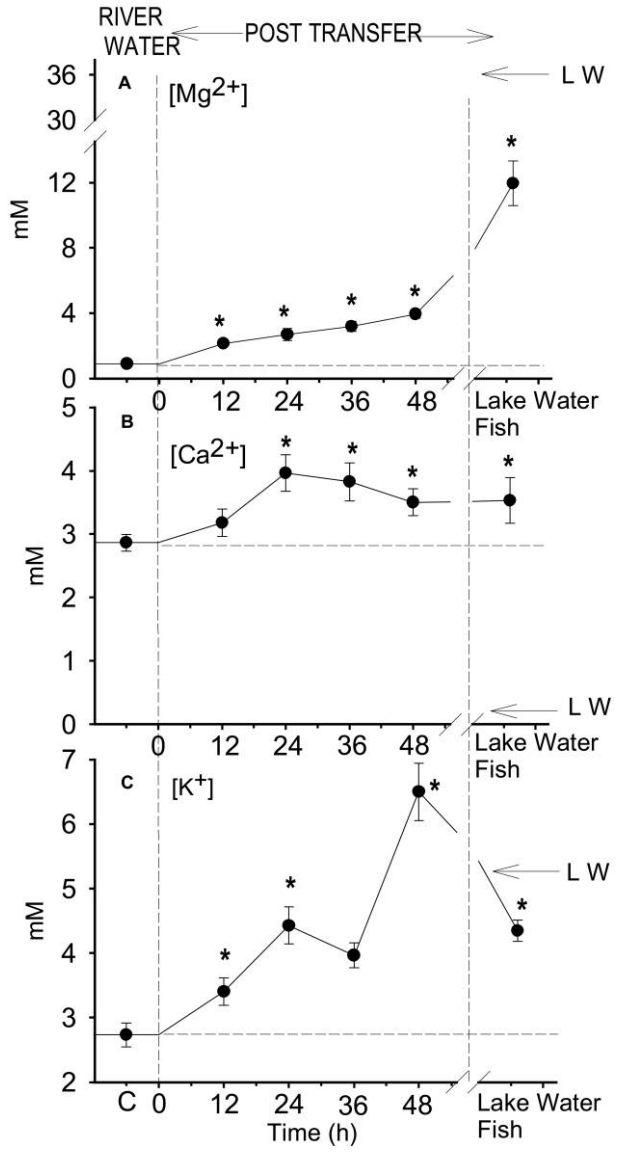

Figure 9. Plasma concentrations of total magnesium ($[Mg^{2+}]$; *A*), total calcium ($[Ca^{2+}]$; *B*), and total potassium ($[K^+]$; *C*) in the blood of naked carp obtained by terminal sampling in series 2. Data are shown for fish acclimated to river water (*far left*), fish acclimated to lake water (*far right*), and fish at various times after transfer from river water to lake water ($N = 6$–7 for all groups). The concentration of the moiety in lake water is indicated by an arrow (*LW*) to illustrate the extent of equilibration between plasma and the external environment. Note the broken scale indicating the true concentration of total $[Mg^{2+}]$ in lake water. Means ± 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from the value in the river water–acclimated fish.

only 12 h, naked carp allowed most plasma ions and osmolality to rise significantly, and the responses persisted or increased by 36–48 h (Figs. 3, 4, 8, 9). The "final" values measured in long-term lake water–acclimated fish (Table 3) should be viewed with some caution, because these animals were collected from Lake Qinghai by gill netting. Although every effort was made to sample only the healthiest fish, it is possible that these animals were more stressed than the river water fish collected by seine. It is therefore reasonable to assume that the true "final" plasma values lie between the 48-h posttransfer values and the measured values from lake water–acclimated fish (Figs. 8, 9). On this basis, it is apparent that the "final" values for osmolality, $[Na^+]$, $[Cl^-]$, and $[K^+]$ are all approximately equilibrated with the external lake water; the overall response appears to be one of osmoconformity rather than osmoregulation. Thus, the naked carp behaves similarly to stenohaline freshwater cyprinids that tolerate moderate salinities (Lahlou et al. 1969; Maceina et al. 1980; Hegab and Hanke 1982; De Boeck et al. 1997, 2000). This pattern is very different from euryhaline freshwater teleosts such as the rainbow trout (*Oncorhynchus mykiss*; Shehadeh and Gordon 1969; Bath and Eddy 1979; Richards et al. 2003), where

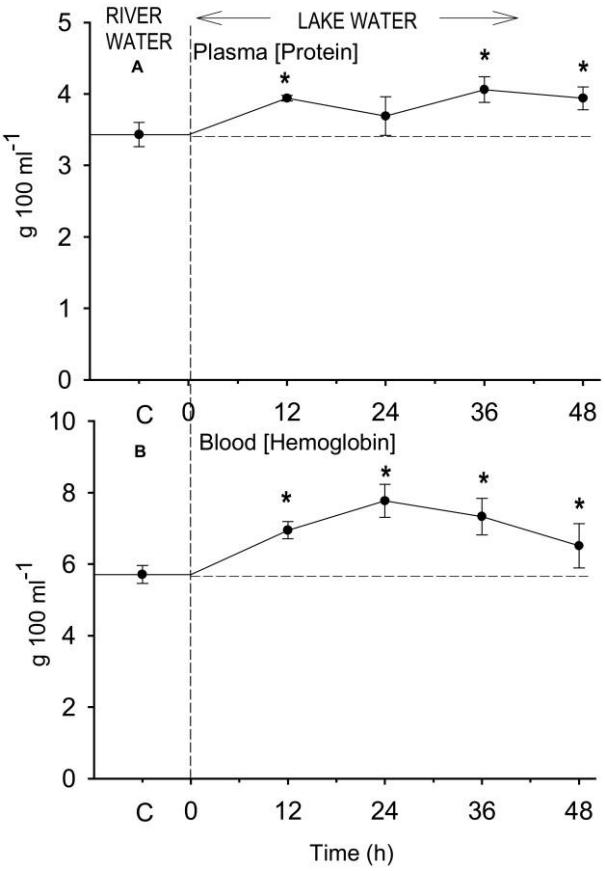

Figure 10. Concentrations of plasma protein (*A*) and whole blood hemoglobin (*B*) in the blood of naked carp obtained by terminal sampling in series 2. Data are shown for fish acclimated to river water (*far left*) and fish at various times after transfer from river water to lake water ($N = 6$ for all groups). Means ± 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from the value in the river water–acclimated fish.

