J Membrane Biol (2013) 246:183–188
DOI 10.1007/s00232-012-9518-3

ORIGINAL ARTICLES

Environmental Regulation of Mitochondria-Rich Cells in *Chalcalburnus tarichi* (Pallas, 1811) During Reproductive Migration

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Received: 22 August 2012 / Accepted: 15 October 2012 / Published online: 3 November 2012

Abstract *Chalcalburnus tarichi* is an anadromous cyprinid fish that has adapted to extreme conditions (salinity 22 ‰, pH 9.8 and alkalinity 153 mEq $\times$ l^{-1}) in Lake Van in eastern Turkey. Changes in immunoreactivity of Na^+/K^+-ATPase in gill tissue and osmolarity and ion levels in plasma were investigated in *C. tarichi* during reproductive migration. Physicochemical characteristics and ion levels in Lake Van were high compared freshwater. Plasma osmolality and plasma ion concentrations ([Na^+], [K^+] and [Cl^-]) increased after transfer from freshwater to Lake Van. The mitochondria-rich (MR) cells of the gill were stained in both filament and lamellar epithelia of *C. tarichi* by immunocytochemistry with a specific antiserum for Na^+/K^+-ATPase in river fish samples. Density and area of MR cells were decreased in lake-adapted fishes. These results indicated that freshwater acclimation capacity is correlated with the size and distribution of MR cells in *C. tarichi*, in contrast to many teleost fishes.

Keywords Mitochondria-rich cell · Na^+/K^+-ATPase · Osmoregulation · Lake Van · *Chalcalburnus tarichi*

Introduction

Euryhaline fish possess the capacity to live at various salinity concentrations. Fishes are adapted to different salinity environments with a number of changes in osmoregulatory organs (Evans et al. 2005). Basic tissues in fish osmoregulation are gastrointestinal epithelium, kidney and gill. The gill is an important tissue in osmoregulation, acid base regulation and ion regulation and can contain three different cell types: mitochondria-rich (MR) or chloride cells, pavement cells and mucous-secreting cells. MR cells have Na^+, K^+, ATPase they are present in extensive tubular system continuous with the basolateral membrane and numerous mitochondria in cytoplasm (Wilson and Laurent 2002). Mucous cells serve to protect the gills against pathogenic microorganisms and parasitic infection with mucous secretion (Dezfuli et al. 2010). The MR cell has vital importance in osmoregulation due to transport of Na^+ and Cl^- from the water into body or vice versa in different salinity environments. The MR cell displays a series of changes after adaptation to high-salinity environments. These changes are characterized by hypertrophy, increases basal–lateral cell surface area and Na^+/K^+-ATPase content and location in gills (Karnaky et al. 1976; Uchida et al. 1996; Hirai et al. 1999).

Lake Van is the largest soda lake in the world, and *Chalcalburnus tarichi* is endemic to the lake. Extreme conditions are found in the lake such as high salinity (0.22 %), pH 9.8 and alkalinity (153 mEq $\times$ l^{-1}) (Danulat and Kempe 1992). *C. tarichi* has unique characteristic that live in this extreme water and is an important protein source in eastern of Turkey. The *C. tarichi* population decreased due to overfishing in the reproductive period, destruction of spawning habitat and abnormalities in the gonads.

C. tarichi is an anadromous fish and migrates to freshwater inlets for annual spawning from April to July. Fishes return to Lake Van for feeding and growth. Then, the fertilized eggs undergo embryonic development in the freshwater, and juveniles stay there for 2–3 months before migrating to the lake (Danulat and Selçuk 1992). They reach sexual maturity at 3 years of age (Unal et al. 1999). Little is known about the osmoregulation physiology of

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C. tarichi (Danulat 1995; Arabacı et al. 2001). To date, no study has been conducted on the size and abundance of MR cells and Na^+/K^+-ATPase enzyme immunolocalization in *C. tarichi*.

