Effects of salinity and plasma prolactin on chloride cells in the gill of *Chalcalburnus chalcoides*

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Abstract

Gill chloride cells and prolactin hormone are of high importance in the adaptation of euryhaline fish. Guldenstati (*Chalcalburnus chalcoides*, 1772), an adromous fish, migrates from the Caspian Sea to rivers to have a more successful reproduction. The present study was aimed to evaluate the changes in the number and size of *C. chalcoides* gill chloride cells as well as to determine the relationship of its plasma prolactin with water salinity. Eighty-four individual *C. chalcoides* were collected from river (Lale Roud; 0.4 ppt), Lale Roud estuary (3.75 ppt), and Caspian Sea (9.71 ppt). The sampling was lasted for a-12 month period in 2014. The highest (1349 ± 152) and lowest (881 ± 37) number of gill chloride cells were observed in the animals collected from the Caspian Sea and in the river (Lale Roud), respectively. However, plasma prolactin demonstrated the highest level in *C. chalcoides* caught from the river (0.89 ± 0.02 ng ml^{-1}), but the lowest amount (0.70 ± 0.03 ng ml^{-1}) in the ones collected from the Caspian Sea.

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Overall, these results suggest that *C. chalcoides* migration across its habitats is an energy consuming ecological behavior, and the fish consumes high energy just after breeding and while returning to the Caspian Sea.

Keywords: Salinity, Chloride cells, Prolactin, *Chalcalburnus chalcoides*

Introduction

Hydromineral regulation is of utmost importance in fish. Gills and gut, and particularly their chloride cells, are the main organs for regulation of water and ions in fish (Bradshaw and McCormick 2006). The concentration of the main blood plasma ions, especially Na^+, Cl^-, Ca^{2+}, and Mg^{2+} readily reflects hydromineral regulation in fish. Plasma osmolarity in these animals living in freshwater is generally about 300 to 325 mOsmol L^{-1} and thus far above the ambient freshwater osmolarity, whereas in the seawater-dwelling fish it is about three times lower than seawater with 950 to 1050 mOsmol L^{-1} (Di Giulio and Hinton 2008). Hence, the osmotic and ion gradient across the branchial epithelia leads a

driving force for passive water and ion movements between fish body and ambient water. The outflow of ions and osmotic uptake of water in freshwater fish as well as inflow of ions and osmotic water loss in seawater fish are effectively regulated via the integumental epithelia permeability to water and ions (Marshall and Grosell 2006). Due to their key function in ion transport activity, the gill chloride cells are characterized by an extremely well-developed endoplasmic canalicular system with the basolateral membrane and containing membrane-bound, ion-translocating enzymes such as Na^+/K^+- and Ca^{2+}-ATPase which plays a crucial role in cells ion exchange, as well as different types of exchangers and postulated ion channels in the apical and basolateral membranes (Charoenphandhu, Limlomwongse and Krishnamra 2006).

Being hyperosmotic with respect to their environment, freshwater fish inevitably eliminate large volumes of diluted urine and retain salt (Bayly 1972). High urine flow rates following a high glomerular filtration rate and body osmolarity are controlled by a number of pituitary and non-pituitary hormones (such as prolactin, growth hormone, arginine vasotocin, angiotensin, and cortisol) controlling the organs involved in osmotic regulation to maintain the balance of hydro-minerals in different environments with different salinity (Haruta, Yamashita and Kawashima 1991, Mancera and McCormick 2007, Di Giulio and Hinton 2008).