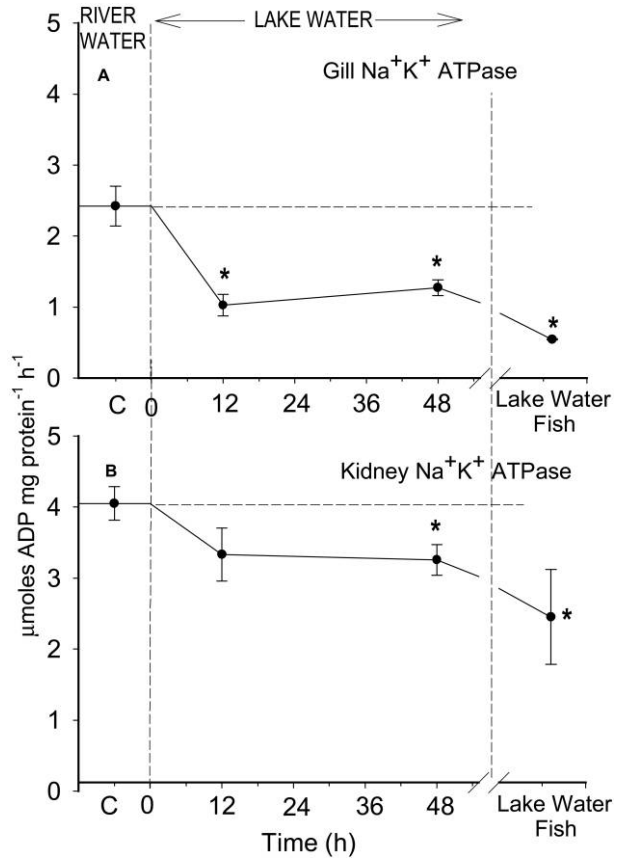

Figure 11. Na^+,K^+-ATPase activities in gill (*A*) and kidney (*B*) of naked carp obtained by terminal sampling in series 2. Data are shown for fish acclimated to river water (*far left*), fish acclimated to lake water (*far right*), and fish 12 and 48 h after transfer from river water to lake water ($N \geq 6$ for all groups). Means ± 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from the value in the river water–acclimated fish.

plasma $[Na^+]$, $[Cl^-]$, and osmolality are quickly brought down to levels lower than those in the environment.

When the Na^+ and Cl^- gradients between plasma and external environment are greatly reduced or eliminated after equilibration with lake water, we would anticipate that the activity level of Na^+,K^+-ATPase, the major ion motive enzyme in the gills (McCormick 1995; Marshall and Grosell 2005), might be turned down to reduce ATP turnover. This is exactly what was observed in the naked carp, with 50%–60% reductions by 12 h and 70% reductions in the longer term (Fig. 11*A*). Wang et al. (2003) reported a comparable difference between naked carp collected from river water versus lake water. Interestingly, this did not occur in the euryhaline rainbow trout after transfer to 14 ppt seawater (Richards et al. 2003), but there the situation is complicated by isoform switching in preparation for full seawater adaptation. We do not know whether more than one isoform occurs in the naked carp.

As part of the equilibration with external lake water over the first 48 h after transfer, there appears to be a net loss of water from the plasma space that causes an 18%–25% hemoconcentration in the naked carp (Fig. 7). This change can account for most of the measured increases in plasma osmolality (Fig. 8*A*), $[Na^+]$ (Fig. 8*B*), $[Cl^-]$ (Fig. 8*C*), and $[Ca^{2+}]$ (Fig. 9*C*). A similar hemoconcentration due to extracellular dehydration has been seen in the euryhaline rainbow trout (Bath and Eddy 1979; Eddy and Bath 1979) after transfer to increased salinity.

Note that plasma $[Ca^{2+}]$ was regulated toward the upper range of values reported in most teleosts (Holmes and Donaldson 1969) and at levels far higher than in either river water or lake water (Table 1), in keeping with its critical importance in cardiac and neuromuscular function. Ca^{2+} is particularly interesting because this ion is actually in higher concentration in river water than in lake water, yet plasma levels increased by about 22% after transfer to the latter, supporting the hemoconcentration explanation. A substantial portion of plasma

