Osmoregulatory capabilities of Zander (*Sander lucioperca*) fingerlings in different salinities of the Caspian Sea

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Abstract

Osmoregulation capabilities of two size groups (1 and 2g) of zander , *Sander lucioperca*, fingerlings were investigated survival rate, plasma osmolarity, sodium (Na^+), chloride (Cl^-) ion concentrations and cortisol level within 0, 6, 24, 72 and 240 h after abrupt transfer from freshwater to 7 and 12‰ salinity. Also, some hematological parameters were measured at 240h. The plasma osmolarity and ion concentrations (Na^+, Cl^-) increased immediately after the transference to 7 and 12‰ salinity, reaching maximum at 72h in 1g and at 24 h in 2g fingerlings, and significantly decreased at 240 h, while the freshwater control group maintained almost constant during 10 days which showed lower than the salinity groups. The pattern of cortisol level changes was similar in two size groups after exposure to different salinities and it reflected stress of handling. There were no significant differences between hematological values of two salinity treatment groups than the freshwater control at 240h. The result showed, not only 2g zander fingerlings acted better than 1g in faced with salinity but also the fingerlings weighing 1g could survived and tolerate Caspian Sea water salinity up to 12‰. It is suggested that the release of zander weighing from 1g would help to restocking management of this species in the southern Caspian Sea.

Keywords: *Sander lucioperca*, size, salinity, Caspian Sea, osmoregulation, cortisol

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Introduction

Sander lucioperca (Percidae, Teleostei) is one of the most important semi-anadromous (Craig, 2000), valuable and economic fish in the Southern Caspian Sea. Declining the stocks due to overfishing in the previous decades led to its restocking by Iran fisheries organization from 1990s (Abdolmalaki and Psuty, 2007). At the present, millions of zander fingerling with different sizes, reproduced as a semi artificial propagation way and release into the water resources entrance to the Caspian Sea every year. Understanding about whether difference in size can caused to differences in their ability to cope with stress of after release, especially water salinity, is very important. Some osmoregulation studies during smoltification of salmonids showed a size-dependent development of its endocrine and ionregulatory pathways (Bilton et al., 1982; Beckman et al., 2003).

The ability to regulate plasma ions in the face of changing external salinity is an inevitable need for fish that move between fresh water and seawater as part of their normal life history such as anadromous fish (McCormick, 2001) and their effective osmoregulatory mechanisms maintain an osmotic and ionic balance under different salinity conditions (McCormick et al., 1989; Lee et al., 1996).

Several studies have been carried out on osmosis process and the change of hematological and osmotic indices in teleost especially in Salmonids but study on osmoregulation in zander is very few and limited to assay salinity tolerance (Neacsu et al., 1981; Craciun et al., 1982; Brown et al., 2001). It is expressed that optimal salinity for the zander is probably about 6 ‰ (Craig, 2000). It has been detected that abrupt transfer to high saline water caused to disturb plasma ion balance. In this case, ATPase activity decreased, sodium increased in the cells and osmotic pressure is too high, may leading to death (Neacsu et al., 1981). However, gradually transfer to high salinity result in increased ATPase activity to pump out salts, therefore osmotic equilibrium is restored (Craciun et al., 1982). The physiological effects of saline water on mature zander showed that it has a high level hypo-osmoregulatory. It can tolerate a gradual increase in salinity rising to 29-33 ppt up to saline environment and have good tolerance of sudden transfer to salinities of 8 and 16 ppt (Brown et al., 2001). However, it has not been seen an investigation on the physiological characteristics and plasma ion regulation abilities in fingerlings of zander prior to seawater migration. Also, in recent years, despite of much release of zander fingerlings to the rivers leading to the Caspian Sea, the catches have remained still low. It is appear that direct releasing of them into the estuaries or Caspian Sea water can be one of the ways which can help to management of zander restocking in southern part of Caspian Sea. Therefore, information of osmoregulatory capabilities of different size of these fingerlings would provide a better understanding of the life history and the osmoregulatory physiology of this species and could be used to develop the restock management strategies. The main objective of

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this study was to investigate the effect of size on salinity tolerance and to examine alterations in the short term in fingerlings of zander transferred directly from freshwater to estuary and Caspian Sea water. Hence we studied the effects of three salinity (<0.5 ‰ freshwater , 7 ‰ estuary water and 12‰ Caspian Sea water) on hematological parameters, plasma osmolarity, Na^+, Cl^- and circulating level of cortisol in two sizes of zander, *Sander lucioperca*.

Material and methods

Specimens of zander fingerlings in two sizes of body mass (group 1: weight=1.09 ± 0.01 g, total length= 60.1 ± 0.3 mm and group 2: weight=2.03 ± 0.03 g, total length=69.7 ± 0.4 mm, mean± SE) were supplied from Dr. Yousefpour Fish Hatchery Center, Siahkal (Guilan, Iran). The experimental studies were performed in Inland Water Aquaculture Institute in Bandar Anzali (Guilan, Iran). Fish were preserved in local well water at 18±0.5°C for 1 week before the experiments began. One thousand and three hundred fifty fish (group 1= 900 pcs, group 2=450 pcs) were randomly subjected to three different salinities (<0.5 ‰ = aerated freshwater (FW), 7 ‰ and 12 ‰=Caspian seawater (CW) with 3 replicates for each treatment (nine 100 l fiberglass tanks for each size group) during 10 days. Fish density was considered 0.99 g/l as 100 pcs for group1 and 50 pcs for group 2 were replaced in each tank. Salinity of 7‰ was obtained by mixing Caspian Sea water with well water. Salinity of water was checked with a light refractometer (ATAGO, Japan).

