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Osmoregulation Ability in Different Sizes of Caspian Trout (*Salmo trutta caspius*)Parrs, with the Same Age, Following Direct Transfer from Fresh Water to the Caspian Sea Water

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ABSTRACT

In order to determine the effects of fish size and weight on its salinity tolerance, the chloride cells (CCs) immunolocalization changes were examined in Caspian salmon (*Salmo trutta caspius*) parrs, with the same age (about 2 years old) but different types (type A: 4.88 g, 8.36 cm; type B: 14 g, 11.84 cm; type C: 24.05 g, 14.08 cm). Fish survival rate, blood osmolality, gills CCs histological and immunohistochemical changes were investigated following their transfer from freshwater (FW) to the Caspian Sea water (CSW). The survival rate increased in larger sizes and blood osmolality showed a tendency to increase in parallel with salinity. After 10 days in CSW, some abnormalities were observed in gill structure such as: lamellae cohesion, lamellae rupture and separated lamella from filament epithelium that were shown in all types. These abnormalities in type B were less than the other types. Gill CCs were observed on the gill filament and lamellae. In direct transfer of Salmon parrs to the CSW, changes in number, sectional area, and the surface occupied by CCs in the gill tissue were observed in all the three types. In the type B, the number of CCs did not change, however, in the type C, they decreased, while in the type A, there was significant increase. But in the CSW, the occupied surface by gill CCs in the type C, was reduced significantly compared to other types. According to the present results, among the Salmon parrs with the same age, the fish with type A lacked osmoregulation, while the type B had better compatibility with the CSW. However, after reaching the size of the type C and considering osmultification, it is probable that the type B fish would become compatible with the fresh water environment and they will not have osmoregulation ability in saline water for the long term.

Keywords: Chloride cell, Immunolocalization, Salinity tolerance, *Salmo trutta caspius,*.

INTRODUCTION

When fish are exposed to different salinity, they begin to adapt through osmoregulation, which results in the maintenance of their blood osmolality within a narrow range of ~ 285-360 mosm kg^{-1}. Previous studies have demonstrated that the osmoregulation ability is influenced by the size of the fish and heavier sizes cause salinity tolerance development and osmoregulatory ability (McCormick and Saunders, 1987; Hoar, 1988; Robert, 2000). Many studies showed positive correlation between osmoregulation ability, sea water tolerance, and migration ability with body size (McCormick and Saunders, 1987; Hoar, 1988; Halvorsen *et al.*, 1993; Arnesen *et al.*, 1998; Robert, 2000). Thus, fish size is an important factor in salinity tolerance and its weight and length have much more significance than age in salinity tolerance and osmoregulation mechanism (Conte *et al.*, 1996). Generally, most anadromous salmonids such as Sockeye (*Oncorhynchus nerka*), Coho (*O. kisutch*), Chinook (*O. tshawytscha*), Masu (*O. masow*),

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Steelhead trout (*O. mikiss*) and Atlantic salmon (*Salmo salar*) migrate to the sea water (SW) after parr-smolt transformation, which involves morphological and physiological changes for pre-adaptation to SW entry in these sizes (Hoar, 1988; Boeuf, 1993). Chloride cells (CCs) secrete ions in sea water-adapted fish and in FW-adapted fish, absorb ions and maintain the acid-base balance (Wood and Marshall, 1994; Evans *et al*., 2005). CCs have been reported in the gill filaments and gill lamellae of several FW and SW-acclimated teleost species (Uchida *et al*., 1996, Shikano and Fujio, 1998, 1999). Shikano and Fujio (1998) demonstrated functional difference between two CCs types by using immunohistochemical localization of Na^+, K^+-ATPase pump. Na^+, K^+-ATPase is a key enzyme of ion transport located in the basolateral membrane of CC.

This enzyme produces the ionic and electronic slope in the salt secretion in the hyperosmotic environment and the ion absorption in hypoosmotic environment. This pump runs the sodium ions away from the inside to the outside of the cell membrane (inside the blood) and, vice a versa, pumps the potassium ions from the blood into the cell. This pump usually exists in all cells, but the density of that in cells involved in the osmoregulation, i.e. Ionocytes cells, is very much (Uchida *et al*., 1996; Shikano and Fujio, 1998 and 1999; Eldon, 2003; Evans *et al*., 2005)

Salmo trutta caspius, is an anadromus fish that has lived in western and southern parts of the Caspian Sea. The life cycle of Salmon fishes are as the follow: (Laird and Needham,
1988).

Egg → Alevin → Fry → Parr → Smolt → Adult → Spawner

Its breeding has occurred in FW but spending most of its life (about 3-5 years) in the Caspian Sea. After maturity, they migrate to the river for spawning. Since the last two decades, several millions of *Salmo trutta caspius* (same age but different size) have been annually produced and released into the Caspian Sea by the Iranian hatchery centers (Kiabi *et al*., 1999; Niksirat and Abdoli, 2009). Although some studies investigated osmoregulation in *Salmo trutta caspius* but they did not pay attention to the effect of size and weight in osmoregulation ability in these fishes with the same age. High economic value, and being at the risk of extinction, has made these fishes as good samples for research with regard to many problems in releasing and high maintenance cost of these Salmon parrs, before releasing. Therefore, the purpose of this study was to investigate the effect of fish sizes on osmoregulation ability. Also, salinity stress was investigated in different types of fishes with the same age in order to evaluate their abilities to tolerate salinity stress and cope with new environment.