The main objective of the present study was to investigate ionic changes and osmolality in plasma and quantitative changes of MR cells in gill tissue during reproductive migration from freshwater to the brackish water of Lake Van.

Materials and Methods

Fish

Sexually mature *C. tarichi* (18.2 ± 0.9 cm, 76.2 ± 8.2 g) were captured in two different sampling areas during the reproduction period (Karasu River and Lake Van). All experimental procedures followed national animal care regulations. Water pH, temperature, salinity, dissolved oxygen and specific electrical conductivity were measured in the two different sampling areas with a multiparameter device (Orion 5 Star; Thermo, Barrington, IL). The fish were anesthetized with MS–222 (100 mg/l) and killed by spinal transection. Blood was collected from the caudal vessels using a heparinized syringe and centrifuged at 15,000×*g* at 4 °C for 10 min. After centrifugation, plasma was transferred to Eppendorf tubes and stored prior to analysis. Plasma and water $[Na^+]$, $[K^+]$, $[Cl^-]$ and $[Ca^{2+}]$ were determined using an autoanalyzer (Cobas Integra 800; Roche, Mannheim, Germany).

Osmolality Measurements

Plasma and water sample (10 µl) osmolality was measured with a vapor pressure osmometer (Wescor 5520; Wescor, Logan, UT).

Immunocytochemical Detection of Gill MR Cells

Gills were dissected and immediately fixed in 4 % paraformaldehyde in 0.1 M phosphate buffer. Fixed gills were preserved in sucrose solution (4 %) at 4 °C until use. Tissues were cryosectioned (7 µm) and placed on poly-L-lysine-coated glass slides.

Sections were washed in PBS and incubated for 10 min with a blocking solution containing 1 % bovine serum albumin and 0.1 % gelatin in PBS. Slides were incubated overnight at room temperature with the specific monoclonal mouse antibody raised against the α-subunit of chicken Na^+/K^+-ATPase (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA). Slides were washed in PBS and then incubated with the secondary antibody (1:100), anti-mouse IgG produced (1:1,000) in goat (Alexa Fluor 488; Invitrogen, Carlsbad, CA), for 2 h under dark conditions in a humidified chamber. All slides were mounted with a coverslip and viewed with a digital camera attached to a microscope (Leica, Wetzlar, Germany). Negative control sections were incubated under the same conditions without primary antibody.

The abundance of MR cells was determined for each fish by counting all strongly stained, immunoreactive cells of the filaments and lamellae along a 500-µm length of filament from both Karasu River and Lake Van branchial gill samples. Sectional MR cell size (μm^2) for each fish was determined by measuring 30 cells per fish, using an image-analysis program (Leica).

Statistics

Data were expressed as mean ± SEM. The significance of differences between the river and lake groups was analyzed using Student's *t* test. Significance was indicated at $P < 0.05$.

Results

During the reproductive migration of fishes, differences were observed in the physicochemical characteristics of sample waters. These water characteristics are summarized in Table 1. Ion concentrations of sample water are shown in Table 2. Ion analysis results indicated that ion levels were significantly higher in lake water than river samples.

Osmoregulation

The osmolality of Karasu River water samples (81 ± 0.5 mOsmol kg^{-1}) was significantly lower compared to Lake Van (557 ± 3 mOsmol kg^{-1}). The plasma osmolality of Karasu river samples (296 ± 3.986 mOsmol kg^{-1}) was significantly higher than Lake Van samples (477.625 ± 5.294 mOsmol kg^{-1}). The osmolality of plasma samples increased 1.6-fold in lake-adapted fish. The results are shown in Fig. 1.