Water temperature, pH and dissolved oxygen were monitored daily and their mean value were 18 ± 0.17°C, 7.85 ± 0.01 , 6.4 ± 0.1 mg l^{-1}, respectively. Water of each tank was completely renovated every day. The fish were exposed to a daily photoperiod of 12-h and they were fed two times a day at 2% of their body weight with live food (*Daphnia* and Chironomidae) during experimental period and a week before that. Mortality was recorded every day for calculating the survival rates in the end of fresh water and salinity challenges to determine the levels of salinity tolerance.

At intervals of 0, 6, 24, 72 and 240 h after starting trials, fish were randomly netted (group 1: n=15, group 2: n=10), anaesthetized and sampled. The first sampling, at 0 h, was done before exposing the fry to the 7S and CW environments. Within 1 min, blood was rapidly collected by cutting the caudal peduncle with surgical blade and blood was collected capillary tube coated with heparin. For plasma extracting, blood samples from each replicate of treatments pooled and then centrifuged (5 min 5500 rpm). Plasma was separated and immediately stored in -20º C until analysis of osmoregulatory (osmolarity, Na^+ and Cl^-) and hormonal (cortisol) parameters. A sample of blood was used to hematological assays at the end of experiment (240h).

Plasma osmolarity was measured using cryoscopy method by an osmometer (Roebling, Germany) on duplicates. Cortisol was determined by radioimmunoassay (RIA) by use of RIA kit from Immunotech (France, Czech Division, Prague, Czech Republic) according to Rottlant et al. (2001) and expressed as ng ml^{-1}. Intra-assay and inter-

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assay c.v. were 5.8 and 9.2 %. Chloride concentration was determined using a colorimetric method using Technicon autoanalyser (Technicon, New York, USA) and Sodium was measured by flame photometry (Fc 180 Clem, Sao Paulo, Brazil) according to Burtis et al. (2006).

Red blood cell counting was performed by hemocytometer over cell suspended in Rees-Escher´s solution. For determination of hematocrit (Hct), the sample was collected in capillary tubes and centrifuged for 3 min at 4500 × g and expressed as percent packed cell volume (Rehulka, 2000). Hemoglobin (Hb) concentration was determined spectrophotometrically at 540 nm (Cyanmethemoglobin method). Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was calculated using standard formula (Blaxhall and Daisley, 1973).

Factorial analysis of variance (3-way ANOVA) was used to test for size, treatment salinity and time effects. Two way analysis of variance (2-way ANOVA) was used to test for differences between salinity treatments in different time points within each weight group. t-Test was used to determine differences in hematological parameters between two size groups at 240 h after starting experiments at the same salinity. Tukey's HSD multiple comparison test was used to identify significant differences ($p<.05$) between means. Data are presented as means ± SE.

Results

The survival rates in all treatments of two size group were higher than 95%. There were no significant differences among survival rates in two size groups (Table 1).

Table 1: Survival rates of zander (*Sander lucioperca*) fingerlings in fresh water and direct transfer to saline water during 10 days

Size group	Salinity treatments		
	Freshwater (<.05‰)	7 ‰	Caspian Sea water (12 ‰)
1	97.7± 0.3	98.7± 0.3	99± 0.57
2	97.7± 0.67	99± 0.6	98± 0.6

Values are means±standard erorr

Either of two size groups maintained constantly their plasma osmolarity, Na^+ and Cl^- concentrations in freshwater during experiment. Both of salinity challenges (7‰, CW) and duration of their exposure affect on plasma osmolarity, Na^+ and Cl^- concentrations in both of two size group ($p<.05$).

In size group 1, plasma osmolarity levels slightly increased after 6, 24 and 72 h of both 7 ‰ and CW exposure as their values were significantly higher than FW values at time 72h ($p<.05$), then decreased to new levels with no significantly differences between them at 240h. In spite of plasma osmolarity value of

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CW were significantly higher than FW at 240 h, the value of 240h after exposure to 7‰ had not significantly different to FW level at the same time (Figure 1A). The values of plasma Na^+ concentration slightly peaked at 72h after exposure to 7‰ and CW salinities then decreased at 240h without any significant differences between them. Despite the appearance of small changes in the value of plasma Cl^- concentrations after transfer to different salinities in this experiment, there were no any significant differences between different time sampling in each treatment and also between FW, 7‰ and CW treatments in each time intervals within 10 days acclimation period, similar to Na^+ ($p>.05$) (Fig. 1C, E).