MATERIALS AND METHODS

Sampling

This experiment was performed in Shahid-Bahonar Propagation and Culture Center in Kelardasht, Iran. 180 individuals of parr fishes about 2 years old i.e. fishes that had spent 2 summers but not 2 years after hatching, with different types were selected (Table 1).

Blood Osmolality

Following the end of culture period, the remaining alive fishes were anesthetize by the Clove flour (concentration 350 ppm) and the blood samples were taken from about eight fishes in every replicate (three replicate for each type) by cutting the tail stem with the help of the non-Heparinized hairy tube. Then, for blood coagulation and serum production, the test tubes were kept in refrigerator ($_{+}4^{o}C$) for 4 hours. After that, the tubes were centrifuged at the rate of 1000×g for five minutes, and the resulting serums were kept in a container having liquid nitrogen, until the time of experimentation (Krayushkkina *et*

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Table 1. Different type of *S. trutta caspius* parrs used in the study.

	Type A	Type B	Type C
Weight (g)	4.87±0.92	14±0.51	24.05±0.63
Length (cm)	8.36±0.66	11.84±0.47	14.08±0.57

Values are presented as the mean ± S.D for each weight and length.

al., 1999). The osmolality content of the blood serum was measured by osmometer (OsmoTech, England), in mOsm kg^{-1}.

Histological Study

For histological and immunohystochemical studies, 36 fish gills (12 for each type and 6 for each salinity) were immersed into Bouin's fixative for 48 hrs, washed and dehydrated in an ascending of ethanol and then embedded in paraplast (Sigma, 060K19271). Following embedment in paraplast, transversal and longitudinal sections of 4 µm were cut on a Leitz Wetzlar microtome (Wetzlar, Germany) and collected on glass slide and stained with Haematoxylin-Eosin (Martoja and Martoja-Pierson, 1967; Khodabandeh *et al*., 2005, 2009).

Immunohystochemical Study

For immunohystochemical studies, the sections were collected on poly-L-lysine glass slides. Then, they were dehydrated. The sections were washed in PBS (phosphate buffered saline) for 10 min and incubated in A solution (250cc PBS+2.18 g NaCl+0.01 mM Tween 20 (pH= 7.3)) for 10 minutes and B solution (50% PBS+50% Skimmed milk (Regiler) for 20 minutes. Then, the sections were washed in PBS. Immunolocalization of the Na^+, K^+-ATPase was performed through immunofluorescence light microscopy using a mouse monoclonal antibody IgGα5 (Hybridoma Bank, University of Iowa, USA) raised against the α-subunit of the chicken Na^+, K^+-ATPase (Takeyasu *et al*., 1988). This antibody, diluted in PBS to 20 µg mL^{-1}, was placed on the sections and incubated for 3hrs at room temperature in a moist chamber. The slides were washed in PBS and then incubated with the secondary antibody anti-mouse IgG produced in goat, conjugated with the fluorochrome fluorescent (FITC: fluoresce in isothiocyanate conjugated; Merck Germany) diluted 1:100 in PBS for 2hrs under dark conditions (Khodabandeh, 2007; Khodabandeh *et al.*, 2009). For obtaining the images of the tissues, we used fluorescent microscope (Nikon TE2000S) with the appropriate filter set (filters of 450-490). CCs number calculating, their sectional area and surface occupied measuring were done by using of at least 10 immunohistochemistry photos, taken with magnification of 40X from each type and each salinity. These images were analyzed using Image Tool (2, 0) software. With multiplication of the number of cell in the surface of one mm^2 by the average of space in the same surface and multiply by 1000, the percentage of the occupied surface by the CCs was measured.

Statistics

By using SPSS software version 11.5, the data were analyzed and the Excel 2003 was used for drawing of the diagrams. First, the normal distribution of the data was tested with the test of Shapiro-Wilk and the homogeneity of the variances was examined with the test of Leven. The analysis of the data was investigated by One Way ANOVA (for comparison of the average of the treatments) and in order to have comparison among the treatments, for homogenous variance, the Duncan test, and for the heterogeneous variances, the DanetT3 test, was used. The comparison of each treatment was done before and after transferring to the CSW with the use of independent *T*-test (Independent samples T-Test).

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RESULTS

Blood Osmolality

Blood osmolality was measured after 10 days and showed a tendency to increase in parallel with salinity (Table 2). Survival rate of fishes was recorded, too, in FW and following their transfer to CSW in each type. The survival rate in A, B and C type was 75, 92, and 100%, respectively.

Histological Analysis

The four pairs of *S. trutta caspius* holobranches correspond to the general organizational plan of the gills of teleosts (Figure 1-A). Each holobranch consists of two hemibranches carrying numerous branchial filaments. Lamellae, parallel to each other, are perpendicular to the filaments (Figures 1-B and 1-C). A cross section of a filament reveals the location of the cartilaginous branchial spine as well as the blood vessels. The afferent and efferent arteries are located near the trailing and leading edges of the filament, respectively. The central venous sinus occupies a truly central position. At the lamellae level, the epithelium is formed by two cellular layers of pavement cells. In the center of each lamella, numerous vascular spaces are located, which are maintained wide open by pillar cells (Figure 1-D). The filaments are covered by an epithelium which comprises several well differentiated pavement and CCs (Figures 1-E and 1-F).

