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Unusual physiology of scale-less carp, *Gymnocypris przewalskii*, in Lake Qinghai: a high altitude alkaline saline lake

Yuxiang S. Wang[a,*], Richard J. Gonzalez[b], Marjorie L. Patrick[c], Martin Grosell[a], Chuanguang Zhang[d], Qiang Feng[d], JiZeng Du[e], Patrick J. Walsh[a], Chris M. Wood[f]

[a]*Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL, USA*

[b]*Department of Biology, University of San Diego, 5998 Alcala Park, San Diego, CA, USA*

[c]*Department of Cell Biology and Neuroscience, University of California, Riverside, CA 92521, USA*

[d]*Institute of Zoology, Chinese Academy of Sciences, Beijing, PR China*

[e]*Division of Physiology and Neurobiology, Department of Biotechnology, Zhejiang University, Hangzhou, PR China*

[f]*Department of Biology, McMaster University, Hamilton, Ontario, Canada*

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Abstract

The scale-less carp (*Gymnocypris przewalskii*) inhabits Lake Qinghai located on the Qinghai–Tibet plateau (elevation, 3200 m) in western China. The lake waters are alkaline (pH 9.4, titratable alkalinity = 30 mmol l^{-1}), Mg^{2+}-rich (18.7 mmol l^{-1}), Ca^{2+}-poor (0.30 mmol l^{-1}) and saline (9‰). These fish make annual spawning migrations into freshwater rivers. We investigated the physiology of nitrogen excretion and ionoregulation of fish from the lake and river. Fish from both waters were ammonotelic, although ammonia–N excretion rates were lower in lake fish (175 vs. 344 μmol kg^{-1} h^{-1}, $P<0.05$) resulting in unusually high levels of ammonia in blood plasma (2.23 vs. 0.32 mmol l^{-1}), bile, liver, muscle and brain. Exposure to 0.4 mmol l^{-1} total ammonia in lake water ($[NH_3]=0.16$ mmol l^{-1}) killed fish within 8 h. River fish survived exposure to 1.0 mmol l^{-1} total ammonia in river water at pH 8.0 ($[NH_3]=0.023$ mmol l^{-1}) for 24 h suggesting high ammonia tolerance in lake fish. High glutamate dehydrogenase and glutamine synthetase activities in tissues probably allow the fish to alleviate ammonia toxicity by amino acid accumulation. Neither lake nor river fish relied on urea excretion to remove excess N. Urea–N excretion rates were below 20 μmol kg^{-1} h^{-1} for both groups, and levels of urea in plasma and tissues were moderate. When exposed to elevated ammonia, urea–N excretion increased slightly (~50 μmol kg^{-1} h^{-1}) and liver and muscle urea levels increased in the river fish. Plasma ion levels were within the range typical of cyprinids, but river fish had significantly higher plasma $[Na^+]$ and $[Cl^-]$ and lower $[K^+]$ than fish from the lake. During 48-h lake-to-river water transfer, plasma Na^+ and Cl^- levels rose significantly. Significantly higher Na^+/K^+-ATPase activity in the gills of river fish may be related to the higher plasma ion levels. Plasma $[Mg^{2+}]$ and $[Ca^{2+}]$ were tightly regulated despite the great differences in the lake and river water levels.
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Keywords: *Gymnocypris przewalskii*; Lake Qinghai; Nitrogen excretion; Ionregulation; Glutamine synthetase; Glutamate dehydrogenase; Ammonia; Urea; Scale-less carp

*Corresponding author. Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6. Tel.: +1-613-533-6134; fax: +1-613-533-6617.

E-mail address: yuxiangw@biology.queensu.ca (Y.S. Wang).

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1. Introduction

The scale-less carp (*Gymnocypris przewalskii*, Family Cyprinidae) resides in the alkaline (pH 9.1–9.5) and saline waters (9‰ total salinity) of Lake Qinghai, which is located, at an elevation of 3200 m above sea level, on the Qinghai-Tibet plateau in western China. Unlike most stenohaline cyprinids, *G. przewalskii* undergo an annual spawning migration between the saline lake water and freshwater rivers (Guong and Hu, 1975). The spawning fish migrate to the rivers during March–July, and return to the lake upon the completion of reproduction (Walker et al., 1996). The water chemistry of the lake and the transition from lake water to river water pose significant challenges for nitrogenous waste excretion and ion balance.

In most water-breathing teleost fish, nitrogenous waste is excreted as ammonia, mainly via the diffusion of non-ionic NH_3 across the gills via the so called 'proton trap' mechanism (Randall and Wright, 1987). However, when the pH of ambient water is at or above the p*K* of ammonia (pH 9–10), the outward transepithelial NH_3 gradient at the gills is greatly reduced, thus impeding diffusive nitrogen excretion. Consequently, ammonia accumulation occurs in the fish (Evans and Cameron, 1986), which can be fatal (Wood, 1993).