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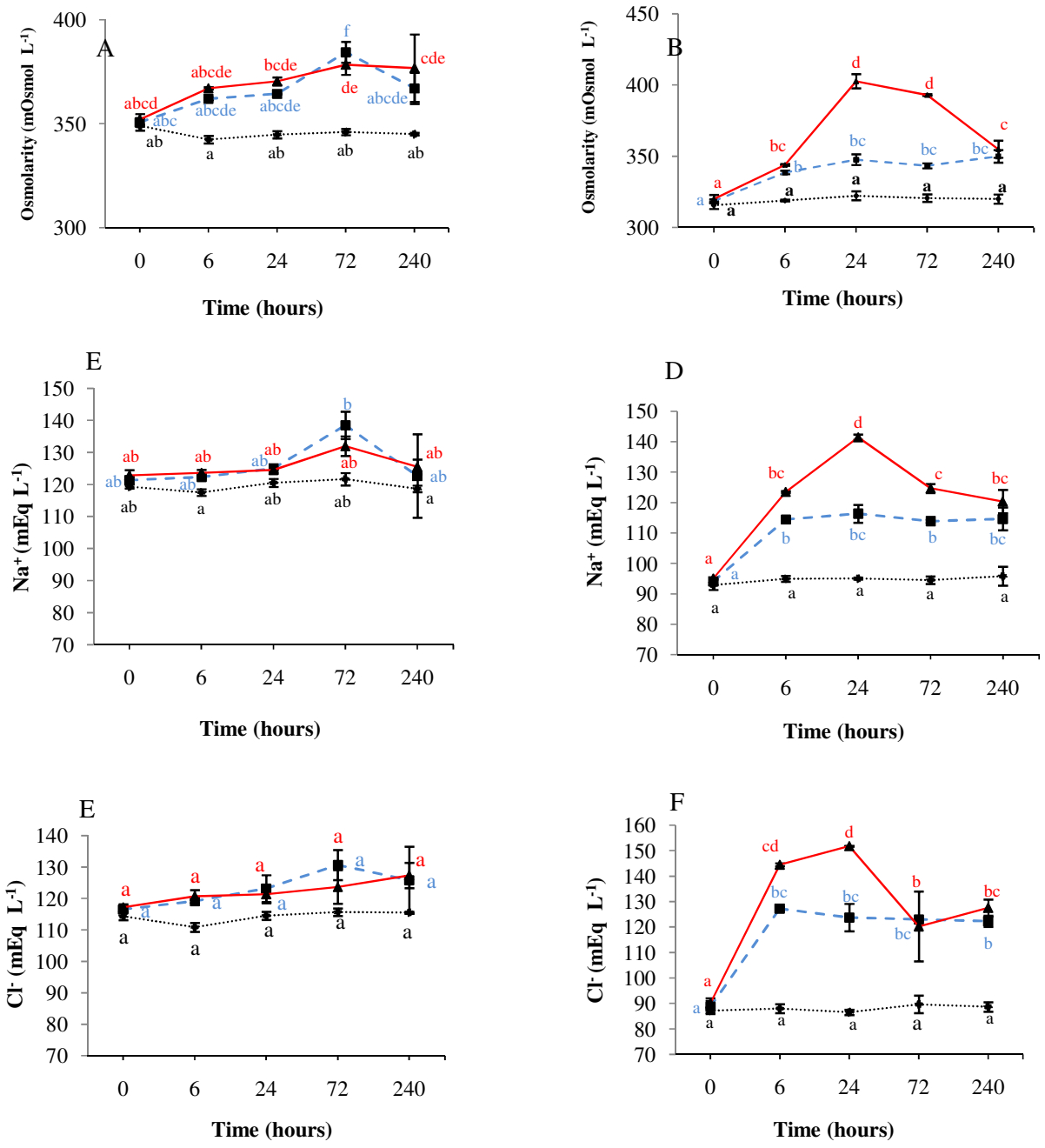

Figure 1: Plasma osmolality, sodium, and chloride concentrations in 1 g (A, C, E) and 2 g (B, D, F) zander (*Sander lucioperca*) exposed to <.05‰ (.....), 7‰ (---▪---) and 12‰ (‾◆‾) at 0, 6, 24, 72 and 240 h. Values are presented as means±standard erorr. Means with different letters are significantly different (*p<.05*, 2-way ANOVA, Tukey HSD multiple comparison tests).

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In size group 2, plasma osmolarity, Na^+ and Cl^- concentrations increased significantly following 6h of both 7‰ and CW exposure relative to FW values in the same time. In CW challenge, all of indices peaked at 24h, and then significantly decreased at 240h while there were not any significant differences from 6 h to 240 h, in 7‰ challenge. The value of three indices had not significant different at 240 h after transfer to 7 and 12 ‰ salinity treatments ($p<.05$) (Fig. 1B, D, F).

A significant interaction between the effect of size and salinity treatment was observed in case of plasma osmolarity and ion concentrations ($p<.05$). Thus the effect of salinity acclimation on plasma osmolarity and the regulation of plasma Na^+ and Cl^- was dependent on fish size within this size range, As the values and the pattern changes of the plasma osmolarity, sodium and chloride concentrations were different in two weight groups (Figures 1A, B, C, D, E, F).

Plasma cortisol levels were highest at the beginning of the experiment in both weight and then were dropped off significantly to the lowest level in all three treatments (FW, 7‰ and CW) at 6h ($p<.05$). Values slowly increased to 240 h, with no significant differences between time intervals (24, 72 and 240h) in all treatment of group 1 and 7‰ treatment of Group 1. But In CW salinity challenge of group 1, cortisol value significantly increased From 6 to 72 h, and then slowly declined to the end of acclimation period.