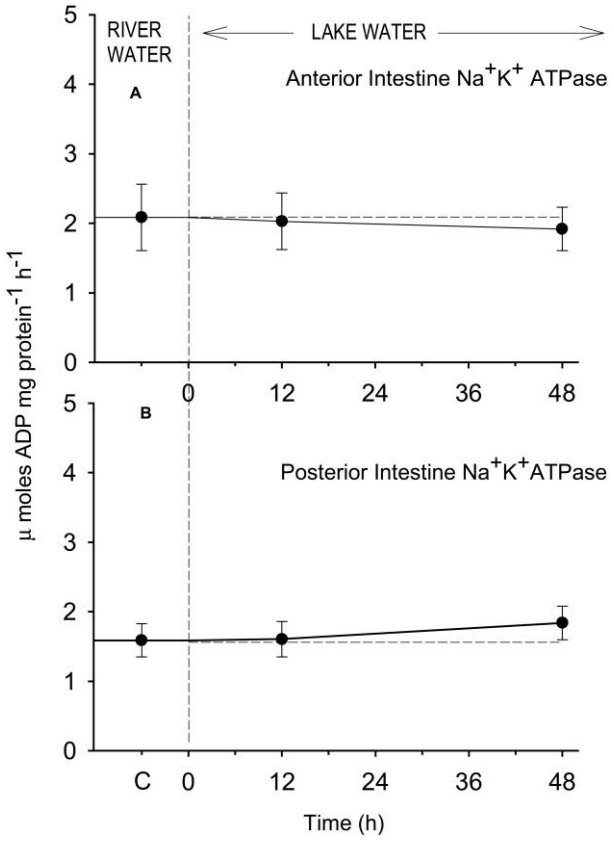

Figure 12. Na^+,K^+-ATPase activities in anterior intestine (*A*) and posterior intestine (*B*) of naked carp obtained by terminal sampling in series 2. Data are shown for fish acclimated to river water (*far left*) and fish 12 and 48 h after transfer from river water to lake water ($N \geq 6$ for all groups). Means ± 1 SEM. There were no significant differences ($P \leq 0.05$) from the values in the river water–acclimated fish.

Ca^{2+} appears to be protein bound in teleosts (Hickman and Trump 1969) and therefore would increase in proportion to the rise in protein concentration.

The two ions for which the hemoconcentration explanation clearly does not hold are $[K^+]$ (Fig. 9*C*) and $[Mg^{2+}]$ (Fig. 9*A*), with increases of 138% and 335%, respectively, on transfer to lake water. K^+ is usually assumed to be in dietary surfeit, with excess intestinal uptake being lost by diffusion and secretion through gills and kidney, respectively. Active K^+ uptake across the gills at a rate comparable to efflux can apparently occur in the freshwater rainbow trout, but uptake exceeds efflux when the fish are acclimated to 22 ppt seawater (Eddy 1985). Therefore, the simplest explanation for the sharp rise in plasma $[K^+]$ is that it simply equilibrates with the external lake water, reaching a level that is still within the normal range reported for teleosts, at least marine teleosts (Holmes and Donaldson 1969).

Equilibration with lake water is clearly not the explanation for the observed response in plasma $[Mg^{2+}]$; indeed, had this occurred, plasma $[Mg^{2+}]$ would have increased to 36 mmol L^{-1} (Table 1), and the fish would have undoubtedly died. Even in full-strength seawater containing 54 mmol L^{-1} $[Mg^{2+}]$, euryhaline and stenohaline marine teleosts normally keep this ion below 3 mmol L^{-1} in the blood plasma to avoid its toxic effects on neuromuscular function (Holmes and Donaldson 1969; Beyenbach 2000; Marshall and Grosell 2005). In the naked carp in lake water, plasma $[Mg^{2+}]$ is clearly regulated but stabilizes at a value as high as 12 mmol L^{-1} (Fig. 9*A*), a significant portion of which is probably protein bound rather than free in the plasma (Oikari and Rankin 1985). Nevertheless, there may well be a chronic sublethal impact on the animals' health. Hegab and Hanke (1982) reported a similarly large increase in plasma $[Mg^{2+}]$ (to 8 mmol L^{-1}) in the stenohaline common carp during exposure to 15 ppt seawater. The classic study of Oikari and Rankin (1985) on rainbow trout illustrated the primary role of the kidney in Mg^{2+} excretion, with negligible capacity for Mg^{2+} excretion across the gills. However, as with K^+, there is apparently a Mg^{2+} uptake mechanism on the gills to cope with times of dietary Mg^{2+} deficiency (Flik et al. 1993; Bijvelds et al. 1998). In the naked carp in lake water, it is probable that Mg^{2+} loading occurs by diffusion across the gills and also possibly across the intestine (if drinking occurs; see below) and that the high plasma set point is determined by the kidney's ability to excrete Mg^{2+} (Oikari and Rankin 1985; Beyenbach 2000).

In euryhaline fish, drinking plays a key role in maintaining water balance in saline environments (Marshall and Grosell 2005) and would be briskly activated in freshwater rainbow trout transferred to a salinity comparable to lake water (Shehadeh and Gordon 1969; Bath and Eddy 1979; Eddy and Bath 1979). Furthermore, Lahlou et al. (1969) reported that drinking took place when a stenohaline cyprinid, the goldfish, was acclimated to comparable salinity, so this likely occurs in the naked carp. Indeed the presence of fluid in the anterior intestine of lake water–acclimated fish, but not in river water–acclimated fish, suggests that this is the case. Interestingly, $[Mg^{2+}]$ and $[Ca^{2+}]$ were higher in intestinal fluid than in plasma (Table 3), suggesting the (partial) exclusion of these divalents from absorption, as in marine teleosts (Marshall and Grosell 2005), though the compositional data were confounded by the presence of food in the gut contents. Organic molecules from digested food probably accounted for the high osmolality in the intestinal fluid. Intestinal Na^+,K^+-ATPase activity did not change after transfer to lake water, at least in the short term (Fig. 12). Samples from long-term lake water–acclimated fish did not survive transit, but Wang et al. (2003) earlier reported no differences in intestinal Na^+,K^+-ATPase activity levels in naked carp collected from river water versus lake water.