In general, most teleosts are vulnerable to elevated internal ammonia levels (Person-Le Ruyet et al., 1995). However, some fish species have evolved various mechanisms to tolerate or avoid very high environmental and internal ammonia. Species such as the Lake Magadi tilapia (*Alcolapia grahami*), toadfish (Batrachoididae) and air-breathing mudskipper (*Heteropneustes fossilis*) are capable of converting ammonia to less toxic urea via the ornithine urea cycle, then excreting a part or all of their nitrogenous waste as urea (Randall et al., 1989; Mommsen and Walsh, 1989; Saha and Ratha, 1994). Mudskippers (*Periophthalmus cantonensis*) and toadfish can also tolerate high internal ammonia by converting ammonia to free amino acids in the brain, the tissue most sensitive to ammonia intoxication, through an especially powerful glutamate dehydrogenase/glutamine synthetase detoxification system (Wang and Walsh, 2000; Danulat and Kempe, 1992; Iwata, 1988). In some fish, urea synthesis by uricolysis has been identified as the ureogenic pathway used to eliminate excess ammonia (see reviews by Anderson, 1995; Wright and Land, 1998). Others, such as mudskippers, gobiid fish and mangrove killifish can utilize partial amino acid catabolism to generate ATP while using alanine as the temporary ammonia sink, thus no ammonia is released into the system (Ip et al., 2001; Lim et al., 2001; Iwata et al., 2000). Furthermore, it has been suggested that an active NH_4^+ transport mechanism via Na^+/K^+-ATPase and Na^+/H^+ exchanger may be employed to facilitate ammonia excretion when the transepithelial electrochemical gradient alone cannot cope with the need for ammonia excretion (Randall et al., 1999; Wilson et al., 2000).

The challenges for ion regulation are somewhat different. While in lake water, the fish are only slightly hyperosmotic to their surroundings, but upon moving to the freshwater rivers they become strongly hyperosmotic. Consequently, Lake Qinghai water should pose less of a challenge for ion balance in comparison to river water. Yet, little is known about the mechanisms of ion regulation utilized by *G. przewskii* while living in Lake Qinghai or during the period of migration into freshwater.

Since very little is known about how *G. przewskii* excretes nitrogenous waste(s), the main goal of our research expedition was to examine nitrogen metabolism in *G. przewalskii* in saline and alkaline Lake Qinghai, and during migration up a less alkaline freshwater river. A second goal was to describe the ion regulatory changes G. *przewalskii* experiences during migration from the lake to freshwater. To address these questions we traveled to Lake Qinghai in the summer of 1998 and conducted experiments on lake and river fish.

2. Materials and methods

2.1. Collection and maintenance of experimental fish

Our field station was set up on the bank of the Buha River near Bird Isle in Lake Qinghai (West Shore). Using gill nets, local fishermen collected adult lake fish from more than 1 km off the west shore of Lake Qinghai. The fish were transported to the field laboratory within 2–4 h of collection in large containers filled with Lake Qinghai water. Adult *G. przewalskii* fish were also collected from the Buha River approximately 15 km upstream from the inlet to Lake Qinghai using a cast net, and transported to the field lab in containers filled with river water.

The mature river and lake fish (15–25 cm, 70–220 g) of both sexes, 20–30 fish at a time, were kept in holding tanks filled with 60 l of unfiltered, aerated river and lake water, respectively. Water was changed in the holding tanks every 24 h. The experiments were conducted under natural photoperiod and ambient temperature ($\sim$15 °C).

To establish the physiological profile of scaleless carp living in the lake and while migrating up the freshwater Buha River, blood and tissue samples were taken from both lake and river fish. Blood samples of river fish were taken immediately upon capture via caudal puncture using 1-ml heparinized plastic syringes (sampled fish were returned to the river). Lake fish were sampled 12 h after transport to allow for recovery. Because a limited number of lake fish were available, some of the fish were kept in a holding tank after blood sampling for later experiments. Tissue samples were collected from a separate set of lake and river fish. To collect tissue samples, fish were terminally anaesthetized using MS-222 (1 g l^{-1}), then muscle, heart, liver, brain, intestine, gill, kidney and bile were removed from each fish. Samples were freeze-clamped and stored in liquid nitrogen, and were shipped in a liquid nitrogen dry shipper (-80 °C) back to laboratories at University of San Diego, University of Miami and McMaster University for further analysis of metabolites and enzyme activity.

2.2. Water chemistry

We analyzed the water composition in Lake Qinghai water and the Buha River. Water pH, salinity, dissolved O_2 content, titratable alkalinity and temperature measurements were conducted on site, and ion analyses were carried out later on water samples The concentrations of Cl^-, Na^+, Mg^{2+}, Ca^{2+} and K^+ in both lake and river water were determined.

2.3. Experimental design

2.3.1. Nitrogenous waste excretion by G. przewalskii in Lake Qinghai and Buha River water

To describe the patterns of nitrogen excretion of lake fish and river fish, ammonia–N and urea–N excretion rates were measured in six lake fish and six river fish. Each fish was held in an individual polyethylene container filled with 2–4 l of aerated water. The fish mass to water volume ratio was always less than 50 g l^{-1} of water. Water was replaced every 12 h. Water samples (10 ml) were taken at 0, 12, 24 and 36 h for the lake fish and at 0, 8, 18 and 24 h for the river fish. The flux rates were calculated based on the change in concentration of ammonia–N or urea–N in the water over the given period. In addition, ammonia–N was measured in blood plasma, liver, muscle and brain, while urea–N was assayed in plasma, muscle and liver of both groups at the end of the test. Glutamate dehydrogenase (GDH) and glutamine synthetase (GSase) activities were measured in liver, muscle and brain samples.

Rate of O_2 consumption was measured in river fish. Three fish were kept in individual 10-l 'Jerry' cans filled with the river water. The fish were allowed to acclimate in aerated water for at least 2 h after being transferred to the can, and then aeration was stopped and the chamber was sealed. The water O_2 was measured over several 1-h intervals using a YSI-15 submersible O_2 electrode. Oxygen consumption rates were calculated based on the difference between the initial and final dissolved O_2 content measurement in the water over the given period. Repeat measurements were made after a short re-aeration period.