Cortisol changes were independent of salinity and weight. Each of these factors had no significant effect on cortisol levels. No significant interaction between the effect of size and salinity treatment was observed about cortisol concentration ($p>.05$) (Fig. 2).

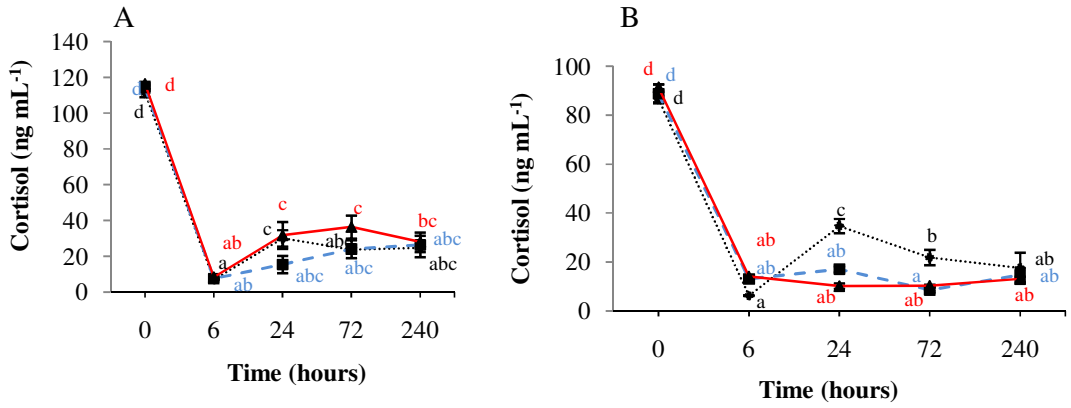

Figure 2: Changes in plasma cortisol in two size groups of Zander (*Sander lucioperca*) (1 g ,A, and 2 g ,B) exposed to <.05‰ (.....), 7‰ (---▪---) and 12‰ (¯◆¯) at 0, 6, 24, 72 and 240 h. Values are presented as means±SEM. means with different letters are significantly different (*p<.05*, 2-way ANOVA, Tukey HSD multiple comparison tests).

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Hematological parameters

In both weight groups, there were no significant differences between three salinity challenges about any of the hematological values ($p>.05$). In 7 ‰ treatment, the mean values of Hct and RBC of 1g zander fingerlings were significantly higher than 2g zander fingerlings ($p<.05$). In CW, the mean values of Hb and Hct of size group 1 were significantly higher than size group 2 ($p<.05$) (Table 2).

Table 2: Hemoglobin concentration (g/dl), Hematocrit (%), Red Blood Cell Count (10^3cells/mm^3), MCV (fl: femtolitr), MCH (pg: picogram) and MCHC (%) in 1 and 2 g Zander (*Sander lucioperca*) fingerlings at 240 h after transfer from Fresh water to Fresh water or to 7 and 12‰ salinity treatments from Caspian Sea water.

Hematological Parameter	Size group	Salinity treatments		
		Fw(<0.05‰)	7 ‰	CW(12‰)
Hb(g dl^{-1})	1	2.69 ± 0.18	3.18 ± 0.31	3.15 ± 0.02*
	2	2.79 ± 0.29	2.3 ± 0.18	2.73 ± 0.04*
Hct %	1	38 ± 2	41 ± 1*	37.5 ± 1.5*
	2	30.5 ± 1.5	30 ± 0.0*	30.5 ± 0.5*
RBC(10^3cells mm^{-3})	1	1585 ± 175	1920 ± 100*	1700 ± 230
	2	1425 ± 115	1370 ± 10*	1415 ± 5
MCV(fl)	1	241.3 ± 14.0	213.9 ± 5.9	223.5 ± 21.4
	2	214.6 ± 6.8	219 ± 1.6	215.6 ± 2.8
MCH(pg)	1	17.3 ± 3.1	16.5 ± 0.8	18.8 ± 2.4
	2	19.51 ± 0.2	16.8 ± 1.1	19.3 ± 0.1
MCHC (%)	1	7.1 ± 0.9	7.7 ± 0.6	8.4 ± 0.3
	2	9.00 ± 0.2	7.7 ± 0.4	9.00 ± 0.00

Values are presented as means±standard erorr. An asterisk indicates a significant difference between two size group in a column ($p<.05$).

Discussion

Both size groups of zander fingerlings were able to tolerate both Caspian Sea water salinity challenges test. Salinity tolerance ability of euryhaline teleosts can specify generally with the survival rate after direct or piecemeal transfer between different salinity environments (Hiroi and McCormick, 2007; Kang et al., 2010). High survival rate of both

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size groups of zander fingerlings in this study showed that this species weighing 1 to 2 g is able to tolerate different salinity of Caspian Sea water up to 12 ‰.

Brown's study (2001) on the physiological effects of saline water on adult zander from waters of Great Britain indicated that this fish can tolerate direct transition to brackish waters with salinity 8 and 16 ppt. Increased plasma osmolarity, Na^+ and Cl^- concentrations was observed after abrupt transfer of 1g zander fingerlings to 7 and 12 ‰ salinity challenges as this increase continued for 72 h and then decreased at 240 h. However, in 1g zander fingerlings that faced with the challenges of two Caspian Sea water salinity (7 and 12%), plasma osmolarity and ions concentrations significantly increased at 6h exposed to salinity treatments and peaked at 24h then decreased until the end of the experiment at 240 h.