Structural Damages

All of the filament and lamellae in type A of the fish's gill in FW were normal (Figure 2-A), but, following their release in CSW, some abnormalities in gill structure were observed such as: lamellae rupture (Figure 2-B), lamellae cohesion (Figure 2-C), and separated lamellae from filament epithelium (Figure 2-D). In type B, all of the filament and lamellae were normal (Figure 2-E), but lamellae rupture (Figure 2-G) and lamellae cohesion (Figure 2-F) appeared after fish's transfer to CSW, though they were less than in the other types. In the case of type C in FW, all of the filament and lamellae were normal, similar to the other types (Figure 2-H); however, after their introduction to CSW: lamellae rupture (Figure 2-I), separated lamellae from filament epithelium (Figure 2-J), and lamellae cohesion (Figure 2-K) were observed, similar to type A.

Immunolocalization of Na^+, K^+-ATPase

Immunofluorescence microscopy was used for the localization of Na^+, K^+-ATPase as the fixation and staining process provides good antigenicity. The negative control section of the sample from FW (type A), deprived of primary antibodies, showed no specific immunofluorescence (not shown). Auto-fluorescence was observed in blood cells (Figure 3-A). In cross section of the filament, the CCs were seen near the central sinus venous. In both FW and CSW samples, fluorescent cells were

Table 2. Blood osmolality (mOsm kg^{-1}) of different types of *S. trutta caspius* parrs in freshwater (FW) and Caspian Sea water (CSW.)

	A type	B type	C type
FW	264±3.46a	258.5±20.7[a]	276±1.2A
CSW	497.66±8.5b	368.33±24[b]	373±13B

[a, b] Values are presented as the Mean±S.E for each type between FW and CSW. Different letters of each column indicate significant difference (P< 0.05).

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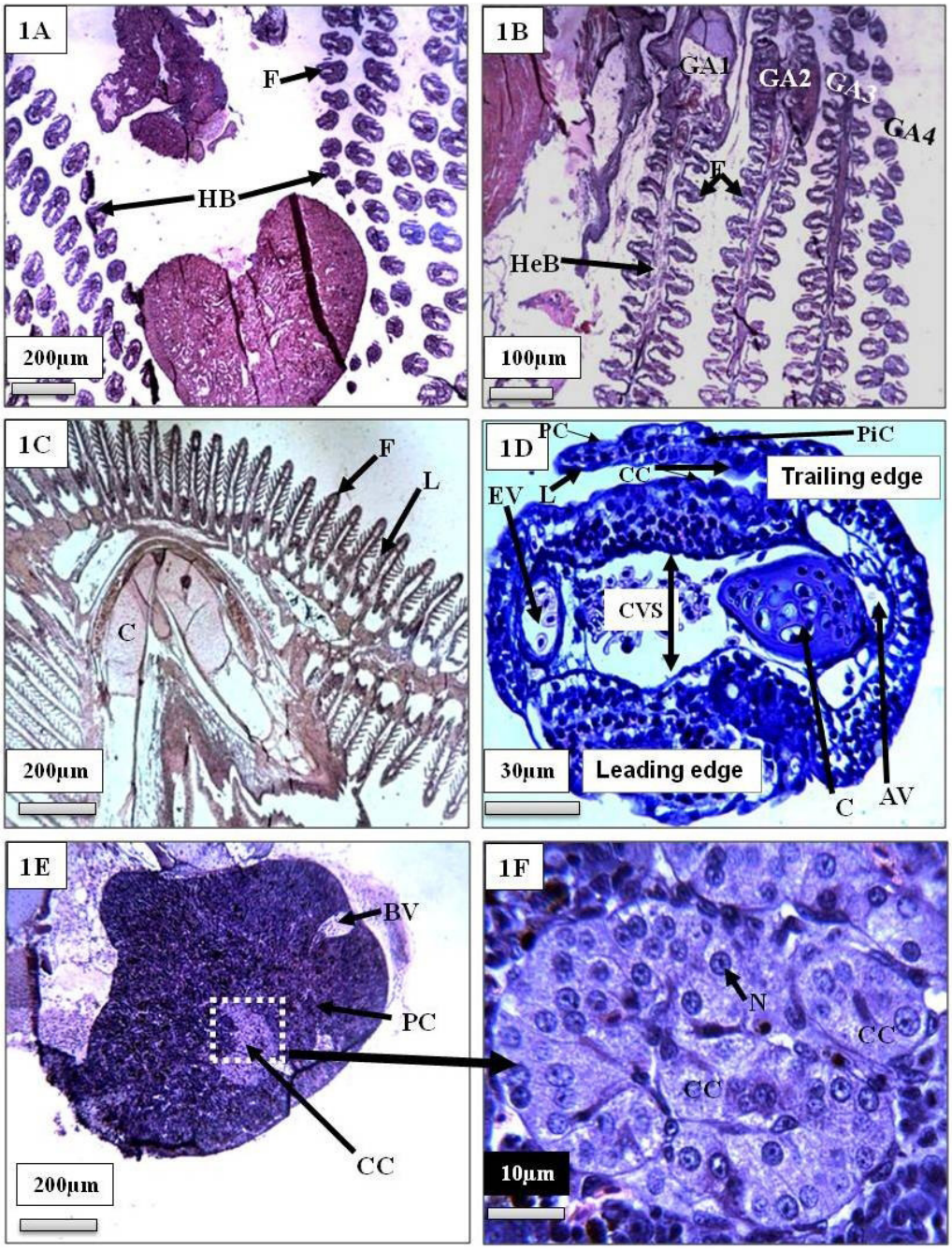