2.3.2. Ammonia challenge

To examine the effect of high ammonia exposure, six lake fish and six river fish were challenged with elevated water ammonia. Lake fish were exposed to 0.4 mmol l^{-1} in lake water (pH 9.2), while river fish were exposed to 1.0 mmol l^{-1} of total ammonia in river water (pH 8.0) for 24 h. At the end of the exposure period, blood, liver, muscle and brain samples were collected and analyzed for ammonia and urea concentrations. The brain tissue was also assayed for GDH and GSase activity. Ammonia–N excretion rate was not measured in fish exposed to high ammonia because the combination of high ammonia background in the water and low ammonia excretion rate compromised the assay sensitivity.

2.3.3. Ionoregulation of G. przewalskii in Lake Qinghai and Buha River water

To characterize the ionoregulatory status of lake and river fish the concentrations of Cl^-, Na^+, Mg^{2+}, Ca^{2+}, K^+ and total protein were determined in plasma of both groups; hematocrit was also measured. Gill and intestinal tissue was also assayed for Na^+/K^+-ATPase activity. In addition,

bile was collected from the gallbladder and assayed for osmolality, bile acids, total ammonia and total CO_2.

2.3.4. Lake to river water transfer

To assess the physiological response of lake fish to the drop in salinity and pH during migration into freshwater rivers, 30 lake fish were transferred to 100-l river water after being held for 24 h in lake water in the lab. At 0, 6, 18, 30 and 48-h intervals after the transfer five fish were removed, and blood samples were taken by caudal puncture. Hematocrit and plasma ion and protein levels were measured.

2.4. Analytical methods

Water pH was measured using an Orion model 266 pH electrode, and dissolved O_2 content was monitored using a YSI-15 dissolved O_2 electrode corrected for water temperature and barometric pressure. Water salinity was estimated using a refractometer (American Optical). Titratable alkalinity was measured by titrating 10 ml water samples, that had been aerated with CO_2-free air, with 0.02 mol l^{-1} HCl until the end point pH 4.0 was reached.

Blood samples were centrifuged at $9000\times g$ for 2 min to obtain plasma. One part of the plasma sample (200 μl) was immediately stored in liquid nitrogen for later analysis of ammonia and urea. Plasma protein concentration was estimated by refractometry (American Optical), while the rest of the plasma samples were used for ion analysis.

All cations (except Cu) were analyzed after appropriate dilutions using atomic absorption spectroscopy (Varian AA-1275, AAS). Cl^- concentration in the water, plasma and bile samples was determined using the colorimetric assay of Zall et al. (1956). Total Cu concentration in bile was determined by graphite furnace AAS (Varian AA-1275 with GTA-95 atomizer) using 10–20 μl injection volumes, N_2 gas and standard operating conditions as described by the manufacturer. The total concentration of bile acids and ammonia was determined using Sigma kits (450-A and 171-A) modified for microplate reader use. Osmolality of bile was measured using a Wescor-5100C vapor pressure osmometer. The total CO_2 concentration of the bile was analyzed using a Corning 965 carbon dioxide analyzer.

Urea was measured using a modified diacetylmonoxime–thiosemicarbizide colorimetric assay (Price and Harrison, 1987), and water ammonia was measured using the indophenol blue method (Verdouw et al., 1978). Ammonia concentration in plasma and various tissue samples was analyzed using Sigma NAD/glutamate dehydrogenase kits. Tissue ammonia and urea were extracted by homogenizing frozen tissue in 6% ice-cold perchloric acid (1:4 w/v) with either sonication (Kontes Micro Ultrasonic Cell Disrupter) or by grinding in glass homogenizer. The homogenate was then centrifuged at $9000\times g$. The supernatant was immediately neutralized with saturated Tris buffer before the assays.

Maximal Na^+/K^+-ATPase activities in tissues were measured using the phosphomolybdate colorimetric assay of Holliday (1985) with Bonting's reagent (Bonting, 1970). Tissue samples ($\sim$100 mg) were homogenized in 5 ml of ice-cold homogenization buffer (250 mmol l^{-1} sucrose, 6 mmol l^{-1} EDTA) with a glass homogenizer. The homogenate was centrifuged at $2000\times g$ for 10 min at 4 °C and the supernatant used for the Na^+/K^+-ATPase activity assay. Protein content of this tissue extract was assayed using the Bradford method (Bradford, 1976). Enzyme activities were expressed as μmol P mg^{-1} protein h^{-1} at 24 °C.

For the analysis of maximal enzyme activity of GDH and GSase, tissue samples were homogenized and sonicated using an ultrasonic tissue disrupter. Tissue samples were homogenized on ice in 4× the volume of homogenization buffer (20 mmol l^{-1} K_2HPO_4, 10 mmol l^{-1} HEPES, 0.5 mmol l^{-1} EDTA, 1 mmol l^{-1}dithiothreitol, 50% glycerol, pH 7.5). The homogenate was centrifuged at $14\,000\times g$, 4 °C for 1 min. The supernatant was assayed for enzyme activities as previously described (Barber and Walsh, 1993), and activities are expressed as μmol product g^{-1} wet tissue min^{-1} at 24 °C.

2.5. Statistical analyses

All data are expressed as means $\pm$ 1 standard error of mean (S.E.). The *F*-test was used to test the homogeneity of variances between samples in lake fish and river fish or treatment and control groups. When appropriate, Student's two-tailed *t*-test was used if comparisons were made between two groups Single factor ANOVA followed by Scheffe's test was performed to test the difference

Table 1
Water electrolytes and other physico-chemical conditions in Lake Qinghai and Buha River in comparison to typical seawater and river water

	Cl^-	Na^+	Mg^{2+}	Ca^{2+}	K^+	Salinity	DO	TA	pH
Lake Qinghai[a]	98.5	88.0	18.7	0.30	2.51	9	3	30.1	9.2
Buha River[b]	2.4	0.26	0.82	1.19	0.12	–	3.5	2.8	8.0
Seawater[c]	548	470	53.6	10.2	9.9	33	7–9	2.3	7.9–8.0
World River[d]	0.22	0.27	0.17	0.37	0.59	–	–	1.0	7.8–8.0

Units for all ions and titratable alkalinity (TA) are mmol l^{-1}. Units for dissolved O_2 (DO) are mg l^{-1} and units for salinity is parts per thousand.