Prolactin is an essential hormone to control hydro-mineral balance in freshwater fish. The main role of prolactin is epithelial permeability to ions and water, especially in gills, intestine and kidney epithelial tubes (Bonga 1997, Khalil, Hashem, Ibrahim and Mousa 2012). This hormone is of high important function in adaptation of euryhaline fish during migration from seawater to freshwater ecosystems (Manzon 2002). Guldenstati (*Chalcalburnus chalcoides*, 1772), an adromous fish, migrates to upstream and rivers to achieve more successful reproduction (Bagherian and Rahmani 2009, Coad 1996, Keivany, Nasri, Abbasi and Abdoli, 2016). *C. chalcoides*has a high food value and economical position in the provinces surrounding the southern basin of the Caspian Sea. However, based on the reported information by International Union for Conservation of Natural Resources (IUCN), this fish species has been considered as vulnerable one (Kiabi, Abdoli and Naderi 1999). In spite of its valuable ecological and high economical position in the Caspian Sea and the provinces sounding the southern part of this lake, up to now, no study has been evaluated its gill infrastructural and blood hormonal changes during migration between freshwater and the Caspian Sea water. Hence, the present study was aimed to evaluate the changes in the number and size of *C. chalcoides* gill chloride cells as well as to determine the relationship of its plasma prolactin with water salinity in different salinities.

Materials and Methods

For this study, 84 healthy individuals of *C. chalcoides* (average total length: 16.5 ± 4 cm; average weight: 45.69 ± 0.27 gr) were collected from three locations across its annual migration way (i.e., river, estuary, and Caspian Sea, with

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salinity of 0.4, 3.75, and 9.71 ppt, respectively). These locations are in the southern cost of the Caspian Sea and Lale Roud in Chamkhaleh, Guilan province (fig. 1). The sampling was lasted during a-12 month period in 2014; from the river, in May, June, July and August; from the estuary, in April and September; and from the sea, in October, November, December, January, February, and March. Some physicochemical parameters of water, including salinity, dissolved oxygen, pH, and temperature, at the sampling sites are showed in Table 1.

Figure 1.Sampling locations from the southern coast of the Caspian Sea, Chamkhale, Guilan, Iran.

Table 1. The mean value of air temperature as well as water physiochemical parameters at the sampling location in river (Lale Roud), estuary and the Caspian Sea

Month	Sampling environmental	Air temperature (°C)	Water temperature (°C)	Dissolved oxygen (mg l^{-1})	pH	Salinity (ppt)
		Mean ± SD				
April	Lale Roud Estuary	15.75 ± 0.00	13.61 ± 0.00	7.40 ± 0.00	8.10 ± 0.00	4.00
May	River	20.13 ± 0.00	19.08 ± 0.00	7.50 ± 0.00	8.30 ± 0.00	0.3
Jun		25.08 ± 0.00	24.48 ± 0.00	9.60 ± 0.00	8.40 ± 0.00	0.5
Jul		26.02 ± 0.00	25.50 ± 0.00	9.20 ± 0.00	8.30 ± 0.00	0.4
Aug		28.04 ± 0.00	27.39 ± 0.00	9.00 ± 0.00	8.20 ± 0.00	0.4
Sep	Lale Roud Estuary	26.16 ± 0.00	24.55 ± 0.00	8.10 ± 0.00	7.90 ± 0.00	3.50
Oct	Caspian Sea	22.49 ± 0.00	19.56 ± 0.00	9.00 ± 0.00	8.00 ± 0.00	9.50 ± 0.00
Nov		19.28 ± 0.00	17.84 ± 0.00	8.10 ± 0.00	8.20 ± 0.00	10 ± 0.00
Dec		14.21 ± 0.00	11.78 ± 0.00	8.40 ± 0.00	8.00 ± 0.00	9.00 ± 0.00
Jan		9.75 ± 0.00	9.00 ± 0.00	8.60 ± 0.00	8.00 ± 0.00	9.1 ± 0.00
Feb		11.65 ± 0.00	10.27 ± 0.00	8.22 ± 7.50	8.20 ± 0.00	10.50 ± 0.00
mar		12.41 ± 0.00	10.79 ± 0.00	8.20 ± 0.00	8.30 ± 0.00	10.20 ± 0.00

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Blood plasma prolactin and gill chloride cellanalysis

The collected fishes were anesthetized with clove extract (100 ppm), and their blood was obtained from the caudal vein using 5 cc syringes. The blood samples were then transferred into heparinized tubes and centrifuged for 8 minutes at 4000 g to separate plasma. The plasma prolactin hormone measured according to the protocol described by Wheeler &Hutchinson (2006). To determine the size and number of gill chloride cells, the fish gills were dissected and fixed in 10% formalin for 48 hours. After dehydration, clarification, and paraffin immersion, gill paraffin blocks were prepared, and then 5µm sections were prepared and stained with hematoxylin-eosin (H&E). The number and size of chloride cells were observed using light microscopy and photographed.