Figure 1. Hematoxylin-Eosin staining of cross and sagittal sections of parr fish's gill**.** (1A): The four pairs of *S. trutta caspius* holobranches correspond to the general organizational plan of the gills of teleosts. (1B): Each holobranch consists of two hemibranches and each hemibranch consist of two rows of filament. (1C): Sagittal section of the gill, numerous branchial filaments exist on gill arc and lamellae parallel to each other are located perpendicularly on the filaments. (1D): Crass section of gill filament. (1E, 1F): Pavement cells and CCs in gill epithelium. Abbreviations: AV: Afferent Vessel, BV: Blood Vessel, C: Cartilage, CC: Chloride Cell, CVS: Central Sinus Venous, EV: Efferent Vessel, F: Filament, GA: Gill Arch, HB: Holobranch, HeB: Hemibranch, L: Lamellae, MC: Mucus Cell, N: Nucleus, PV: Pavement Cell, PiC: Pillar Cell.

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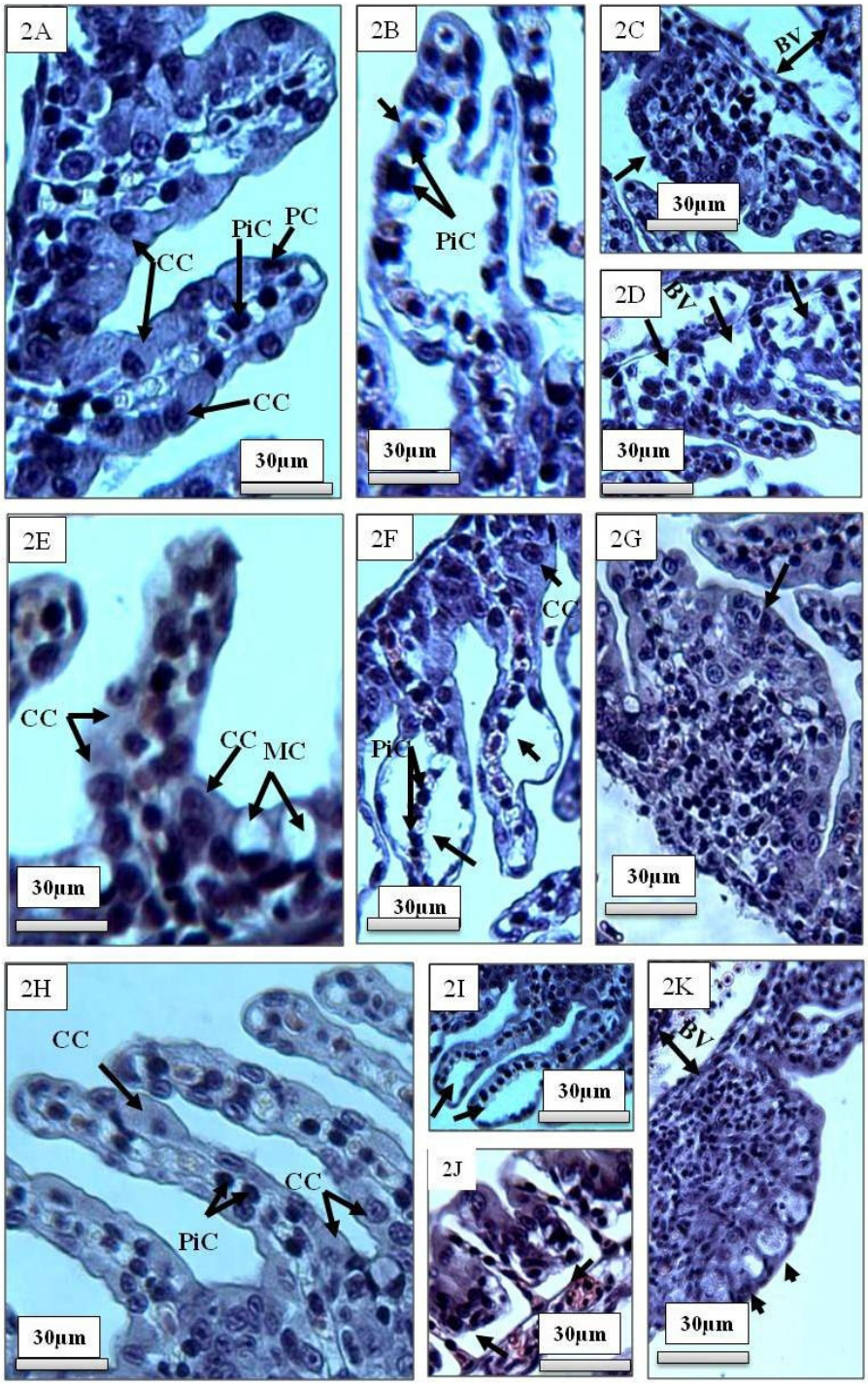