Plasma osmolarity levels increased after faced with Caspian Sea water salinities (7 and 12%) in both size groups. This result was mainly reported in most fish that have been studied, including *Oncorhynchus mykiss* (Morgan and Iwama, 1996), *Acipenser oxyrinchus* (Altinok et al., 1998), *Acipenser persicus* (Jabbarzadeh Shiadeh et al., 2000), *Oreochromis niloticus* (Fontainhas-Fernandes et al., 2001), *Oreochromis mossammbicus* (Sardella et al., 2004), and *Acipenser sinensis* (He et al., 2009). Such changes can be attributed to changes in the water content of the blood due to environmental salinity changes (Plaut, 1998). When fish enter to hyperosmotic or isosmotic environment, dehydrated trough facilitated diffusion and increase plasma ion concentrations. Increase of drinking is a compensatory mechanism for temporary blood dilution. Then other osmoregulatory mechanisms serve to return steady state in hydromineral homeostasis (Martinez-Alvarez et al., 2002).

In euryhaline fish, there are two periods to establish balance in body ions and water after transfer to hypertonic or hypotonic media: 1- The adaptive period, with changes in osmotic parameters, and 2- the chronic regulatory period, where these parameters again reach homeostasis (Holmes and Donaldson, 1969; Maetz, 1974). In the present study, it was found that acclimation in both of two size groups of zander fingerlings includes two physiological stages, too: Critical stage (72h for 1g and 24h for 2g) and stabilization stage. In 1g zander fingerlings, changes of plasma ions level were slight but acute period for plasma osmolarity changes was 72h while this period in 2g zander fingerlings was 24 h. The only investigation of these processes in zander reported decreased branchial Na^+, K^+-ATPase 24 h after transfer to 8–12 ppt water, and recovery over the next 2 days (Craciun et al., 1982).

The loss of water from gill surface trough osmotic way is a critical problem in the beginning of abrupt transfer from FW to brackish water (or sea water) (Cataldi et al., 1999; McKenzie et al., 1999; Martinez-Alvarez et al., 2002). In CW (or 7%), 2g zander fingerlings lose water more than 1g because of their larger body and more gill surfaces, thus plasma osmolarity and ion concentrations (Na^+, Cl^-) more increase. The critical period was reported in Gulf of Mexico

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sturgeon (*Acipenser oxyrinchu*s) and juvenile Chinese sturgeon (*Acipenser sinensis*) 24 h after transfer (Altinok et al., 1998; He et al., 2009). As well as, it was discovered that the highest blood sodium concentration of rainbow trout and euryhaline killifish, *Fundulus heteroclitus* and the highest blood osmolarity of euryhaline killifish occurred 24 hours after transfer to seawater (Marshall et al., 1999; Al-Jandal and Wilson, 2011).

Critical time interval (72h for 1g and 24h for 2g) until the end of tests (240h) is the start of regulation and steady period because of decrease of blood osmotic parameters. Critical stage or duration of temporary increase in plasma osmolarity and electrolyte levels of 2g size groups (24h) were less than 1 g (72h). Thus, physiological compensatory mechanisms of 2g size groups for hydromineral equilibrium establishment in body were start earlier than 1g zander fingerlings. It was reported that plasma osmolarity of mature zander (150–450 g, from Grand Union canal, UK) significantly increased at 24 h after abrupt transfer to 16 ppt salinity water but its value significantly increased to 5 days later day 6 (Brown et al., 2001).

The results of current research revealed that the value of plasma osmolarity in CW and 7% treatments significantly was higher than FW in both of two size groups at 240 h, supported by He et al. (2009). They discovered that after exposure to brackish water (10%), the serum osmolarity levels of Chinese sturgeon peaked at 24h and then decreased significantly at 216h and remained constant until 480h but that value at 216 h until 480h was significantly higher than serum osmolarity levels of fresh water control fish at the same time. They concluded that serum osmolarity increased in crisis period and decreased significantly, reaching a new steady (He et al., 2009). Thus, if the treatment duration was considered more than 10 days (>240h) in our study, there was probability that the values were closer to FW or would be stable.

The trend of ion concentration changes in 2g fingerlings was similar to their osmolarity trend. On the other hand, except of plasma osmolarity changes in size group 1, trend of ionic changes in this group were lower than size group 2, after two salinity treatments. Also, there were not any significant changes between ion concentration values of two salinity challenges and FW in 1g zander fingerlings during the test. These results show that 1g and 2g zander fingerlings can tolerate Caspian Sea water salinity up to 12 %.

Significant effect of body weight on plasma osmorality, Na^+ and Cl^- levels in the results of this study show that body weight/size of zander fingerlings is an effective factor on their adaptation to the Caspian Sea environment. It is appeared that despite the survival rate of both size group were high exposed to salinity, the osmoregulatory mechanisms in the body of bigger fingerlings acted better than the smaller size of them. Allen and Cech (2007) also reported a body size of fish effect in hyperosmotic adaptability. This point can be found the other research that the body size of fish have a positive effect on their osmoregulatory capability in both of freshwater and seawater (Conte and Wagner, 1965; Halvorsen et al., 1993). Jonassen et al. (1997) concluded that seawater tolerance of

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tilapia fingerlings, *Oreochromis spilurus spilurus,* increased with the size of fish and the acclimation time (Jonassen et al., 1997). Jackson (1981) also reported rate and amplitude of the increase in plasma osmosis concentration of rainbow trout related to size as increase in body size causes increase in adaptation ability (Jackson, 1981).