Table 1 Physicochemical characteristic of sampling waters

Parameter	River	Lake Van
pH	8.68	9.42
Temperature (°C)	14.6	23.5
Salinity (‰)	0.2	17.2
Dissolved oxygen	10.45	6.00
Conductivity (mS cm^{-1})	0.499	28.08
Saturation (%)	126.1	98.6

Table 2 Ion concentrations in Karasu river and Lake Van water

Ion concentration (mmol l^{-1})	River	Lake Van
Na^+	0.468 ± 0.008	296.032 ± 6.194
K^+	0.113 ± 0.001	8.783 ± 0.287
Mg^{2+}	0.389 ± 0.002	2.794 ± 0.451
Ca^{2+}	0.087 ± 0.001	0.365 ± 0.042
Cl^-	1.001 ± 0.129	178.919 ± 4.253

Values are mean ± SEM ($n = 8$)

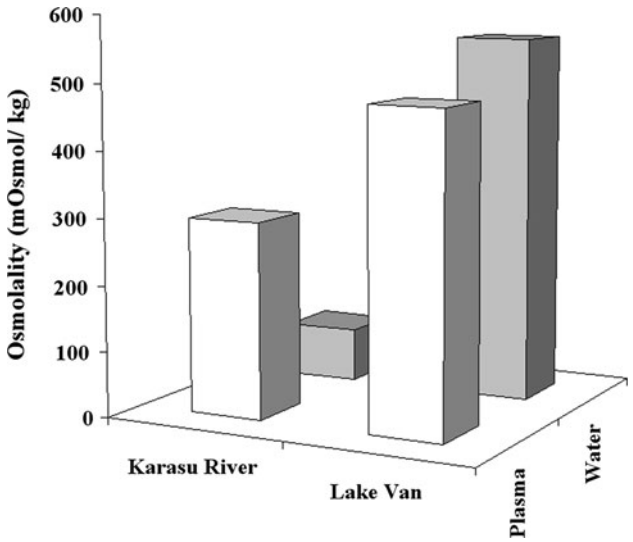

Fig. 1 Blood and water osmolality (mOsmol kg^{-1}) in Karasu river and Lake Van samples. Values are means ± SEM, $n = 8$, $^*P < 0.05$

Table 3 Plasma ion changes in *C. tarichi* from river and lake groups

Plasma ion concentration (mmol l^{-1})	River	Lake Van
Na^+	164.333 ± 0.667	225.333 ± 3.363*
K^+	0.753 ± 0.033	6.650 ± 0.706*
Ca^{2+}	12.880 ± 0.563	13.700 ± 1.181
Cl^-	131.500 ± 0.846	141.83 ± 2.271*

Values are mean ± SEM ($n = 8$)

* $P < 0.05$

Plasma $[Na^+]$, $[K^+]$, $[Cl^-]$ and $[Ca^{2+}]$ Levels

Plasma ion concentrations were measured in plasma of river- and lake-adapted fish (Table 3). Plasma ions of *C. tarichi* were elevated during reproductive migration from river to lake. Plasma $[Na^+]$, $[K^+]$ and $[Cl^-]$ levels were significantly higher in lake samples, but $[Ca^{2+}]$ was not different compared to river samples.

Immunocytochemical Detection of Gill MR Cells

Immunofluorescence staining of gill epithelium of *C. tarichi* in river- and lake-adapted fishes is shown in Fig. 2. Na^+/K^+-ATPase immunofluorostain was not observed in negative controls (data not shown). The distribution of MR cells was observed only in primary filaments and was absent in lamellae of lake samples. MR cells were usually round or flat in shape in freshwater-adapted fish. Flat MR cells disappeared in lake water-adapted samples. The quantitative analysis of lamellar MR cells in the river and lake samples is shown in Table 4. However, there were no differences in MR cell numbers in filaments. The MR cells in lake-adapted fish were smaller than those in river-adapted fish.