[a] Lake Qinghai water sampled from the shore near the Bird Isle, July, 1998.

[b] Buha river water sampled during July, 1998.

[c] Typical seawater (Schmidt-Nielsen, 1997).

[d] Typical river water (Wetzel, 1983).

between the control and subsequent experimental points in the time-trial experiment. Significance was accepted at $P<0.05$ throughout.

3. Results

3.1. Water in Lake Qinghai and Buha River

Among the major ions in Lake Qinghai, Na^+, K^+ and Cl^- were 15–25% of their concentrations in seawater, Mg^{2+} was approximately 35% of seawater levels and Ca^{2+} was extremely low (Table 1). Water pH was high, as was titratable alkalinity. In contrast, with the exception of Ca^{2+}, ion levels of Buha River water were much lower than levels in lake water.

3.2. Nitrogen metabolism, excretion and ammonia tolerance in the river and lake fish

Lake fish had very high plasma ammonia–N concentrations, which were almost seven times higher than plasma levels in river fish (Table 2). Ammonia–N levels were also significantly higher in liver, muscle and brain tissue of lake fish. In contrast, no significant differences were observed between river fish and lake fish in urea concentrations in plasma, liver and muscle. When challenged with 0.4 mmol l^{-1} total ammonia at pH 9.2 ($[NH_3]=0.16$ mmol l^{-1}) all six lake fish died within 8 h. River fish, on the other hand, exposed to 1 mmol l^{-1} total ammonia at pH 8.0 ($[NH_3]=0.023$ mmol l^{-1}) survived until sampled at 24 h. In river fish, high ammonia exposure resulted in a three-fold increase in plasma, five-fold increase in liver, two-fold increase in muscle and 61% increase in brain ammonia–N concentrations measured at 24 h (Table 2).

Consistent with higher ammonia concentration in plasma and tissues of lake fish, their rate of ammonia–N excretion was 50% lower than excretion rates of river fish (Table 3). Urea–N excretion was not detectable in either group (i.e. less than 20 μmol-N kg^{-1} h^{-1}). However, urea–N excre-

Table 2
Ammonia–N and urea–N concentrations in plasma, liver, muscle and brain of *G. przewalskii* collected from Lake Qinghai or Buha River, and in river fish exposed to high ambient ammonia (HAE: 1 mmol l^{-1}) in river water for 24 h

		Plasma	Liver	Muscle	Brain
Ammonia	Lake fish	2.23±0.81*	9.84±1.34*	10.91±0.60*	6.97±0.25*
	River fish	0.32±0.05	5.51±0.73	8.78±0.60	4.94±0.19
	HAE river fish	1.33±0.24*	27.94±1.12*	16.28±1.61*	7.98±0.17*
Urea					
	Lake fish	5.87±0.68	0.33±0.01	1.02±0.09	
	River fish	5.45±0.48	0.32±0.01	1.35±0.25	
	HAE river fish	7.97±2.16	3.14±0.16*	2.26±0.21*	

Values are expressed as means±S.E. in mmol N kg^{-1} for liver, muscle and brain, and mmol N l^{-1} for plasma ($N=8$, 11 and 4 for river, lake and HAE fish, respectively).

*Denotes significant difference ($P<0.05$) from the corresponding values in river fish.

Table 3
Ammonia excretion rates in *G. przewalskii* collected from the lake water or Buha River

Time (h)	Lake fish	Time (h)	River fish
1–12	403±41	1–8	656±73*
12–24	194±49	8–18	527±47*
24–36	175±20	18–24	344±52*

Values are expressed as Mean±S.E. in μmol N kg^{-1} h^{-1} ($N=5$).

*Denotes significant difference ($P<0.05$) from the corresponding values in lake fish.

tion rate in river fish exposed to elevated ambient ammonia was detectable at 47.3±8.5 μmol kg^{-1} h^{-1}. The rate was equivalent to approximately 15% of the ammonia–N excretion rate in non-ammonia exposed river fish.

GSase and GDH activities were not significantly different in liver, muscle and brain of river and lake fish (Table 4). However, after 24 h exposure to 1 mmol l^{-1} ammonia, the activities of both enzymes had climbed by approximately 20% in the brains of river fish.

Oxygen consumption rate of three river fish was measured at 5.10±0.22 mmol kg^{-1} h^{-1}. Simultaneous measurement of rate of ammonia–N excretion was 344±52 μmol kg^{-1} h^{-1}. Together these numbers yield an ammonia–N excretion:O_2 consumption ratio (Ammonia Quotient) of 0.067.

3.3. Ionoregulation in the river and lake fish

Despite the generally much higher ion levels in lake water, plasma [Cl^-] and [Na^+] of lake fish were 18 and 8% lower, respectively, than levels in river fish (Table 5). Plasma [Mg^{2+}] and [Ca^{2+}] in lake fish were comparable to those in river fish, but plasma [K^+] in lake fish was almost three times higher than in river fish. Plasma protein concentrations, which in both river and lake fish were relatively high compared with other teleosts, were slightly, but significantly higher in lake fish. Haematocrit in both groups was similar in both groups and close to 40%.

Correlated with the elevated ion levels in river fish was a 67% greater gill Na^+/K^+-ATPase activity in river fish relative to lake fish (Table 6). In contrast, Na^+/K^+-ATPase activity in intestines and kidney did not show significant differences between the two groups.