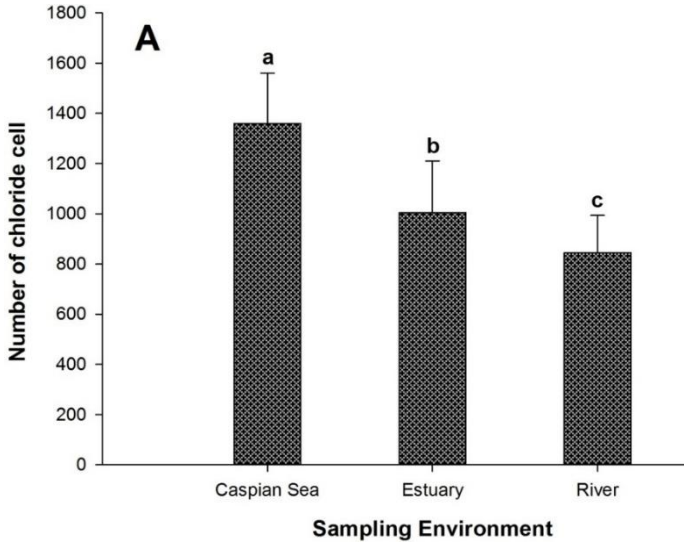
Statistical Analysis

Data statistical analyses were conducted with SPSS software (SPSS, version. 22). All results are presented as the mean ± standard deviation (S.D.). Significant differences were determined using one-way ANOVA, followed by Tukey test to compare the differences between the fish groups ($P<0.05$), and also the relationship between the number and size of chloride cells with plasma prolactin and water salinity were determined using Pearson correlation test ($P<0.01$).

Results

Size and count of chloride cells

Statistical analysis showed significant differences between the numbers of gill chloride cells in *C. chalcoides* in the three different water salinities ($P<0.05$, fig. 2A), with the highest (1349 ± 152) and lowest (881 ± 37) number in the Caspian Sea and in the river (Lale Roud), respectively. Likewise, gill histological analysis demonstrated the same pattern forchloride cell size in the sampling environments, the largest and smallest in the highest (Caspian Sea) and lowest (Lale Roud) salinities, respectively (Tukey, $P<0.05$; fig. 2B and 3).



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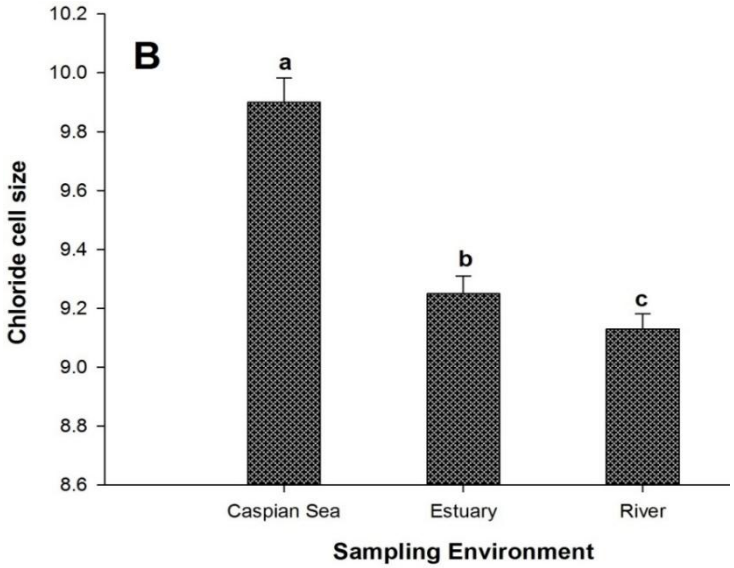

Figure 2. The average number (A) and size (B) of *C. chalcoides* gill chloride cells three different water salinities (Caspian Sea, estuary, and river). Different letters indicate statistically significant differences.