Cortisol is a key multifunctional hormone involved in physiological homeostasis of fish especially in the early stages of growth. It can be noted these functions include the response to stress, regulation of the energetic metabolism and its associated processes such as osmoregulation, migration and growth (McCormick, 1995, 2001; Mommsen et al., 1999; Laiz-Carrio et al., 2002). In current study, high levels of cortisol just after transfer and rapidly decrease suggest that cortisol played its role in response to handling stress due to transition from stock tank (in resting time) to 100 l fiberglass tanks in the first moments of the experiment. The patterns of its changes from 6h to 240h in both size groups exposed to three test salinity (FW,7%, CW) suggest that the role of environmental compatibility. In both size groups of fingerlings, the values of cortisol in 7‰ and CW treatments were at a similar level and very close to the value in FW control at 240h. Many studies have shown that under acute stress the plasma level of cortisol can easily shoot up many folds to enhance the mobilization of energy reserves and metabolic rate (Wendelaar Bonga, 1997; Flik et al., 2006). Although, cortisol is often referred to as a Seawater-adapting hormone due to its osmoregulatory role in sea water, but it has been recognized its contribution to hydromineral regulation in freshwater fish, too (McCormick, 2001).Under longer-term of stressful situations, the fish seem to adapt to the stress and plasma cortisol and its clearance rate fluctuate within the range considered 'normal' for a particular species (Wendelaar Bonga, 1997). Brown et al. (2001) reported that cortisol concentrations of mature zander on day 6 after exposure to three salinity treatment (FW 8 and 16 ppt) was higher than the values on day 1 (at 24h) without any significant differences between them. In the present study was detected that the cortisol level of all treatments at 240 h had not any significant different compared to the level at 24h in all salinity challenges, except FW control in 2g zander fingerlings. But the establishment of cortisol concentrations in the same level at 240h in two size groups seems that because of adaptation by zander fingerlings to stressful condition.

In current study that was found no significant difference between hematological values of two salinity treatment groups than freshwater as control group. Hematological indices (especially Hct and MCHC) remain unchanged after acclimation to salt waters has been observed in mature zanders (Brown et al., 2001), sturgeon, *Acipenser naccarii* (Martinez-Alvarez et al., 2002), juvenile hybrid mozambique tilapia (*Oreochromis mossambicus* × *O. urolepsis hornorum*), (Sardella et al., 2004), rainbow trout (Shepherd et al., 2005).

Martínez-Álvarez et al. (2002) discovered the value of Hct, Hb and RBC in *Acipenser naccarii* increased with increasing

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water salinity but returned to early values when the fish remain in 35% salinity at 20 days (Martinez-Alvarez et al., 2002). In present study, sampling for blood analyzing was not done at intervals of 0, 6, 24, 72h. It appears that salinity exposure of zander fingerlings (1 and 2g) first caused to increase the blood parameters but recovered close to values of FW control. It is possible that variation in values of blood indices has occurred at the onset of exposure to stress specially salinity stress. When the fish could be overcome environmental stressful stimuli and was adapted to new condition, it is expected that the blood indices recovered. In a hyperosmotic environment, first the fish would lose water passively and concentrations of blood-cell elements increase. Then increase of water ingestion compensates temporarily dilution of the blood parameters. Finally, the hematological parameter would return to initial values (or the values in hypoosmotic control environment) as a result of the effect of osmoregulatory mechanisms for re-establishment the extracellular fluid volume.

In this study, some hematological parameters (Hb, Hct, RBC and MCV) of 2g zander fingerlings were significantly higher than 1g fingerlings in salinity treatments. It probably revealed the weight of the fingerlings exert some degree of influence on the hematological parameters in acclimation to salinity challenges. The effect of size on blood indices in salinity conditions reported in *Acipenser transmontanus* (Mojazi Amiri et al., 2009) and *Tilapia guineensis* (Akinrotimi et al., 2010, 2012).

In conclusion, the results of the ionic, hormonal and blood indices showed that although 2g zander fingerlings acted better than 1g in faced with salinity, however both two size groups (1g and 2g) of zander fingerlings could tolerate the Caspian Sea water salinity up to 12‰ and survived in this range of salinity and so, osmoregulatory mechanisms in their body can be evolved a relatively large extent. Thus, the fingerlings of zander can be released to the Caspian Sea water close to estuaries. Also, this result has provided valuable information for restock management of zander in south of Caspian Sea.

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References

Abdolmalaki, S. and Psuty, I., 2007. The effects of stock enhancement of pikeperch (*Sander lucioperca*) in Iranian coastal waters of the Caspian Sea. *ICES Journal of Marine Science*, 64, 973–980. (in Persian).

Akinrotimi, O. A., Uedeme-Naa, B. and Agokei, E. O., 2010. Effects of acclimation on

[DOR: 20.1001.1.15622916.2014.13.2.2.4]

hematological parameters of *Tilapia guineensis* (Bleeker, 1862). *Science World Journal*, 5(**4**), 1-4.