Renal Function

In river water, naked carp exhibited UFRs (Fig. 5) and ion excretion rates (Figs. 6, 7) typical of freshwater teleosts fitted with bladder catheters (Hickman and Trump 1969; Wood and Patrick 1994). The slightly lower urinary ion concentrations in

Table 4: Proximate composition of naked carp collected from river water and lake water in series 2

	N	Red Muscle	White Muscle	Liver
Lipid (μg mg^{-1}):				
River	≥8	209.2 ± 31.0	21.7 ± 3.4	114.7 ± 18.7
Lake	5	347.1 ± 33.2	44.5 ± 5.0*	253.4 ± 15.6*
Carbohydrate (μg mg^{-1}):				
River	≥8	8.0 ± 2.0	6.2 ± 2.1	91.0 ± 7.7
Lake	5	10.6 ± 3.3	5.6 ± 1.7	22.5 ± 4.8*
Protein (μg mg^{-1}):				
River	≥8	134.3 ± 10.1	141.5 ± 7.1	112.5 ± 5.5
Lake	5	102.1 ± 5.8*	136.5 ± 3.2	86.8 ± 4.1*

Note. Data are means ± 1 SEM.

* $P \leq 0.05$ relative to corresponding value in river water fish.

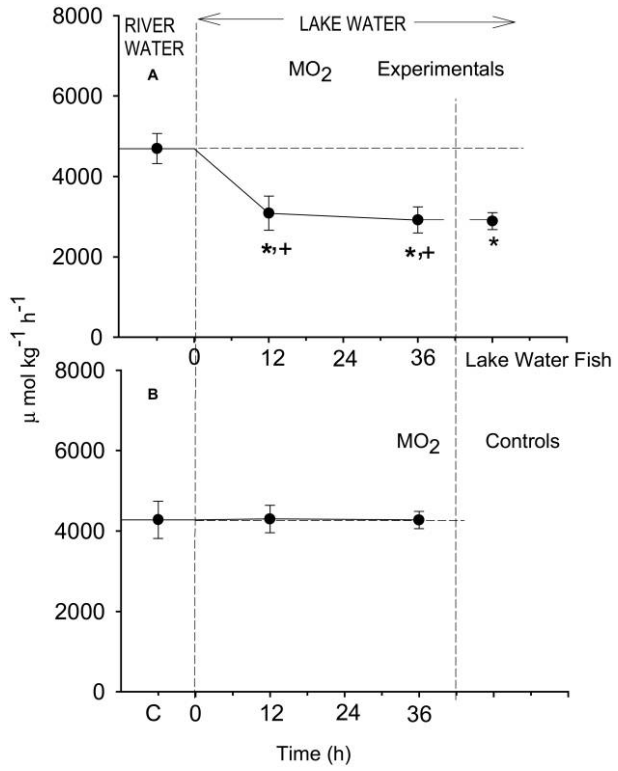

Figure 13. *A*, Resting rates of O_2 consumption (Mo_2) of naked carp acclimated to river water (*far left*) and at 12 and 36 h after transfer to lake water ($N = 6$). Data for a separate group of lake water–acclimated carp ($N = 5$), tested in lake water, are shown at far right. *B*, Similar measurements on a control group ($N = 4$) of carp acclimated to river water and transferred to river water. Means ± 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from respective pretransfer value in the river water–acclimated fish. Cross indicates significantly different ($P \leq 0.05$) from simultaneous value in the control group.

samples taken by spot sampling (Table 3) are also typical, reflecting the normal reabsorptive role of the urinary bladder (Curtis and Wood 1991). Our catheterization technique essentially negated any opportunity for ion reabsorption by the urinary bladder and may have contributed to the paradoxical result that renal excretion rates for most ions were higher in river water than in lake water. Provided that the fish do not drink, the UFR provides an index of osmotic water flux (Hickman and Trump 1969). Therefore, the dramatic reduction in UFR on transfer to lake water (Fig. 5) indicates a virtual elimination of water entry, in accord with the elimination of the osmotic gradient between lake water and blood plasma (Fig. 8*A*). While urinary concentrations of osmolytes increased greatly, the fall in UFR was the overwhelming feature, and renal excretion rates of most electrolytes fell greatly (Figs. 6, 7). Three previous studies on stenohaline freshwater teleosts exposed to elevated external salinity (goldfish: Lahlou et al. 1969; channel catfish: Norton and Davis 1977; Prussian carp: Elger et al. 1984) reported similar but less dramatic changes in renal function.

In the naked carp, the overall clearance rate of osmolytes fell from 1.15 mL kg^{-1} h^{-1} in river water to 0.10 mL kg^{-1} h^{-1} after 36–48 h in lake water, and the rate of free water clearance (the difference between the UFR and the clearance rate of osmolytes; for calculation details, see Guyton and Hall 2000) dropped from 3.64 to −0.02 mL kg^{-1} h^{-1}. In other words, the kidney changed from an organ that excreted water at a greater rate than salt in river water to one that conserved water relative to salt in lake water. Very likely, this was accomplished by a decrease in glomerular perfusion, as reported by Elger et al. (1984) for the Prussian carp (*Carassius auratus gibelio*) transferred to 12 ppt salt water, and could be followed by a loss of glomeruli (Ogawa 1961; Elger and Hentschel 1981). Clearly, renal function is depressed when the fish are in lake water, and the observed 30% decrease in kidney Na^+,K^+-ATPase activity, which was initiated within 48 h posttransfer (Fig. 11*B*), is in accord with this trend.

The single exception is renal Mg^{2+} excretion, which appears to be maintained after transfer to lake water (Fig. 7*A*). The