Table 4
Glutamine synthetase (GSase) and glutamate dehydrogenase (GDH) activities in liver, muscle and brain of *G. przewalskii* collected from Lake Qinghai or Buha River, and in river fish after high ambient ammonia exposure (HAE)

		Liver	Muscle	Brain
GSase				
	Lake fish	2.04±0.51	ND	66.76±6.22
	River fish	1.59±0.48	0.022±0.005	62.25±3.94
	HAE river fish	N/A	N/A	76.17±2.28*
GDH				
	Lake fish	120.48±14.62	3.55±0.53	5.68±0.11
	River fish	116.20±13.19	4.75±0.92	5.21±0.06
	HAE river fish	N/A	N/A	6.28±0.17*

Values are expressed as means±S.E. in μmol g^{-1} min^{-1} ($N=8$ for river and lake fish, $N=4$ for HAE fish). *Indicates significant difference ($P<0.05$) from the corresponding values in river fish. ND indicates not detectable. N/A indicates not available.

Table 5
Plasma electrolytes, urea, protein and haematocrit in *G. przwalskii* collected from Lake Qinghai and Buha River

	Cl^-	Na^+	Mg^{2+}	Ca^{2+}	K^+	Urea-N	Protein	Hct %
Lake Qinghai fish	97.1* (2.9)	128.9* (3.1)	1.14 (0.10)	3.44 (0.15)	2.63* (0.36)	5.87 (0.68)	9.8* (0.2)	38.7 (3.2)
Buha River fish	118.7 (2.6)	140.7 (2.8)	0.96 (0.05)	3.25 (0.11)	0.90 (0.17)	5.45 (0.48)	8.8 (0.3)	38.3 (0.8)

Values are Means (S.E.). $N=10$ and 14 for lake fish and river fish, respectively. Units for all ions and urea–N are mmol l^{-1}. Units for plasma protein are g 100 ml^{-1} and units for hematocrit (Hct) are%.

*Denotes significantly different from the corresponding values in river fish ($P<0.05$).

Table 6
Na^+–K^+ ATPase activities in the gills, kidney and intestines of *G. przewalskii* collected from Qinghai Lake and Buha River

	River fish	Lake fish
Gills	3.67±0.57	2.19±0.58*
Intestines	2.88±0.66	2.39±0.22
Kidney	9.63±0.76	8.48±0.36

Values are expressed as means±S.E. (μmol P mg^{-1} protein h^{-1}, $N=6$).
*Denotes significantly different from the corresponding values in river fish ($P<0.05$).

The concentrations of Mg^{2+} and K^+ were approximately 30% higher in the bile of lake fish than river fish, but all other ions were similar (Table 7). Bile acid concentrations were two-fold higher in lake fish. It is also interesting to note that the concentration of ammonia in the bile of fish collected from the lake was more than three times higher than in river fish.

The transfer experiment reinforced the unexpected differences in plasma ion levels between lake and river fish. Upon transfer of lake fish into river water, plasma Na^+ and Cl^- levels steadily rose (Fig. 1a) and after 48 h were 10–15% higher than initial levels. In contrast, plasma K^+ levels fell sharply (Fig. 1b) during the first 6 h of transfer, but then recovered over the remaining 48 h. Plasma Ca^{2+} and Mg^{2+} levels did not vary significantly over the 48-h period. Plasma hematocrit did not increase significantly during the transfer, while plasma protein was only significantly elevated after the 48-h transfer (Table 8).

4. Discussion

4.1. Nitrogenous waste excretion

Our results indicate that living in lake water poses a significant challenge for N-waste excretion in *G. przewalskii*. Fish in both lake and river environments are clearly ammonotelic since virtually all of their N-waste was excreted as ammonia. However, there were significantly lower rates of ammonia excretion in lake fish, which is likely due to inhibition of ammonia excretion by alkaline lake water. Diffusion is considered the predominant pathway of nitrogenous waste elimination, and is facilitated by a 'proton-trap' in the apical boundary layer, which acts to maintain the outward gradient (Randall and Wright, 1987). In high pH water the 'proton-trap' is short-circuited and the NH_3 gradient between fish blood plasma and water is much reduced, thus impeding NH_3 diffusion (Wood, 1993).

It is also interesting to note that despite the high pH, lake fish were still able to excrete ammonia. The key to understanding why there may be a relatively low buffering capacity of lake water. This is illustrated by comparison of the water chemistries of Lake Qinghai and Lake Magadi, home to the Lake Magadi tilapia (*Alcolapia grahami*). Lakes Magadi and Qinghai have similar high pH values, but the buffering capacity of Lake Magadi is approximately 10-fold higher based on titratable alkalinity. Consequently, Lake Magadi tilapia must excrete all their N-waste as urea (Randall et al., 1989; Walsh et al., 1993), while *G. przewalskii* can still excrete NH_3. Thus, it would seem that the combination of high pH and high buffering capacity is the key to negating the proton-trap mechanism of ammonia diffusion and driving a switch to ureotely.

Probably because of decreased rates of ammonia excretion, lake fish showed very high levels of ammonia in plasma (over 2 mmol l^{-1}) as well as in tissue. The high plasma ammonia could, at least in part, be due to puncture sampling (Wood, 1993), but since the same puncture sampling was used for river fish, the difference between the two is probably real. It is also possible that lower ammonia levels in river fish were at least partially due to low food intake during spawning migration, but given the higher rates of excretion in river fish this does not seem likely to be the main cause of differences in internal ammonia levels. It seems

Table 7
Blood haematocrit (Hct) and plasma protein concentration (PP) in *G. przewalskii* during 48-h exposure to river water

Post-transfer time (h)	0	6	18	30	48
Hct (%)	30.0±2.4	29.4±1.7	31.4±1.3	32.2±1.7	34.0±1.4
PP (g 100 ml^{-1})	7.77±0.58	7.37±0.40	8.01±0.57	7.97±0.34	9.15±0.66*

Values are means±S.E. ($N=5$). *Denotes significantly different from the corresponding values at 0 h.