Akinrotimi, O. A., Agokei, E. O. and Aranyo, A. A., 2012. Changes in Blood Parameters of *Tilapia guineensis* Exposed to Different Salinity Levels. *Journal of Environmental Engineering and Technology*, 1(**2**), 4-12.

Al-Jandal, N. and Wilson, R. W., 2011. A comparison of osmoregulatory responses in plasma and tissues of rainbow trout (*oncorhynchus mykiss*) following acute salinity challenges. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, 159(**2**), 175-181.

Allen, P. J. and Cech, J. J., 2007. Age/Size effects on juvenile Green sturgeon (*Acipenser medirostris*), oxygen consumption, growth & osmoregularion in saline environments. *Environmental Biology of Fishes*, 79, 211-229.

Altinok, I., Sara, M. G. and Frank, A. C., 1998. Ionic and osmotic regulation capabilities of juvenile Gulf of Mexico sturgeon, *Acipenser oxyrinchus* de Sotoi. *Comparative Biochemistry and Physiology*, 120, 609-616.

Beckman, B. R., Larsen, D. A. and Dickhoff, W. W., 2003. Life history plasticity in Chinook salmon: relation of size and growth rate to autumnal smolting. *Aquaculture*, 222, 149-165.

Bilton, H. T., Alderdice, D. F. and Schnute, J. T., 1982. Influence of time and size at release of juvenile Coho salmon (*Oncorhynchus kisutch*) on returns at maturity. *Canadian Journal Fisheries and Aquatic Sciences*, 39, 426-447.

Blaxhall, P. C. and Daisley, K. W., 1973. Routine hematological methods for use with fish blood. *Journal of Fish Biology*, 5, 771-781.

Brown, J. A., Moore, W. M. and Quabius, E. S., 2001. Physiological effects of saline waters on zander. *Journal of Fish Biology*, 59, 1544-1555.

Burtis, C. A., Ashwood, E. R. and Bruns, D. E. 2006. Tietz textbook of clinical chemistry (4th ed.). Saunders. 2448P.

Cataldi, E., Barzaghi, C., Di,Marco, P., Boglione, C., Dini, L., McKenzie, D. J., Bronzi, P. and Cataudella, S., 1999. Some aspects of osmotic and ionic regulation in Adriatic sturgeon *Acipenser naccarii*. I: ontogenesis of salinity tolerance. *Journal of Applied Ichthyology*, 15, 57-60.

Conte, F. P. and Wagner, H. H., 1965. Development of osmotic and ionic regulation in juvenile coho salmon *Oncorhynchus kisutch*. *Comparative Biochemistry and Physiology*, 18, 1-15.

Craciun, V., Craciun, M., Neacsu, I. and Trandafirescu, I., 1982. Na super(+) - K super(+) pump activity in the zander during acclimatisation to salinity conditions in the Black Sea. In: Craig, J.F., (Eds.), Percid fishes systematics, ecology and exploitation. Blackwell Science, UK, pp.112-113.

Craig, J. F., 2000. Percid fishes: systematics, ecology and exploitation. Blackwell Science, UK, 352P.

Flik, G., Klaren, P. H. M., Van der Burg, E. H., Metz, J. R. and Huising, M. O., 2006. CRF and stress in fish. *General Comparative Endocrinology*, 146, 36-44.

Fontainhas-Fernandes, A., Russell-Pinto, F., Gomes, E., Reis-Henriques, A. and Coimbra, J., 2001. The effect of dietary sodium chloride on the physiological response of (*Oreochromis noliticus*) to salinity acclimation. *Fish Physiology and Biochemistry*, 23, 307-316.

[DOR: 20.1001.1.15622916.2014.13.2.2.4]

He, X., Zhuang, P., Zhang, L. and Xie, C., 2009. Osmoregulation in juvenile Chinese sturgeon (*Acipenser sinensis Gray*) during brackish water adaptation. *Fish Physiology and Biochemistry*, 35, 223-230.

Hiroi, J. and McCormick, S. D., 2007. Variation in salinity tolerance, gill Na^+/K^+-ATPase, $Na^+/K^+/2Cl^-$ cotransporter and mitochondria-rich cell distribution in three salmonids Salvelinus namaycush, Salvelinus fontinalis and Salmo salar. *Journal of Experimental Biology*, 210, 1015-1024.

Halvorsen, M., arnesen, A. M., Nilssen, K. J. and Jobling, M., 1993. Osmoregulation ability of anadromous arctic charr, *Salvelinus alpinus* (L), migrating towards the sea. *Fish Management and Aquaculture*, 24, 199-211.

Holmes, W. N. and Donaldson, E. M., 1969. The body compartments and the distribution of electrolytes. In: Hoar, W.S. and Randall, D.J., (Eds.), Vol. 1, Fish physiology. Academic Press, New York (NY), pp.1–89.

Jabbarzadeh Shiadeh, S. M., Mojazi Amiri, B., Abtahi, B. and Nazari, R. M., 2000. Study on the changes of some physiological factors during osmoregulation of juvenile Persian sturgeons (*Acipenser persicus*). *Iranian Journal of Fisheries Sciences*, 2, 61-74. (in Persian).