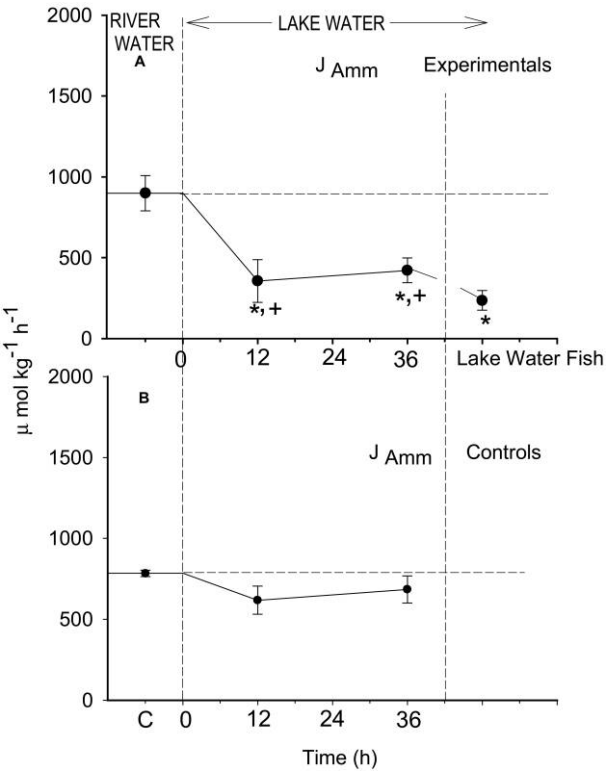

Figure 14. *A*, Resting rates of ammonia-N excretion (J_{Amm}) of naked carp acclimated to river water (*far left*) and at 12 and 36 h after transfer to lake water ($N = 6$). Data for a separate group of lake water–acclimated carp ($N = 5$), tested in lake water, are shown at far right. *B*, Similar measurements on a control group ($N = 4$) of carp acclimated to river water and transferred to river water. Means ± 1 SEM. Asterisk indicates significantly different ($P \leq 0.05$) from respective pretransfer value in the river water–acclimated fish. Cross indicates significantly different ($P \leq 0.05$) from simultaneous value in the control group.

Table 5: Rates of oxygen consumption (M_{O_2}) and ammonia-N excretion (J_{Amm}) immediately after exhaustive exercise in naked carp acclimated to lake water and river water in series 3

	N	M_{O_2} (μmol kg^{-1} h^{-1})	J_{Amm} (μmol kg^{-1} h^{-1})
River water acclimated	10	17,303 ± 1,479	2,136 ± 287
Lake water acclimated	5	7,820 ± 629*	717 ± 166*

Note. Data are means ± 1 SEM.
* $P \leq 0.05$ relative to corresponding river water–acclimated value.

teleost kidney can excrete Mg^{2+} by tubular secretion (Bijvelds et al. 1998; Beyenbach 2000) and can rapidly change over from net tubular Mg^{2+} reabsorption to net secretion under conditions of Mg^{2+} loading (Oikari and Rankin 1985). Therefore Mg^{2+} excretion can continue or even rise despite large reductions in glomerular filtration rate, a pattern commonly observed when euryhaline teleosts such as salmonids migrate into seawater (Hickman and Trump 1969; Beyenbach 2000). The observed rise in plasma [Mg^{2+}] over the first 48 h after transfer to lake water was about 3 mmol L^{-1} (Fig. 8*A*). If we conservatively estimate that this extra Mg^{2+} was distributed in the extracellular space alone, then the initial Mg^{2+} loading rate was about 16 μmol kg^{-1} h^{-1}, whereas the observed renal Mg^{2+} excretion rate during this period was only about 25% of this figure (Fig. 7*A*). It is therefore not surprising that the "final" plasma [Mg^{2+}] may eventually stabilize at a level as high as 12 mmol L^{-1} in lake water–acclimated carp (Fig. 8*A*; Table 3). This eventual stabilization must reflect either a reduction of the net Mg^{2+} loading rate and/or an increase in the renal Mg^{2+} excretion rate in the longer term.

N-Waste Excretion

A recurrent theme in studies on the responses of fish to high environmental pH has been the occurrence of inhibited ammonia-N excretion, accompanied by elevated internal levels of ammonia-N (reviewed in Wood 1993; Wilkie and Wood 1996). This occurs because alkalinization of the boundary layer at the gill surface effectively decreases the PNH_3 gradient for diffusive NH_3 efflux. The responses of the naked carp after transfer to alkaline lake water (Fig. 14) clearly fit this pattern, in agreement with our earlier studies on this fish (Wang et al. 2003). However, the naked carp does not appear to increase urea-N excretion in compensation, in contrast to many other species that tolerate alkaline lake water (Randall et al. 1989; Danulat and Kempe 1992; Wilkie et al. 1993; Wright et al. 1993), nor does it eventually restore ammonia-N excretion after a transient inhibition in contrast to the rainbow trout (Wilkie and Wood 1991; Wilson et al. 1994). Rather, *Gymnocypris przewalskii* appears to simply tolerate very high plasma ammonia levels (Table 3) and to "permanently" turn down ammonia-N production to levels less than 30% of those seen in river water–acclimated fish (Fig. 14).

In this regard, there are likely two important contributing factors. The first is a parallel decrease in M_{O_2} to about 60% of river water levels (Fig. 13), so overall metabolic rate is reduced, thereby constraining decreased ammonia-N production. The second is a selective reduction in the rate of protein oxidation during long-term lake water acclimation. As explained by Lauff and Wood (1996), the contribution of protein oxidation to total aerobic oxidation rate can be calculated from the ratio of the AQ to 0.27, the theoretical maximum for the oxidation of fish protein (Van den Thillart and Kesbeke 1978). Thus, river water–acclimated fish, with an AQ of 0.202, supported 75% of their higher M_{O_2} by protein oxidation, while lake water–acclimated fish, with an AQ of 0.081, supported only 30% of their much lower M_{O_2} by protein oxidation. Unlike the rapid initial declines in M_{O_2} and ammonia-N excretion after transfer, this fuel switching was not a function of the lake water exposure in itself, because the AQ of river fish stayed high (0.187, 69% protein oxidation) for the first 36 h posttransfer. Rather, it was likely a reflection of the fuels available. Long-term lake water–acclimated fish are actively feeding and have much larger lipid stores on board (Table 4), whereas river fish are anorexic during their migration and likely rely on protein breakdown and associated gluceoneogenesis (note higher liver carbohydrate; Table 4) to fuel aerobic metabolism.