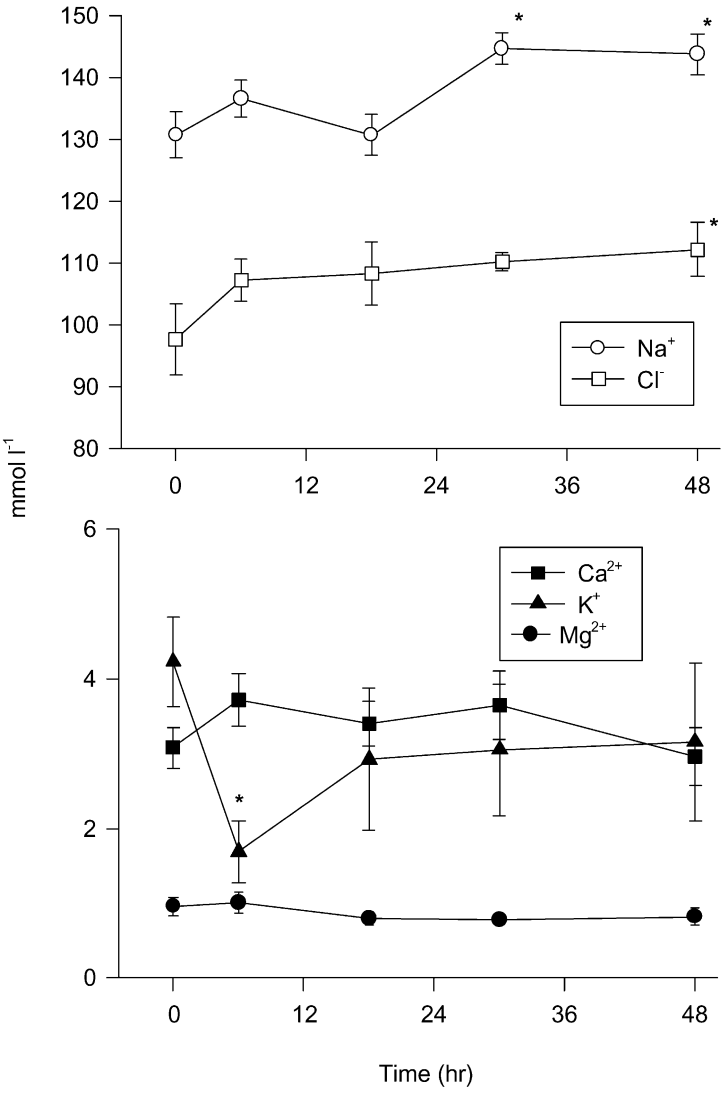

Fig. 1. (a) Plasma Na^+, Cl^- and (b) Ca^{2+}, Mg^{2+} and K^+ concentrations in *G. przewalskii* during 48-h transfer from Lake Qinghai water to Buha River water. Values are expressed as means ± S.E. ($N=5$). *Denotes significant difference from corresponding values before the transfer (0 h).

clear that *G. przewalskii* tolerate very high ammonia in the plasma and tissues while in the lake. A similar strategy has also been observed in another cyprinid, *Chalcalburnus tarichi*, of Lake Van, an alkaline and saline lake in Turkey (Danulat and Kempe, 1992), and in the Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) which lives in similar alkaline circumstances (Wright et al., 1993).

Further evidence that lake fish are 'living on the edge' is the outcome of the high ammonia exposure tests. Within 8 h of exposure to 0.4 mmol l^{-1} ammonia, all lake fish were killed, while river fish survived 24 h in river water plus 1 mmol l^{-1} ammonia. The key to understanding this seeming discrepancy is that, due to the higher pH of lake water, the actual unionized NH_3 concentration of lake water was seven times higher than in river water (0.16 mmol l^{-1} vs. 0.023 mmol l^{-1}). Thus, the gradient for diffusion was much smaller for lake fish. Still, the relatively high ammonia concentration in plasma, liver and muscle in live-caught lake fish and ammonia-exposed river fish indicates that the fish is rather tolerant of elevated

Table 8
Gallbladder bile composition of *G. przewalskii* collected from Qinghai Lake or Buha River

	River fish	Lake fish
Na^+ (mmol l^{-1})	379.0±12.5	410.2±13.4
K^+ (mmol l^{-1})	7.5±0.5	9.9±0.4*
Ca^{2+} (mmol l^{-1})	5.0±0.5	6.2±0.4
Mg^{2+} (mmol l^{-1})	5.2±0.4	6.8±0.4*
Cu (μmol l^{-1})	215±43	245±41
Cl^- (mmol l^{-1})	56.9±3.4	44.6±5.2
Bile acid (mmol l^{-1})	37.3±8.8	90.5±14.5*
Ammonia (mmol l^{-1})	3.2±0.7	10.8±2.0*
Total CO_2 (mmol l^{-1})	<0.2	<0.2
Osmolality (mOsm kg H_2O^{-1})	323.3±10.2	328.1±6.4

Values are means±S.E. ($N=6$). *Denotes significantly different from the river fish ($P<0.05$).

internal ammonia levels and are somehow avoiding toxicity. Another rather ammonia tolerant Cyprinid (*Chalcalburnus tarichi*) from Lake Van had similar elevated levels of ammonia in liver, muscle and brain after exposure to high environmental ammonia (Danulat and Kempe, 1992).