Figure 2. Hematoxylin-Eosin staining of sagittal sections of parr fish's gill in FW and CSW. (2A): Sagittal section of gill filament in type A of parr fishes in FW. (2B): Lamellae rupture, (2C): Lamellae cohesion and (2D): Separated lamellae from filament epithelium after transferred to CSW. (2E): Sagittal section of gill filament in type B of parr fishes in FW. (2F): Lamellae rupture and (2G): Lamellae cohesion after transferred to CSW. (2H): Sagittal section of gill filament in type C of parr fishes in FW. (2I): Lamellae rupture, (2J): Separated lamella from filament epithelium and (2K): Lamellae cohesion after transferred to CSW. Abbreviations: BV: Blood Vessel, CC: Chloride Cell, L: Lamellae, MC: Mucus Cell, PV: Pavement Cell, PiC: Pillar Cell, RBC: Red Blood Cell.

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observed on the epithelia of the filaments and on the lamellae (Figure 3-B).

Number of CCs (cell mm^{-2}), occupied area (%), and sectional area (μm^2) of CCs in gill epithelium was different in each type and each salinity level (Figures 4).

In FW, a lot of CCs were observed in lamellae epithelium of parr fishes (Figures 3-C, 3-E, and 3-G). In type A, the total number of CCs increased on lamellar in CSW samples, but type of this cells decreased. There was no significant difference in occupied area of CCs in gill epithelia in FW and CSW (Figures 3D; 4a; 4b, and 4d).

In type B, CCs number, type, and occupied area decreased on epithelia of lamellae and the number and occupied area of CCs were increased on filament epithelium. There was no significant difference in type of CCs in FW and CSW (Figures 3F; 4a; 4b, and 4d).

In type C, the total number of CCs and their occupied area were decreased when parr fishes were transferred to CSW. These changes were observed in lamellar epithelium and were not significantly differ in epithelia of filament in FW and CSW (Figures 3F; 4a; 4c and 4e).

In FW, there were not different in type and total numbers of CCs of 3 types of parr fish's gill, but after introduced it to CSW, the number of CCs in type C of parr fishes significantly decreased than other types (Figures 4b; 4d, and 4f).

DISCUSSION

Blood Osmolality

Changes in plasma osmolality were observed after 10 days of exposure to altered external salinity. This parameter increased significantly from fish adapted to FW to fish adapted to CSW. Previous studies demonstrated that, after an abrupt change in salinity, an adaptive period involving changes in osmotic variables is expected (review in Laiz-Carrion *et al.*., 2005). In our experiments, transfer from FW to CSW led to changes in plasma osmolality that were significantly different from FW. Almost instant changes in plasma osmolality and/or electrolyte concentration after salinity transfer have also been described by Marshall *et al.* (1999) in *Fundulus heteroclitus*, by Kelly and Woo (1999), in *Sparus sarba,* and by Lin *et al*. (2004a) in *Orechromis mossambicus.* According to this study, there was a direct relationship between survival rate and fish size and weight. There was much mortality in type A of parr fishes, whereas there was no mortality in type C during transfer from FW to CSW. Robert (2000) suggested that fish size was an important factor to salinity tolerance and there was no relationship between salinity tolerance and fish age. Other studies have indicated that pre-adaptation of salmon fish by low salinities can result in increasing salinity tolerance before osmoltification (Salman and Eday, 1987) and direct transferring from FW to hypersaline conditions cause severe dehydration, which might impair the function of Na^+, K^+-ATPase activity and this impairment may cause mortality (Guner *et al*., 2005), similar to the observations of the present study.

Immunolocalization of Na^+, K^+-ATPase and CCs Distribution

Following the transfer of Parrs to CSW, some abnormalities were observed in gill structure such as: lamellae rupture, lamellae cohesion and separated lamellae from filament epithelium. These were significantly less in type B than in the other types. Immunohistochemical localization of Na^+, K^+-ATPase is recognized as a useful marker of CCs in different tissues of fish (review in Khodabandeh, 2007, and Khodabandeh *et al*., 2009). We observed simply recognizable immunofluoresce cells