Discussion

Chalcalburnus tarichi has the highest osmolality among teleost fish. Plasma osmolality in fish migrating from freshwater to saline water was significantly increased (Fig. 1). Similar results were observed in fish transferred in laboratory conditions or migration in the wild from freshwater to saline water (Uchida et al. 2000; Fielder et al. 2007; He et al. 2009). Plasma and tissues of *C. tarichi* contain high urea concentrations, and urea increases in osmolality in lake water (Danulat and Kempe 1992). Therefore, high plasma osmolality of *C. tarichi* is isosmotic with Lake Van water. Freshwater ion concentrations for $[Na^+]$, $[K^+]$ and $[Cl^-]$ were lower compared to lake water. Similarly, the main plasma ion concentrations ($[Na^+]$, $[K^+]$ and $[Cl^-]$) were significantly elevated in lake compared to river water. MR cells in fishes are involved in salt secretion in saline water and ion uptake in freshwater (Hiroi et al. 1998; Evans et al. 2005). *C. tarichi* may take up $[Na^+]$ and $[Cl^-]$ in freshwater via Na^+/H^+ and Cl^-/HCO_3^- exchangers coupled to Na^+/K^+-ATPase, while effluxes of this ion occur in Lake Van during reproductive migration.

Immunohistochemical staining of Na^+/K^+-ATPase has been widely used for the localization of MR cells in gill, kidney, intestine and yolk-sac membrane (Ura et al. 1996; Shiraishi et al. 2001; Nebel et al. 2005). Several studies have shown that abundance, size and location of MR cells are changed depending on the environmental salinity (Karnaky et al. 1976; Uchida et al. 2000; Zydlewski and Mccormick 2001; Fielder et al. 2007). In the present study, MR cells were larger in river- than lake-water samples (Table 4; Fig. 2). Lamellar MR cells were observed only in river samples. In chum salmon and Japanese sea bass, lamellar MR cells disappeared in seawater-adapted fish (Uchida et al. 1998; Hirai et al. 1999). Hirai et al. (1999)