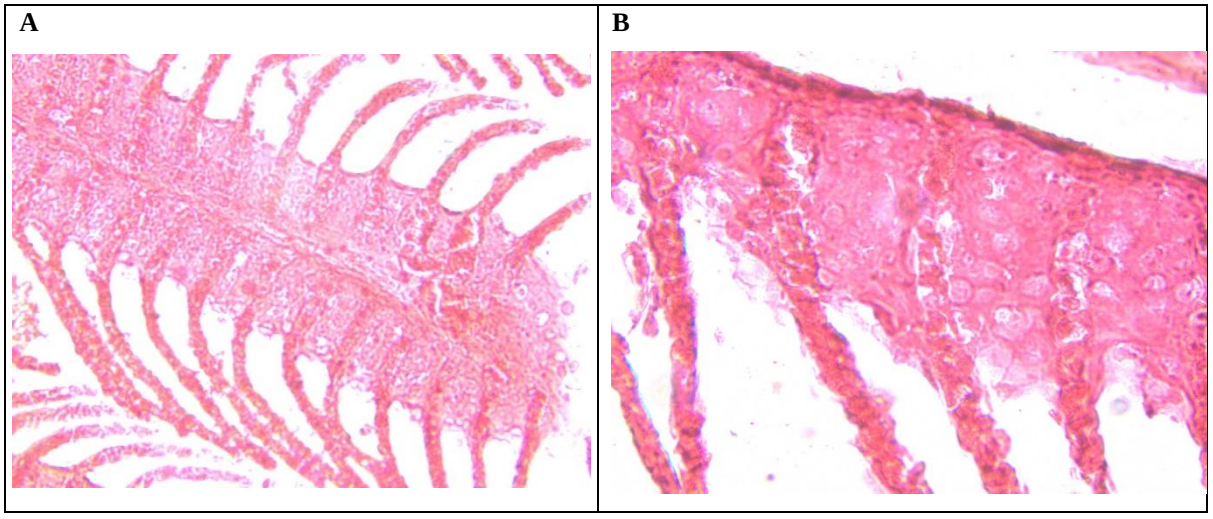

Figure 3.Histological sections of *C. chalcoides* gill caught from river (A)and (B) the Caspian Sea (x 40). H&E staining.

Plasma prolactin hormone

The measurement of plasma prolactin showed the highest level in *C. chalcoides* caught from the river (0.89 ± 0.02 ng ml^{-1}), but the lowest amount (0.70 ± 0.03 ng ml^{-1}) in the ones collected from the Caspian Sea, i.e., the amount of prolactin hormone illustrated the highest and lowest concentrations in the 0.4 and 9.71 ppt, respectively (fig. 5; Tukey, $P<0.05$).

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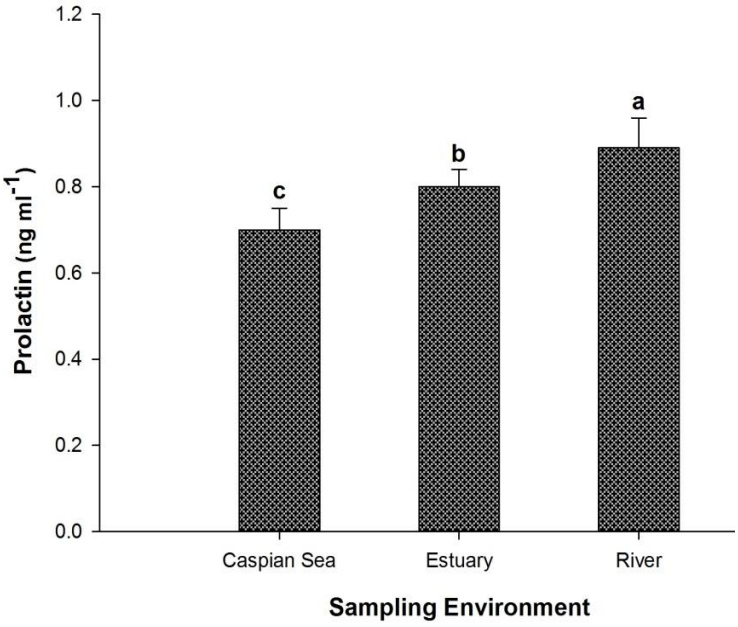