Jackson, A. J., 1981. Osmotic regulation in rainbow trout (*Salmo gairdneri*) following transfer to sea water. *Aquaculture*, 24, 143-151.

Jonassen, T. M., Pittman, K. and Imsland, A. K., 1997. Seawater acclimation of tilapia, *Oreochromis spilurus spilurus* Günter, fry and fingerings. *Aquaculture Research*, 28, 205-214.

Kang, C. K., Tsai, H. J., Liu, C. C., Lee, T. H. and Hwang, P. P., 2010. Salinity-dependent expression of a Na^+, K^+, $2Cl^-$ cotransporter in gills of the brackish *medaka Oryzias* dancena: a molecular correlate for hypo osmoregulatory endurance. *Comparative Biochemistry and Physiology*, 157A, 7–18.

Lee, T. H., Hwang, P. P., Lin, H. C. and Huang, F. L., 1996. Mitochondria-rich cells in the branchial epithelium of the teleosts, *Oreochromis mossambicus*, acclimated to various hypotonic environments. *Fish Physiology and Biochemistry*, 15, 513- 523.

Laiz-Carrion, R., Sangiao-Alvarellos, S., GuzmaLn, J. M., Martin del Rio, M. P., Minguez, J., Soengas, J. L. and Mancera, J. M., 2002. Energy metabolismin fish tissues related to osmoregulation and cortisol action. *Fish Physiology and Biochemistry*, 27, 179-188.

Maetz, J., 1974. Aspects of adaptation to hypo-osmotic and hyperosmotic environments. In: Malins, D.C. and Sargent, J.R., (Eds.), Biochemical and Biophysical Perspectives in Marine Biology. Academic Press, London, pp.1-167.

Marshall, W. S., Emberley, T. R., Singer, T. D., Bryson, S. E. and McCormick, S. D., 1999. Time course of salinity adaptation in a strongly euryhaline estuarine teleost, *Fundulus heteroclitus*: a multivariable approach. *Journal of Experimental Biology*, 202(**11**), 1535-1544.

Martinez-Alvarez, R. M., Hidalgo1, M. C., Domezain, A., Morales, A. E., Garcia-Gallego, M. and Sanz, A., 2002. Physiological changes of sturgeon *Acipenser naccarii* caused by increasing environmental salinity. *The*

[DOR: 20.1001.1.15622916.2014.13.2.2.4]

McKenzie, D. J., Cataldi, E., Di Marco, P., Mandlich, A., Romano, P., Ansferri, S., Adriatic sturgeon *Acipenser naccarii*. II: Morpho-physiological adjustments to hyper osmotic environments. *Journal of Applied Ichthyology*, 15, 61-66.

Mojazi Amiri, B., Bake, D., Morgan, J. D. and Brauner, C. J., 2009. Size dependent early salinity tolerance in two sizes of juvenile white sturgeon, *Acipenser transmontanus*. *Aquaculture*, 286, 121-126.

Mommsen, T. P., Vijayan, M. M. and Moon, T. W., 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Review in Fish Biology and Fisheries*, 9, 211-268.

Morgan, J. D. and Iwama, G. K., 1996. Cortisol induced changes in oxygen consumption and ionic regulation in coastal cutthroat trout *Oncorhynchus mykiss* parr. *Fish Physiology and Biochemistry*, 15, 385-395.

Neacsu, I., Craciun, V. and Craciun, M., 1981. L'équiliere hydro-mineral chez le sandre (*Stizostedion lucioperca*(L.)) transfere de 1'eau douce en milieux mixo-mesohalins et inversement. In: Craig, J.F., (Eds.), Percid fishes systematics, ecology and exploitation. Blackwell Science, UK, pp.112-113.

Plaut, I., 1998. Comparison of salinity tolerance and osmoregulation in two closely related species of blennies from different habitats. *Fish Physiology and Biochemistry*, 19, 181-188.

Bronzi, P. and Cataudella, S., 1999. Some aspects of osmotic and ionic regulation in

Rehulka, J., 2000. Influence of astaxanthin on growth rate, condition, and some blood indices of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 190, 27-47.

Rottlant, J., Balm, P. H. M., Perez-Sanchez, J., Wendelaar-Bonga, S. E. and Tort, L., 2001. Pituitary and interregnal function in Gilthead Sea bream (*Sparus aurata* L., Teleostei) after handling and confinement stress. *General Comparative Endocrinology*, 121, 333–342.

Sardella, B. A., Matey, V., Cooper, J., Gonzalez, R. and Brauner, C. J., 2004. Physiological, biochemical and morphological indicators of osmoregulatory stress in "California" Mozambique tilapia (*Oreochromis mossambicus* × *O. urolepsis hornorum*) exposed to hypersaline water. *Journal of Experimental Biology*, 207, 1399–1413.

Shepherd, B. S., Drennon, K., Johnson, J., Nichols, J. W., Playle, R. C., Singer, T. D. and Vijayan, M. M., 2005. Salinity acclimation affects the somatotropic axis in rainbow trout. *American Journal of Physiology Regulatory Integrative Comparative Physiology*, 288, 1385-1395.

Wendelaar Bonga, S. E., 1997. The stress response in fish. *Physiological Reviews*, 7, 591-265.

[DOR: 20.1001.1.15622916.2014.13.2.2.4]