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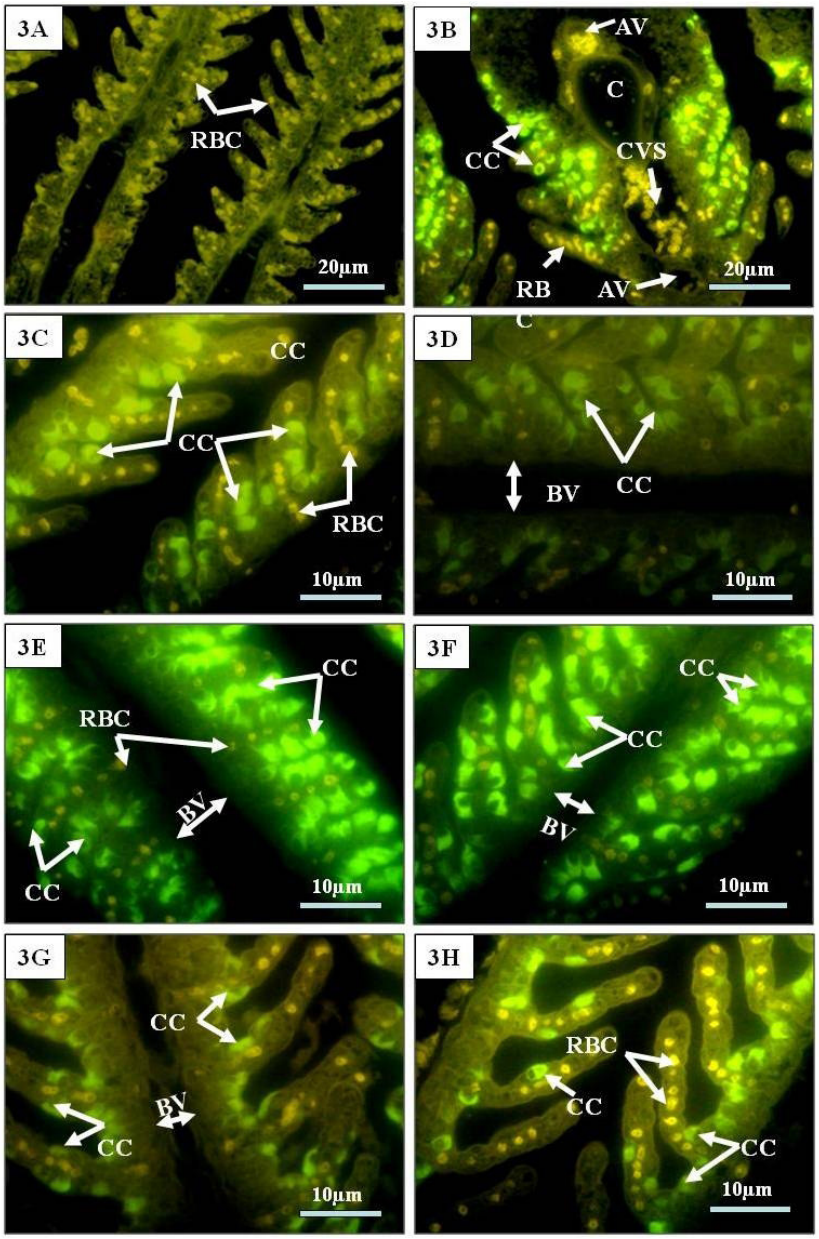

Figure 3. Immunohistochemical staining of cross and sagittal sections of parr fish's gill in FW and CSW. (3A): The negative control section of the sample from FW (type A) deprived of primary antibodies, showed no specific immunofluorescence. Auto-fluorescence was observed in blood cells. (3B): Cross section of filament. CCs were near the central sinus venous. (3C): Type A of parr fishes, and CCs were showed in both filament and lamellae but (3D): CCs acclimated in lamellae after transferred to CSW. (3E): Type B of parr fishes, and CCs were showed in both filament and lamellae but (3F): CCs acclimated in filament after transferred to CSW. (3G): Type C of parr fishes, and CCs were showed in both filament and lamellae but (3H): CCs acclimated in filament and lamellar CCs decreased after transferred to CSW. Abbreviations: AV: Afferent Vessel, BV: Blood Vessel, C: Cartilage, CC: Chloride Cell, CVS: Central Sinus Venous, EV: Efferent Vessel, RBG: Red Blood Cell.

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on the filaments and lamellae of all samples. Previous studies demonstrated that these immunoreactive cells corresponded to CCs (Witters *et al*., 1996; Ura *et al*., 1997; Khodabandeh *et al*., 2009). Uchida *et al*. (1996) also reported that immunoreactivity of Na^+, K^+-ATPase α-subunit in the CCs and gill Na^+, K^+-ATPase activity increased during SW adaptation in chum salmon (*O. keta*). Therefore, precise localization of Na^+, K^+-ATPase is important with respect to further functional identification of ion transport of the CCs during SW or FW adaptation of euryhaline teleosts. In our study, fluorescent CCs observed on the filament and lamellae were the same as reported in previous studies (Uchida *et al*., 1996; Witters *et al*., 1996). We observed that the CCs distribution was altered by external salinity, in contrast with other species such as chum salmon, *O. keta* (Uchida *et al*., 1996), *Lateolabrax japonicus* (Hirai *et al*., 1999), *Chanos chanos* (Lin *et al*., 2003). Many investigators have suggested a role for the lamellar CCs in ion uptake in hypo-osmotic environments on the basis of observations of cell degeneration following transfer from FW to SW and hypertrophication after transfer to deionized water (Shikano and Fujio, 1998).

Significant changes were observed in the number, type, and shape of CCs. In type A of parr fishes, the number of CCs was significantly increased at salinity extremes, but type of these cells decreased; whereas fish acclimated to FW had fewer and larger cells. That occurred in lamellar epithelium of gill. CCs are effective in secreting ions in hypertonic sea water as well as taking up ions in hypotonic FW (Marshall, 2002). Thus, the observed increases in the number of lamellae CCs in CSW may reflect increase in Na^+, K^+-ATPase content and cause increase in blood osmolality and osmoregulation mechanism impairment.