Figure 4. The mean values of plasma prolactin hormone of *C. chalcoides* in different sampling environments. Different letters indicate statistically significant difference.

Plasma prolactin hormone

Table 2 illustrates correlation of both size and count of *C. chalcoides* gill chloride cells with plasma prolactin level and water salinity while migrating between ecosystems with different salinities. Pearson correlation demonstrated a strong positive correlation between the number and size of chloride cells with water salinity, yet a significant negative correlation with plasma prolactin level. In addition, with increase of water salinity a remarkable negative correlation was observed.

Table 2. Correlation of size and count of *C. chalcoides* gill chloride cells with plasma prolactin level and water salinity (Pearson correlation)

	number of chloride cells	size of chloride cells	Prolactin
Size of chloride cell	0.90**		
Prolactin	-0.84**	-0.93**	
Salinity	0.90**	0.98**	-0.94**

** Asterisks show significant correlation and (-) shows negative correlation.

Discussion

Fish gill is considered as the main organ involved in the osmoregulation and maintaining the hydro-mineral balance in hyperosmotic and hyposmotic environments. In some fishes, gill chloride cells have been infrastructurally adapted to fish homeostasis within different

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water salinities; that is, these cells (especially in gills) vitally maintain a biologically suitable hydro-mineral balance between the external and internal body environment following alterations in their size, count, and surface area (Katoh, Hyodo and Kaneko 2003, Laiz-Carrión, Guerreiro, Fuentes, Canario, Martín Del Río and Mancera 2005). Chloride cells have some special structural characteristics such as numerous mitochondria, wide cytoplasmic, and special enzymatic systems of Na^+/K^+ ATPase (Uchida and Kaneko 1996). These unique structural and functional features allow the removal of large volume of monovalent ions against concentration gradient in salt water (Pourkhadje, Abdi, Zolgharnein, Hoseinzade Sahaf and Morovvati 2015). The present study evaluated changes in size and count of gill chloride cells as well as fluctuations in plasma prolactin hormone in *C. chalcoides*, a fish seasonally migrating between river and the Caspian Sea,while migrating between its different seasonal habitats (i.e., rive, estuary, and Caspian Sea). The results demonstrated a significant increase in the chloride cell count when the fish migrate from freshwater (river) to a higher water salinity (Caspian Sea), and also the same pattern was hold true for their size. The highest average count of gill chloride cells was observedin the fish collected from the habitat with the highest salinity (1349 ± 152), whereas it was the lowest in the freshwater habitat (881 ± 37).

To migrate from environments with low salinity or electrolyte, fish need a significant increase in the number of ion carriers in the cell membrane and thus higher enzymatic activities contributed to maintain internal water and electrolyte homeostasis. Therefore, they require increasing the chloride cells for which they are specialized (Zydlewski and McCormick 2001). Hence, the observed alteration in the gill chloride cell count and size can be an adapting physiological phenomenon needed during migration from the hyperosmotic to hyposmotic environments. These data are consistent with that observed studiesin which the migration andosmoregulation of some anadromous and catadromous fish species such as , *Oreochromis mossambicus* (Uchida, Kaneko, Miyazaki, Hasegawa and Hirano 2000), *Oncorhynchus keta* (Uchida and Kaneko 1996), *Rutilus frisii kutum* (Ataimehr, Mojazi, Mirvaghefi, Nezami and Riazi 2010), *Acipenser persicus* (Jabbarzadeh, Abtahi, Mojazi and Nazari 2000), *Cyprinodon variegatus* (Karnaky, Ernst and Philpott 1976), *Epinephelus coioides* (Pourkhadje et al. 2015), *Chalcalburnus tarichi* (Oğuz 2013), *Barbus sharpeyi* (Koohkan 2017) and *Cyprinus carpio* (Azizi, Kochanian, Peyghan, Khansari and Bastami 2011)were evaluated.