The Metabolic Holiday

The M_{O_2} data indicate that the cost of living for the naked carp is 40% lower in lake water than in river water, and that this difference is almost complete within 12 h after transfer (Fig. 13). The magnitude of the response is remarkable. Undoubtedly, a significant portion of the reduction is due to the "osmotic holiday" wherein the carp are allowing plasma osmolality, [Na^+], [Cl^-], and [K^+] to virtually equilibrate with lake water, thereby saving the costs of ion-pumping and volume regulation, and turning down gill and kidney transport functions (Figs. 3–9). The rapid reductions in gill and kidney Na^+,K^+-ATPase activities (Fig. 11) support this interpretation. However there have been many estimates of the cost of iono- and osmoregulation in teleosts (reviewed in Febry and Lutz 1987), and all fall in the range of 2%–20% of resting metabolism, rather than 40%. Indeed, it seems likely that the costs of acid-base regulation would actually increase when the fish enter alkaline lake

Table 6: Rates of oxygen consumption (M_{O_2}) and ammonia-N excretion (J_{Amm}) immediately after exhaustive exercise in naked carp after transfer of river fish to lake water in series 3

	M_{O_2} (μmol kg^{-1} h^{-1})		J_{Amm} (μmol kg^{-1} h^{-1})	
	Controls	Experimentals	Controls	Experimentals
N	4	6	4	6
Pretransfer	14,632 ± 1,711	19,084 ± 2,130	2,061 ± 528	2,187 ± 404
Posttransfer:				
12 h	12,602 ± 672	13,467 ± 2,099*	2,214 ± 359	2,317 ± 923
36 h	10,759 ± 684*	9,915 ± 1,577*	1,487 ± 182*	922 ± 112*,**

Note. Data are means ± 1 SEM. Control fish were transferred from river water to river water at 0 h.

* $P \leq 0.05$ relative to corresponding pretransfer value.

** $P \leq 0.05$ relative to simultaneous control group value.

water, as seen in the Lake Magadi tilapia *Alcolapia grahami* (Wood et al. 2002). Overall, these considerations raise the possibility that additional factors are decreasing metabolic costs of the naked carp in Lake Qinghai water.

We suggest that two factors deserve consideration in future research. The first is the very high level of plasma [Mg^{2+}] in lake water carp (Figs. 4*A*, 9*A*; Table 3). Mg^{2+} is a well-known neuromuscular blocking agent and may thereby reduce baseline activity levels. The second factor is the very high plasma total ammonia level in lake water fish: 2.4 mmol L^{-1} by caudal puncture sampling in this study (Table 3) and 2.2 mmol L^{-1} by the same technique in our earlier study (Wang et al. 2003), more than fourfold greater than in river water–acclimated fish (Table 3; Wang et al. 2003). There is a growing body of evidence that fish grow more efficiently when ammonia levels are moderately elevated (reviewed in Wood 2004), and part of this effect may be due to depressed routine M_{O_2} (Linton et al. 1997). Elevated plasma ammonia appears to decrease swimming performance by depolarizing effects on the muscle membrane potential (Shingles et al. 2001; Wicks et al. 2002). Indeed, postexercise metabolic rate was also depressed in naked carp in lake water, though this result was partially confounded by smaller decreases in the control animals (Tables 5, 6).

If the metabolic holiday in lake water is in fact due to subtle Mg^{2+} and ammonia effects that reduce activity level, the phenomenon may be presently beneficial to the ecology of the species. The naked carp has no piscine predators in Lake Qinghai and is a planktivore (Walker et al. 1996), so there is probably no need for high activity levels for defense and food capture. This is also indicated by low hepatic carbohydrate reserves and high overall lipid reserves in lake water fish. Reduced muscular and ionoregulatory costs may combine to favor growth during the feeding phase of the life cycle in an otherwise severe environment. During the annual spawning migration, when there is a need for enhanced swimming performance, the return to circumneutral river water will correct the high plasma ammonia and Mg^{2+} levels and alleviate the inhibition on activity metabolism. However, if Lake Qinghai continues to dehydrate (see "Introduction"), one wonders how long it will be before beneficial effects turn into toxic effects and the metabolic holiday goes bad.

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Literature Cited

Academia Sinica. 1979. Report on the Comprehensive Expedition to Qinghai Lake. Scientific Publishing House, Beijing. (In Chinese.)

Albers C. 1970. Acid-base balance. Pp. 173–208 in W.S. Hoar and D.J. Randall, eds. Fish Physiology. Vol. 4. Academic Press, New York.

Bath R.N. and F.B. Eddy. 1979. Salt and water balance in rain-

bow trout (*Salmo gairdneri*) rapidly transferred from fresh water to sea water. J Exp Biol 83:193–202.

Bergmeyer H.U. 1985. Methods of Enzymatic Analysis. Academic Press, New York.

Beyenbach K.W. 2000. Renal handling of magnesium in fish: from whole animal to brush border membrane vesicles. Front Biosci 5:d712–d719.

Bijvelds M., J.A. Van der Velden, Z.I. Kolar, and G. Flik. 1998. Magnesium transport in freshwater teleosts. J Exp Biol 201: 1981–1990.

Boutilier R.G., T.A. Heming, and G.K. Iwama. 1984. Appendix: physicochemical parameters for use in fish respiratory physiology. Pp. 403–430 in W.S. Hoar and D.J. Randall, eds. Fish Physiology. Vol. 10A. Academic Press, Orlando, FL.

Cameron J.N. 1971. Rapid method for determination of total carbon dioxide in small blood samples. J Appl Physiol 31: 632–634.

Chen K. 1991. Salt lakes and eolianites in the Qaidam Basin. Pp. 23–24 in L. Tungsheng, ed. Highlights of Quaternary Geology in China. International Union for Quaternary Research, XIII International Congress, Beijing. Press of the University of Science and Technology of China, Hefei.