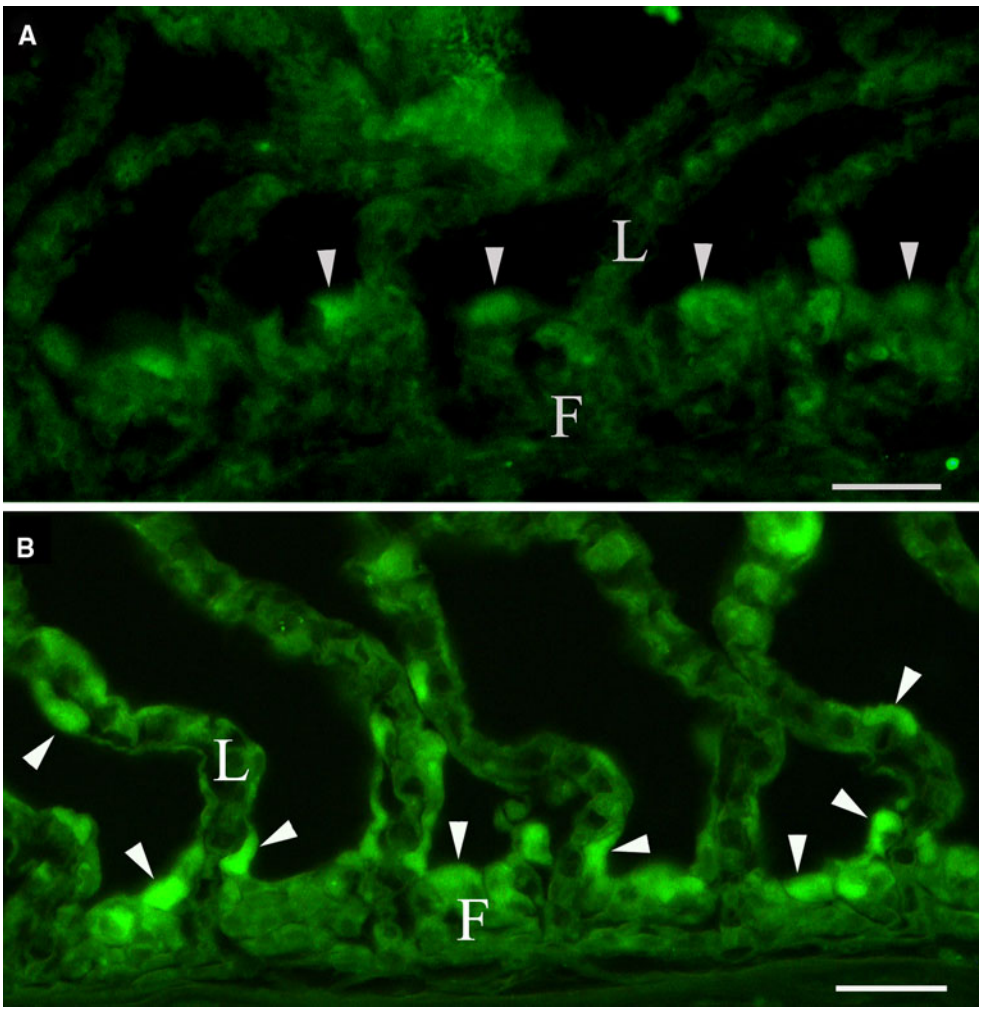

Fig. 2 Sagittal section of gill filament from *C. tarichi*. Fluorescent immunocytochemical stain using a monoclonal antibody against the α-subunit of Na^+/K^+-ATPase in **a** lake- and **b** river-adapted fish. *L* lamellae, *F* filament and mitochondria-rich cell (*arrowhead*). *Scale bar* 25 μm

Table 4 Mitochondria-rich cell number and size in river- and lake-adapted *C. tarichi*

	Filament		Lamellae	
	River	Lake	River	Lake
Number	32.691 ± 1.226	28.115 ± 3.482	7.410 ± 2.727	–
Size (μm^2)	87.567 ± 3.354*	61.716 ± 2.003	70.892 ± 5.390	–

Mitochondria-rich cell numbers are expressed in 500 μm

* Significant difference from the initial value ($P < 0.05$)

explained that lamellar MR cells originated from the filaments and migrated to the lamella during freshwater adaptation. Lamellar MR cells play an important role in Na^+ uptake in freshwater (Avella et al. 1987). In addition, Boyd et al. (1980) showed that low water temperature increases MR cell number on lamellae in the gill. The temperature of Karasu River water was lower than that of Lake Van water (Table 1). MR cell functions are controlled by hormones such as cortisol, growth hormone and prolactin (McCormick 1995; Dang et al. 2000). The size, abundance and distributional changes of MR cells may be affected by these hormones in *C. tarichi*.

Previous studies indicated a positive correlation with Na^+/K^+-ATPase activity or MR cell abundance in gill and external salinity and decreases following migration into freshwater in many teleost fish species (Zydlewski and McCormick 1995; Ura et al. 1996, 1997; Khodabandeh et al. 2009). In contrast, several studies have reported intense MR cell proliferation in the lamellar epithelium and Na^+/K^+-ATPase activity in fish exposed to freshwater (Lin et al. 2003; Wang et al. 2003; Wood et al. 2007; Bystriansky and Schulte 2011). Gill and kidney Na^+/K^+-ATPase of naked carp (*Gymnocypris przewalskii*) declined when fish were transferred from freshwater to alkaline Lake

Qinghai water (Wood et al. 2007). Following freshwater exposure, plasma sodium and chloride levels and total osmolality decreased significantly in Atlantic salmon but Na^+/K^+-ATPase activity increased (Bystriansky and Schulte 2011). Similarly, to these the *C. tarichi* Na^+, K^+, ATPase in the gill MR cells stained strongly and plasma ions ($[Na^+]$, $[K^+]$ and $[Cl^-]$) decreased in river water adapted samples.

In conclusion, plasma is higher in *C. tarichi* than in the other marine teleosts. Filament MR cells were observed in both areas, but lamellar MR cells were only observed in freshwater during reproductive migration. MR cell size was significantly elevated in freshwater, in contrast to reports in the literature.

Acknowledgments I thank Dr. Ertuğrul Kankaya and Emre Erez for water and fish samples.

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