Despite possessing a relatively high tolerance of internal ammonia levels, our data indicate that *G. przewalskii* may utilize a suite of mechanisms to decrease or avoid ammonia toxicity. One possible way of avoiding NH_3 toxicity caused by impaired excretion would be to convert it into the less toxic urea for excretion. However, our results do not indicate that this is happening. We did find considerable levels of urea–N in the plasma, liver and muscle of river and lake fish (Table 2), but there was no detectable urea–N excretion in either group. Interestingly, a low level of urea–N excretion (~50 μmol-N kg^{-1} h^{-1}) was detected as the level of urea–N in tissue compartments rose during exposure of river fish to high ammonia, which raises the possibility of involvement of ureogenic pathways to convert NH_3 to less toxic urea in these fish. We are not able to assess this pathway directly, but our results suggest that it is probably unimportant. We were not able to accurately measure the excretion rate of ammonia–N in the ammonia-challenged river fish, so we do not know the percentage of nitrogen that was eliminated as urea–N, but if total nitrogen excretion was considered unchanged in NH_3-exposed river fish (Table 4), urea–N represents no more than 15% of total excretion. Therefore, it is likely that fish remained predominantly ammonotelic.

Another mechanism is to simply produce less NH_3. The low AQ coupled with a generally low metabolic rate reported earlier are indicative of this strategy. Another possible mechanism is to convert NH_3 to less toxic forms of nitrogenous products such as urea or amino acids. While *G. przewalskii* do not seem to make much urea, it is possible that they may incorporate NH_3 into amino acids. A mechanism involving the enzymes GDH and GSase has been proposed as a way to convert ammonia to amino acids such as glutamine and glutamate (Peng et al., 1998; Iwata et al., 2000; Wang and Walsh, 2000). The extremely high GDH activity in the amination direction in the liver, muscle and brain of *G. przewalskii* would allow the fish to scavenge ammonia and provide glutamate for the subsequent glutamine amidation catalyzed by GSase. Although no significant difference was observed in GDH and GSase between lake fish and river fish, the GDH activities were 5–10 times higher than other teleost fish subjected to conditions unfavorable for ammonia excretion, and liver GSase activity was similar to levels reported in high ammonia tolerant toadfish (Iwata et al., 2000; Wang and Walsh, 2000; Randall et al., 1989). Furthermore, ammonia-exposed river fish did exhibit an increase of activity in both enzymes in the brain.

Still other mechanisms to avoid ammonia toxicity may be important. The large relative mass of muscle in fish and the relatively insensitive nature of the tissue may allow it to act as an ammonia sink to attenuate ammonia levels in other tissues. In fact the highest tissue ammonia levels we found in lake fish were in muscle. Furthermore, the muscle ammonia levels in river fish almost doubled during exposure to 1 mmol l^{-1} ammonia. In addition, the drastically elevated urea–N in muscle suggests that muscle could also serve as a storage site for urea–N synthesized in other tissues, such as liver or possess functional ureogenic capacity to convert ammonia to less toxic nitrogenous products as reported in Lake Magadi tilapia (Lindley et al., 1999).

Ammonia Quotient (0.067) was within the range of 0.06–0.15 reported for other freshwater fish (Lauff and Wood, 1996; Alsop et al., 1999; De Boeck et al., 2000). This value was only 24% of the maximum of 0.27 possible if aerobic metabolism were completely fueled by protein catabolism (van den Thillart and Kesbeke, 1978). The relatively low energy contribution from protein catab-

olism could be partially due to the fact the river fish have very low protein intake during their spawning migration and/or because of their herbivorous feeding habits. The low nitrogen excretion rates in *G. przewalskii* may also simply reflect their low metabolic rates ($\dot{M}O_2 = 5.1$ mmol kg^{-1} h^{-1}). Although no attempt was made to measure O_2 consumption rates in lake fish, judging from the reduced Na^+/K^+ ATPase activity in gills and less challenging transmembrane ion gradient from water to extracellular fluid, we suspect these fish had even lower metabolic rates.

4.2. Ionic and osmotic regulation

Our results show that *G. przewalskii* are able to maintain tight controls of ion levels in both lake and river water. Plasma ion levels of fish collected from Lake Qinghai and the Buha River fell within the range typical of cyprinids and other freshwater teleosts (De Boeck et al., 2000). However, lake fish had ion concentrations much lower than the range characteristic for brackish or salt water species (Holmes and Donaldsen, 1969; McDonald and Milligan, 1992). Cyprinids, in general, are classified as stenohaline freshwater fish and do not usually adapt to elevated salinity well (De Boeck et al., 2000). There are some notable exceptions (Danulat and Kempe, 1992). *G. przewalskii* is among these few cyprinid species that are capable of coping with brackish water. The label of stenohaline on cyprinids may need to be qualified in the future.

In a surprising departure from the expected circumstance, scale-less carp taken during migration up the freshwater Buha River had significantly higher plasma $[Na^+]$ and $[Cl^-]$ and lower $[K^+]$ than fish taken from the isosmotic Lake Qinghai (Table 6) despite the fact that upon entering the Buha River, the outward Cl^- gradient increased from approximately zero to approximately 115 mmol l^{-1}, and the outward Na^+ gradient increased from 40 to 140 mmol l^{-1}. Most euryhaline teleosts tend to have lower plasma Na^+ and Cl^- concentration when moved from brackish to freshwater, due to the increased rates of salt loss by passive diffusion (Postlethwaite and McDonald, 1995; Perry and Laurent, 1989).