Clarke A. and N.M. Johnstone. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. J Anim Ecol 68:893–905.

Curtis B.J. and C.M. Wood 1991. The function of the urinary bladder in vivo in the freshwater rainbow trout. J Exp Biol 155:567–583.

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.

De Boeck G., M. Vanaudenhove, M. Verhoye, J. Van Auderkerke, B. De Wachter, R. Blust, and A. Van der Linden. 1997. Water household of common carp, *Cyprinus carpio*, when submitted to an osmotic challenge as determined by diffusion-weighted magnetic resonance imaging at 7T. Magma 5: 13–19.

De Boeck G., A. Vlaeminck, A. Van der Linden, and R. Blust. 2000. The energy metabolism of common carp (*Cyprinis carpio*) when exposed to salt stress: an increase in energy expenditure or effects of starvation? Physiol Biochem Zool 73:102–111.

Eddy F.B. 1985. Uptake and loss of potassium by rainbow trout (*Salmo gairdneri*) in fresh water and dilute sea water. J Exp Biol 118:277–286.

Eddy F.B. and R.N. Bath. 1979. Ionic regulation in rainbow trout (*Salmo gairdneri*) adapted to fresh water and dilute sea water. J Exp Biol 83:181–192.

Elger M. and H. Hentschel. 1981. The glomerulus of a stenohaline fresh-water teleost, *Carassius auratus gibelio*, adapted to saline water: a scanning and transmission electron-microscopic study. Cell Tissue Res 220:73–85.

Elger M., R. Kaune, and H. Hentschel. 1984. Glomerular intermittency in a freshwater teleost, *Carassius auratus gibelio*, after transfer to salt water. J Comp Physiol 154:225–231.

Febry R. and P. Lutz. 1987. Energy partitioning in fish: the activity-related cost of osmoregulation in a euryhaline cichlid. J Exp Biol 128:63–85.

Flik G., J.A. Van der Velden, K.J. Dechering, P.M. Verbost, T.J.M. Schoenmakers, Z.I. Kolar, and S.E. Wendelaar Bonga. 1993. Ca^{2+} and Mg^{2+} transport in gills and gut of tilapia, *Oreochromis mossambicus*: a review. J Exp Zool 265:356–365.

Guong S.X. and A. Hu. 1975. Observations on the longevity and ovary development of naked carp. Pp. 65–76 in Fish Fauna and Biology of Naked Carp in Lake Qinghai. Scientific Publisher, Beijing. (In Chinese.)

Guyton A.C. and J.E. Hall. 2000. Textbook of Medical Physiology. 10th ed. Saunders, Philadelphia.

Hegab S.A. and W. Hanke. 1982. Electrolyte changes and volume regulatory processes in the carp (*Cyprinis carpio*) during osmotic stress. Comp Biochem Physiol 71A:157–164.

Hickman C.P. and J. Trump. 1969. The kidney. Pp. 91–239 in W.S. Hoar and D.J. Randall, eds. Fish Physiology. Vol. 1. Academic Press, New York.

Holmes W.N. and E.M. Donaldson. 1969. The body compartments and the distribution of electrolytes. Pp. 1–89 in W.S. Hoar and D.J. Randall, eds. Fish Physiology. Vol. 1. Academic Press, New York.

Johansen K., G.M.O. Maloiy, and G. Lykkeboe. 1975. A fish in extreme alkalinity. Respir Physiol 24:156–162.

Lahlou B., I.W. Henderson, and W.H. Sawyer. 1969. Sodium exchanges in goldfish (*Carassius auratus* L.) adapted to a hypertonic saline solution. Comp Biochem Physiol 28:1427–1433.

Lauff R.F. and C.M. Wood. 1996. Respiratory gas exchange, nitrogenous waste excretion, and fuel usage during starvation in juvenile rainbow trout, *Oncorhynchus mykiss*. J Comp Physiol B 165:542–551.

Linton T.K., S.D. Reid, and C.M. Wood. 1997. The metabolic costs and physiological consequences to juvenile rainbow trout of a simulated summer warming scenario in the presence and absence of sublethal ammonia. Trans Am Fish Soc 126:259–272.

Maceina M.J., F.G. Nordlie, and J.V. Shireman. 1980. The influence of salinity on oxygen consumption and plasma electrolytes in grass carp, *Ctenopharyngodon idella* Val. J Fish Biol 16:613–619.

Marshall W.S. and M. Grosell. 2005. Ion transport, osmoregulation, and acid-base balance. Pp. 177–230 in D. Evans and J.B. Claiborne, eds. Physiology of Fishes. 3rd ed. CRC, Boca Raton, FL.

McCormick S.D. 1993. Methods for nonlethal gill biopsy and measurement of Na^+,K^+ ATPase activity. Can J Fish Aquat Sci 50:656–658.

———. 1995. Hormonal control of gill Na^+,K^+-ATPase and

chloride cell function. Pp. 285–315 in C.M. Wood and T.J. Shuttleworth, eds. Fish Physiology. Vol. 14. Cellular and Molecular Approaches to Fish Ionic Regulation. Academic Press, San Diego, CA.

McDonald D.G. and C.M. Wood. 1981. Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri*, at low environmental pH. J Exp Biol 93:101–118.

McGeer J.C. and F.B. Eddy. 1998. Ionic regulation and nitrogenous excretion in rainbow trout exposed to buffered and unbuffered freshwater of pH 10.5. Physiol Biochem Zool 71: 179–190.

Miller G.L. 1959. Protein determination on larger sample sizes. Anal Chem 31:964.

Muir J.F. 1990. Hatchery Development and Spawning Enhancement, Qinghai Lake. Field document for project CPR/88/077, Fisheries Development in Qinghai Province. Food and Agricultural Organization of the United Nations, Rome.