In type B, the number of CCs on filament increased following transfer to CSW, while lamellar CCs decreased markedly. Thus, since increased number of filament CCs may reflect the increase in Na^+, K^+-ATPase content, the filament is probably a significant site for salt secretion in CSW. In contrast with filament CCs, lamellar CCs gradually decreased after transferring to CSW, similar to *O. keta,* and CCs in the lamellar epithelium may have significant roles in the hypoosmotic environments (Uchida *et al.*, 1996). Shikano and Fujio (1998) reported that lamellar CCs degenerated when chum salmon was transferred from FW to brackish water (BW) and reappeared when they were reintroduced to FW. In another study on *O. keta*, they reported an increase in the number of lamellar CCs and a parallel decrease of CCs in the filament during the return of this species to FW for reproduction, which induced a need for hyper-regulation (Uchida *et al.*, 1997). Reciprocally, Ura *et al*. (1997) suggested a decrease or even disappearance of CCs on the lamellae during osmoltification of salmonids. In another study, the CCs of SW-adapted fish showed approximately 2-fold increase in size ($P< 0.05$). Sectional areas of the CCs were found to be the lowest for FW fish (Guner *et al.*, 2005). Cioni *et al.* (1991) observed no increase in the numbers of CCs in SW-adapted *Oreochromis niloticus* and *Oreochromis mossambicus*.

In type C, the number of CCs on the filament did not change following transfer to CSW, while lamellar CCs decreased markedly. According to Franklin (1990), Sockeye salmon (*O. nerka*) with low number of CCs failed to maintain ion balance when transferred to SW, whereas fish with a high density of CCs adapted well to SW. As in tilapia (*O. niloticus*) branchial CCs number decreased after SW exposure and a gradual increase was observed in CCs size. Decreased number of CCs in type C of *S. trutta caspius* fry transferred from FW to CSW caused decrease in the number of lamellar cells, but there were no differences in the size of CCs in the two environments.

In comparison among the three types of parr fishes, differences were observed in the size and total number of CCs in FW, but, after introduction to CSW, the number of CCs in

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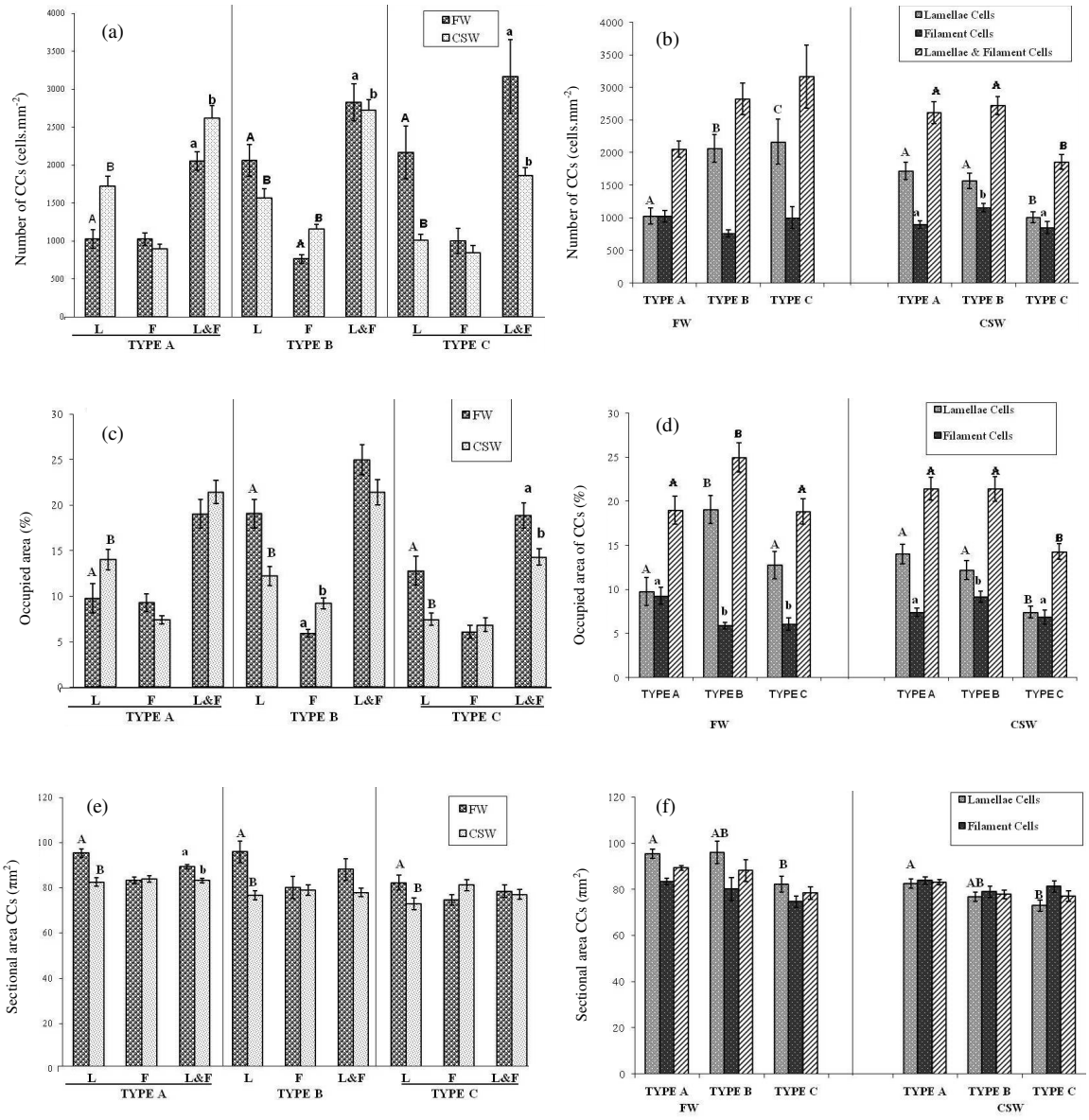