It is not clear whether or not the higher plasma ion levels confer some physiological advantage upon river fish. Perhaps these higher plasma ion levels provide a more suitable internal environment to face the energetic, metabolic and acid–base challenges associated with migration up the river. It is also plausible that the different physiological states of the lake and river (spawning and non-eating) fish may play an important role in dictating the changes in plasma ion concentration, e.g. the hormonal changes (growth hormone and prolactin) in spawning fish can modulate ion balance. Alternatively, the changes in ion levels are the result of adjustments the fish make for ion regulation in the relatively dilute river. One clear ionoregulatory adjustment that may be the cause of the higher plasma Na^+ and Cl^- concentrations is the higher Na^+/K^+-ATPase activity in the gills of fish collected from river. Increased Na^+/K^+-ATPase activity may be necessary to raise rates of ion uptake to counter higher rates of diffusive loss in freshwater.

In parallel to the changes found in the migrating *G. przewalskii*, the transfer experiment showed that the rise in plasma ion levels occurred over 30–48 h of exposure of lake fish to river water (Fig. 1). The coincident rise in plasma protein concentration (Table 7) suggests that in the short-term, there may have been some contraction of plasma fluid volume (perhaps due to diuresis), which helped to elevate plasma Na^+, Cl^- and protein concentrations. Diuresis may also explain the transient drop in plasma K^+ concentration (Fig. 1b). However, this is unlikely to be the long-term situation, because plasma protein concentrations were actually slightly lower in fish collected from the river (Table 6).

Another potential benefit may lie in ammonia excretion. Recently, a series of studies using specific inhibition kinetics and immunolocalization of relevant transporters has indicated a coupling between Na^+ uptake and NH_4^+ excretion in freshwater teleosts (Randall et al., 1999; Wilson et al., 2000; Salama et al., 1999). Specifically, these authors suggest that in mitochondria rich cells, NH_4^+ may replace K^+ in Na^+/K^+ ATPase on the basolateral membrane, and H^+ in the Na^+/H^+ exchangers on the apical membrane. In the present study, the elevated plasma Na^+ concentration shown in the river *G. przewalskii* was concomitant with an elevated ammonia excretion rate suggesting, albeit indirectly, some sort of coupling of this nature. One might question the need for such a route for this fish in a freshwater environment where ammonia elimination via NH_3 diffusion should be rather easy. Nonetheless, considering

that these fish spend most of their lives in high pH and saline water where ammonia excretion via NH_3 diffusion is hindered and Na^+ in the environment is plentiful, Na^+/NH_4^+ exchange via NHE may be a possibility.

While plasma Na^+ and Cl^- concentrations rose, plasma Ca^{2+} levels did not change at all during migration into the freshwater Buha River or during the transfer experiment. This is somewhat surprising considering the unusually low concentrations of Ca^{2+} in the lake and the changes in concentration of these salts as fish move into the river. Like plasma Ca^{2+}, plasma Mg^{2+} concentration also appeared to be tightly regulated. Mg^{2+} levels in the lake water were one-third as high as full-strength seawater and more than 20 times higher than Buha River Water. There is an inward Mg^{2+} gradient for fish in the lake water, and a slightly outward gradient for fish in the river water. Thus, in lake water, the elimination of excess Mg^{2+} may be of critical importance, but that need is virtually eliminated upon moving into the river. Mg^{2+} levels are normally well regulated in both intracellular and extracellular fluid (Grubbs and Maguire, 1987; Flatman, 1991; Bijvelds et al., 1998). In most fish, the low but stable $[Mg^{2+}]$ in the extracellular fluid is normally achieved through the balance between Mg^{2+} uptake through intestinal absorption and branchial uptake, mobilization/deposition in bone and soft tissue and secretion and reabsorption in the kidney (Bijvelds et al., 1998). The latter is thought to be the primary mechanism of homeostasis. Well-controlled Mg^{2+} level in plasma may be the result of rigorous regulation by the kidney through a Na^+/Mg^{2+} exchange mechanism that will excrete excess Mg^{2+} in the urine (Flatman, 1991).

As shown in Table 8, there was little difference in biliary ion concentrations between lake fish and river fish suggesting that the gall bladder of the scale-less carp plays little, if any, role in osmoregulation, as previously reported for the Japanese eel (Hirano and Bern, 1972). A large portion of the accumulated Na^+ ions in the gallbladder bile is likely conjugated to bile acids (Grosell et al., 2000), which explains the low osmolality in comparison to the total ion concentration (Table 3). The asymmetrical Na^+ to Cl^- concentration in the gallbladder bile is in agreement with observations from freshwater rainbow trout along with a wide phylogenetic range of other vertebrates, and is in accord with a recently suggested *trans*-epithelial transport model accounting for a much higher net transport of Cl^- than Na^+ from gallbladder lumen to extracellular fluid in vivo (Grosell et al., 2000). Gall bladder bile volume in rainbow trout depends strongly on feeding status, whereas solute concentrations are largely independent of feeding status (Grosell et al., 2000). With this in mind, the lower bile acid concentration in the river fish may suggest that the bile has resided in the gall bladder for a shorter period than in the lake fish, (i.e. less fluid reabsorption). The high ammonia, K^+ and Mg^{2+} concentrations in the bile of lake fish are consistent with the possible greater fluid reabsorption, as well as with the higher plasma ammonia (Table 2) and K^+ and Mg^{2+} levels (Table 6). The copper concentration in the gall bladder bile is similar to values reported for other teleost fish and much higher than typical plasma Cu levels (10 μmol l^{-1}) confirming the role of hepatobiliary Cu excretion in copper homeostasis (Grosell et al., 2001).

In summary, this first expedition to western China to examine the physiology of the *G. przewalskii* has reaped a wealth of information about the physiology of an unusual fish. Yet as often happens, these findings raise more questions than they answer. Therefore, future studies are in order to clarify many questions generated by this work related to N-metabolism and ion regulation in this fish.

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