Norton V.M. and K.B. Davis. 1977. Effect of abrupt change in salinity of the environment on plasma electrolytes, urine volume, and electrolyte excretion in channel catfish, *Ictalurus punctatus*. Comp Biochem Physiol 56A:425–431.

Ogawa M. 1961. Histological changes of the kidney in goldfish in seawater. Sci Rep Saitama Univ B4:1–20.

Oikari A.O.J. and J.C. Rankin. 1985. Renal excretion of magnesium in a freshwater teleost, *Salmo gairdneri*. J Exp Biol 117:319–333.

Perry S.F. and D.G. McDonald. 1993. Gas exchange. Pp. 251–278 in D.H. Evans, ed. The Physiology of Fishes. CRC, Boca Raton, FL.

Qin B. and Q. Huang. 1998. Evaluation of the climatic change impacts on the inland lake: a case study of Lake Qinghai, China. Clim Change 39:695–714.

Rahmatullah M. and T.R. Boyde. 1980. Improvements in the determination of urea using diacetyl monoxime: methods with and without deproteinization. Clin Chim Acta 107:3–9.

Randall D.J., C.M. Wood, S.F. Perry, H.L. 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.

Richards J.G., J.W. Semple, J.S. Bystriansky, and P.M. Schulte. 2003. Na^+/K^+-ATPase α-isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. J Exp Biol 206:4475–4486.

Shehadeh Z.H. and M.S. Gordon. 1969. The role of the intestine in salinity adaptation of the rainbow trout (*Salmo gairdneri*). Comp Biochem Physiol 30:397–418.

Shi J.-Q. and H.-F. Qi. 2000. The assessment of the scale-less carp resources in Lake Qinghai. Fresh Fish 30:38–40. (In Chinese.)

Shingles A., D.J. McKenzie, E.W. Taylor, A. Moretti, P.J. Butler, and S. Ceradini. 2001. Effects of sublethal ammonia exposure on swimming performance in rainbow trout (*Oncorhynchus mykiss*). J Exp Biol 204:2691–2698.

Soivio A., K. Westman, and K. Nyholm. 1972. Improved method of dorsal aorta catheterization: haematological effects followed for three weeks in rainbow trout (*Salmo gairdneri*). Finn Fish Res 1:11–21.

Van den Thillart G. and F. Kesbeke. 1978. Anaerobic production of carbon dioxide and ammonia by goldfish, *Carassius auratus* L. Comp Biochem Physiol 59A:393–400.

Verdouw H., C.J.A. van Echted, and E.M.J. Dekkers. 1978. Ammonia determination based on indophenol formation with sodium salicylate. Water Res 12:399–402.

Walker K.F., I.G. Dunn, D. Edwards, T. Petr, and H.Z. Yang. 1996. A fishery in a changing lake environment: the naked carp *Gymnocypris przewalskii* (Kessler) (Cyprinidae: Schizothoracinae) in Qinghai Hu, China. Int J Salt Lake Res 4: 169–222.

Wang Y.S., R.J. Gonzalez, M.L. Patrick, M. Grosell, C. Zhang, Q. Feng, J.Z. Du, P.J. Walsh, and C.M. Wood. 2003. Unusual physiology of scaleless carp, *Gymnocypris przewalskii*, in Lake Qinghai: a high altitude saline lake. Comp Biochem Physiol 134A:409–421.

Wicks B.J., R. Joensen, Q. Tang, and D.J. Randall. 2002. Swimming and ammonia toxicity in salmonids: the effect of sublethal ammonia exposure on the swimming performance of coho salmon and the acute toxicity of ammonia in swimming and resting rainbow trout. Aquat Toxicol 59:55–69.

Wilkie M.P. 2002. Ammonia excretion and urea handling by fish gills: present understanding and future research challenges. J Exp Zool 293:284–301.

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

———. 1996. The adaptations of fish to extremely alkaline environments. Comp Biochem Physiol 113B:665–673.

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.

———. 1994. 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). Physiol Zool 67:355–380.

Wilson R.W., P.M. Wright, S. Munger, and C.M. Wood. 1994. Ammonia excretion in rainbow trout *Oncorhynchus mykiss*: the importance of gill boundary layer acidification: lack of evidence for Na^+/NH_4^+ exchange. J Exp Biol 191:37–58.

Wolf K. 1963. Physiological salines for freshwater teleosts. Prog Fish-Cult 25:135–140.

Wood C.M. 1993. Ammonia and urea metabolism and excre-

tion. Pp. 379–425 in D. Evans, ed. The Physiology of Fishes. CRC, Boca Raton, FL.

———. 2004. Is exogenous ammonia a growth stimulant in fish? J Exp Biol 207:2043–2054.

Wood C.M., T.E. Hopkins, and P.J. Walsh. 1997. Pulsatile urea excretion in the toadfish (*Opsanus beta)* is due to a pulsatile excretion mechanism, not a pulsatile production mechanism. J Exp Biol 200:1039–1046.

Wood C.M. and M.L. Patrick. 1994. Methods for assessing kidney and urinary bladder function in fish. Pp. 127–143 in P.W. Hochachka and T.P. Mommsen, eds. Biochemistry and Molecular Biology of Fishes. Vol. 3. Elsevier Science, New York.

Wood C.M., P.W. Wilson, H.L. Bergman, A.N. Bergman, P. Laurent, G. Otiang'a-Owiti, and P.J. Walsh. 2002. Obligatory urea production and the cost of living in the Magadi tilapia revealed by acclimation to reduced salinity and alkalinity. Physiol Biochem Zool 75:111–122.

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.

Wu Y.F. and C.Z. Wu 1992. The Fishes of the Qinghai-Xizang (Tibet) Plateau, China. Sichuan Publishing House of Science and Technology, Chengdu. (In Chinese.)