Figure 4. Gill chloride cell (CCs) changes in Caspian Salmon Parrs, following their transfer from fresh water to the Caspian sea water. (a,b) Number (cell/mm^2) (c,d) Occupied area (%), and (e,f) Sectional area (µm^2). L: Lamellae, F: Filament, L&F: Lamellae and Filament, FW: Freshwater, CSW: caspian sea water; Different letters indicate significant differences ($p<0.05$).

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type C of parr fishes significantly decreased compared to the other types. This decrease in the numbers of lamellar CCs after introduction to CSW can be due to high adaptability of this type to FW. However, the transfer of some FW species to SW (Lee *et al.*, 1996) was followed by a decrease in CCs number, but always with a concomitant loss of osmoregulatory ability. The occurrence of CCs only on gill filaments has been reported in pufferfish, *Tetraodon nigroviridis*, as well as in tilapia, *O. mossambicus*, after acclimatization to FW, BW and SW (Uchida *et al.*, 2000; Lin *et al.*, 2004b). But, in type A and B, number of CCs increased. In type A, this phenomenon occurred in lamellae and was due to increase in osmolality resulting from impaired osmoregulation mechanism, while in type B, it occurred in filament. It consider that type B parrs, prepares to challenge with salinity by developing in its osmoltification process.

In conclusion, in this research, physiologic responses of Caspian salmon (*Salmo trutta caspius*) parrs, to the increase of environmental salinity were studied in three different types. The investigated responses included survival rate, changes of blood osmolality, and gill tissue along with variations in gill CCs indicate the operation of two body systems facing stress salinity. Therefore, with regard to the response of each type, the capability and the osmoregulation ability of that type is identified. As a result, it can be said that high mortality along with osmolality increase, tissue damages, inconsistency and disorder in reproduction of gill CCs in the type A indicates that this type had not reached the osmolality level yet, and was not able to cope with salinity stress of the new environment.Thus, it was forced to accept many changes, but, the reduction of mortality in the heavier types could be attributed to their capability and ability to tolerate salinity. Also, it can be stated that the type has much more importance than age in osmoregulation. According to the author's view, this can lead to more mortality in these Salmon parrs in long term. Probably, this is due to being exposed to fresh water after spending their osmoltification stage. Therefore, according to the present study and considering all that has been said, in terms of osmoregulation, the most appropriate type for releasing in the Caspian Sea is the type B.

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بررسی توانایی تنظیم اسمزی سایزهای مختلف بچه ماهیان دو تابستانه آزاد خزر هم‌سن، در انتقال مستقیم از آب شیرین به آب خزر

ح. رجبی و ص. خدابنده

چکیده

به منظور بررسی اثر اندازه و وزن ماهی در میزان تحمل شوری، تغییرات سلولهای کلراید در ماهی سالمون دو تابستانه هم سن (حدود دو سال) در گروه های مختلف {(نوع A: ۴.۸۸ گرم، ۸.۳۶ سانتی متر)، (نوع B: ۱۴ گرم، ۱۱.۸۴ سانتی متر)، (نوع C: ۲۴.۰۵ گرم، ۱۴.۰۸ سانتی متر)} آزموده شد. میزان بقا و اسمولالیته خون و تغییرات بافت شناسی و ایمونوهیستوشیمی سلولهای کلراید آبششی در طی انتقال آنها از آب شیرین به آب خزر مورد بررسی قرارگرفت. با افزایش اندازه، میزان بقا افزایش یافت و اسمولالیته نیز به موازات افزایش شوری افزایش یافت. پس از ۱۰ روز قرار گرفتن در آب خزر، ضایعاتی در ساختار آبشش همچون: چسبندگی در لا ملاها، پارگی در لاملا و جدا شدن لاملا از فیلامنت در همه گروهها مشاهده گردید. این ضایعات در نوع Bکمترین مقدار را داشت. سلولهای کلراید آبششی در بخش لاملا و فیلامنت آبشش مشاهده شدند. در انتقال مستقیم ماهیان پار سالمون به آب خزر، تغییراتی در تعداد، مساحت و سطح اشغالی توسط سلولهای کلراید در بافت آبششی در هر سه نوع گروه مشاهده شد. اما در آب خزر سطح اشغال سلولهای کلراید در نوع C به طور معنی داری در مقایسه با انواع دیگر کاهش یافت. با توجه به نتایج حاضر، درماهیان پار سالمون هم سن، ماهی نوع A قادر به تنظیم اسمزی نبوده اما ماهی نوع B دارای سازش بهتری با آب خزر بود و پس از رسیدن به اندازه ای مشابه با نوع C به دلیل گذراندن مرحله اسمولتیفیکاسیون، به آب شیرین سازگار بوده و توانایی تنظیم اسمزی در آب شور را در دراز مدت نخواهد داشت.

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