Plasma prolactin level significantly elevated following reduction in water salinity from the Caspian Sea to Lale Roud. Likewise, Pearson correlation test showed a strong inverse correlation between the amount of prolactin hormone with the size and number of gill chloride cells between the fish caught from different salinities; i.e., the higher water salinity and the more size and count of gill chloride cells, the lower amount of plasma prolactin hormone was observed. These results are in

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accord withstudy conducted by Prunet et al (1985) in which *Salmo gairdneri* was transferred from seawater to freshwater, and a significant elevationwas observed in plasma prolactin. Further, daily injections of ovine prolactin into *Oreochromis mossambicus* at a dose of 10 μg g^{-1} caused a significant reduction in the size of chloride cells (Herndon, McCormick and Bern 1991). During the adaptation of euryhaline fish to different salinities, particularly when migrating from saltwater to freshwater, the number and size of chloride cells obviously decrease and, even in some species, these cells are completely destroyed by plasma prolactin hormone; that is, the main role of prolactin in all euryhaline fish is the controlling of water and sodium transfer across the gill epithelium, especially during the migration from sea water to fresh water (Prunet, Boeuf and Houdebine 1985).

Conclusions

The present results, therefore, clearly suggested that *C. chalcoides* migration across its habitats is an energy consuming ecological behavior, and this fish consumes high energy just after breeding and while returning to the Caspian Sea.In addition, water salinity and prolactin hormone can play essential functions in the morphology and distribution of chloride cells.

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تأثیر شوری آب و پرولاکتین پلاسما بر سلول‌های کلراید آبشش ماهی شاه کولی (*Chalcalburnus chalcoides*)

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چکیده

سلول‌های کلراید آبشش و هورمون پرولاکتین اهمیت بسزایی در سازگار شدن ماهیان یوری هالین دارند. ماهی شاه کولی به‌عنوان یک ماهی آنادروموس، از دریای خزر به‌منظور داشتن تکثیر موفق‌تر به رودخانه‌ها مهاجرت می‌کند. مطالعه حاضر جهت بررسی تغییر در تعداد و اندازه سلول‌های کلراید آبشش و همچنین تعیین ارتباط میان پرولاکتین پلاسمای خون ماهی شاه کولی با شوری صورت پذیرفت. در این مطالعه ۸۴ عدد ماهی از رودخانه لله رود، با شوری(ppt ۰/۴)، مصب رودخانه لله رود با شوری (ppt ۳/۷۵) و دریای خزر با شوری (ppt ۹/۷۱) جمع‌آوری گشت. نمونه‌برداری در یک دوره ۱۲ ماهه صورت پذیرفت. بالاترین (۱۵۲ ± ۱۳۴۹) و کمترین (۳۷ ± ۸۸۱) تعداد سلول‌های کلراید به ترتیب در ماهیانی که از دریای خزر و رودخانه جمع‌آوری شده بودند، شمارش گشت. اما پرولاکتین پلاسما بیشترین (۰/۰۲ ± ۰/۸۹ نانوگرم بر میلی‌لیتر) و کمترین (۰/۰۳ ± ۰/۷۰ نانوگرم بر میلی‌لیتر) میزان را در ماهیانی نشان داد که به ترتیب از رودخانه و دریای خزر صید شده بودند. بطور کلی، نتایج بیانگر این است که مهاجرت ماهی شاه کولی در بین زیستگاه‌های آن، یک رفتار اکولوژیک با مصرف بالای انرژی همراه است و این ماهی دقیقاً بعد از تخم‌ریزی و هنگام برگشتن به دریای خزر میزان بالایی انرژی مصرف می‌کند.

کلمات کلیدی: شوری، سلول‌های کلراید، پرولاکتین، ماهی شاه کولی.

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