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Full Length Research Paper

Effect of weight on osmoregulation ability in *Rutilus frisii kutum* fingerlings

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Experiments were conducted to study the downstream migratory behavior and effects of weight on osmoregulation ability of hatchery-reared *Rutilus frisii kutum* fingerlings during adaptation to the seawater. Accordingly, blood osmotic pressure regulation ability in kutum fingerlings with weights of 1, 3, 5 and 7 g in three different salinities, 13 ppt (Caspian Sea salinity), 7 ppt (estuarine area) and 0.3 ppt (freshwater) were assessed. The blood samples were collected before being transferred as control (freshwater) and after exposure to the sea and estuarine water in a period of up to 336 h by a pooling method. The results indicate that only 3, 5 and 7 g kutum are able to adapt to salinity of 7 and 13 ppt since they maintained the osmolarity. In 1 g kutum, the levels of osmotic pressure showed significantly higher values after 48 h until the end of the experiment compared to other weight groups and the respective control level (P<0.05). In conclusion, the results show that osmoregulation systems in 1 g fingerlings were not able to decrease the osmotic pressure. Therefore, they cannot be suggested as suitable sizes to be released into the brackish and marine environments.

Key words: *Rutilus frisii kutum*, osmotic pressure, salinity, weight, Caspian Sea.

INTRODUCTION

Rutilus frisii kutum (Kamenskii, 1901) or Caspian kutum is the most important commercial fish species in the Caspian Sea due to its high value, excellent flesh quality and delicious meat which make it attractive for the fishermen of the countries bordering the Sea. The main habitat of this species is in the southern Caspian Sea, bordering Iran and Azerbaijan (Figure). Kutum is a migratory species that migrates towards freshwater (FW) and lagoons around the Caspian Sea for natural breeding and spawning (Razavi, 1995, 1998; Abdoli, 1999). However, the migration of kutum towards these areas has decreesed very significantly in recent years for several reasons. These include: destruction of natural habitats, overfishing, dam construction, gravel and sand harvest from rivers, oil, Industrial, agriculture and urban pollution, and also ecological changes of the rivers. These factors have led to the destruction or deterioration of the main spawning and nursery grounds of kutum both in the Caspian Sea and its feeder-rivers. The result is a serious depletion in the kutum stocks (Gholami et al., 2007). In the last two to three decades, the stakeholders in aquaculture and

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Abbreviations: ANOVA, Analysis of variance; **CSW,** Caspian Sea water; **DMRT,** Duncan's multiple range test; **DO,** dissolved oxygen; **EW,** estuarine area water; **FW,** fresh water; **IFRO,** Iranian Fisheries Research Organization; **mOsm L^{-1},** milliosmoles per liter.

Figure 1. The Caspian Sea and Sefidrud River location.

also fisheries researchers have been working on the rehabilitation of kutum resources through artificial breeding (Abdolhay, 2010). At present, more than 250 million fingerlings of *Rutilus frisii kutum* produced by artificial breeding are released into the Caspian Sea annually (Bartley, 1995; Shehadeh, 1996; Abdolhay, 1998; Bartley and Rana, 1998; Tahori, 1998; Salehi, 2005, 2008).

Salehi (2008) showed that considering the mean weight of 815 g and mean age of 3.7 years, the recapture coefficient is estimated at 8.3%. Despite annual production of the fingerlings, the fisheries recapture coefficient of kutum is not satisfactory, which can be due to unsuitability of release weight, as well as spatial and temporal factors, namely, lack of information on release sites and release weight. There are no previous comprehensive data to determine the nominal weight of release for kutum. Bartley (1995, 1999) believe that releasing of aquatic fish into natural habitat is a complex process that is related to biological, ecological, economic and social parameters. Osmoregulation ability is also an important factor in determining the suitable release time or weight (Boeuf and Harache, 1982; Sayyad Bourani, 2007; Nikoo et al., 2010). Since fish's blood is always in direct relationship with sea water via gills and gut, ions can therefore transfer between blood and environment (Flik et al, 1985; Boeuf and Payan, 2001). During downstream migration (seawater migration) the level of blood ions increases. Subsequently, the juvenile fish must be able to expel the extra blood ions and keep the blood homeostasis stable (Krayushkina et al., 1999). In this situation, ionic regulation (osmoregulation) system plays a main role in blood homeostasis. Moreover, it should be noted that during the releasing process of cultured fingerling fish into seawater, hypertonicity occurs because seawater is denser than fish blood. However, if the osmoregulation system is well-developed in the fingerlings, they will be able to excrete the excess ions (active transport) at sea to stabilize the ionic equilibrium in blood (Lustek, 2000). Bony fishes have to drink a volume of sea water (about 10% of their body weight) and then physiologically convert

Table 1. Water physical and chemical parameters.

Treatment	pH	Salinity (ppt)	O_2 (mg L^{-1})	NO_2 (mg L^{-1})	NH_4 (mg L^{-1})	CO_2 (mg L^{-1})
Caspian Sea water	8.04 ± 0.09	12.68 ± 0.70	8.27 ± 0.71	0.0068 ± 0.021	0.01 ± 0.004	0.541 ± 0.519
Estuary water	8.03 ± 0.13	7.20 ± 0.73	9.31 ± 0.02	0.0075 ± 0.004	0.013 ± 0.04	0.0
Fresh water	7.90 ± 0.14	0.35 ± 0.03	9.46 ± 1.15	0.012 ± 0.019	0.013 ± 0.009	0.0

Values are expressed as mean ± SE.

that into distilled water. It means that the excess ions are expelled by gills and the kidney (Sayyad Bourani, 2007). In turn, if during the release process the fingerlings are not able to expel the ions due to lack of osmoregulation ability, their blood osmotic pressure will be increased (Nonnotte and Truchot, 1990).

Although, there are a few studies on ionic regulation of kutum (Nikoo et al., 2010; Enayat Gholampoor et al., 2011), this study aimed particularly at analyzing the impact of weight on osmotic regulation ability during seawater adaptation. For this purpose, 1, 3, 5 and 7 g kutum fingerlings recommended by Iranian Fisheries Research Organization (IFRO) were selected for analyzing the level of osmotic pressure during adaptation in estuarine water (EW) and Caspian Sea water (CSW).

MATERIALS AND METHODS

This study was carried out at the Iranian Fisheries Research Center aquatic laboratory. Kutum fry (<1 g) from the IFRO hatchery (Shahid Ansary) in Rasht, Iran, were prepared. They were then transferred to the Sefidrud Fisheries Research Center laboratory in Astaneh, Iran, to be kept in an earthen pond for a period of 11 months until they reached the weight of 1 to 7 g. They were sorted by a manual sorter, weighed by electronic balance and classified into 4 weight groups of 1, 3, 5 and 7 g. The selected weight groups were then transferred into 500 L tanks equipped with inlet-outlet current and aeration system.

After two weeks of acclimatization, the fingerlings were moved to 100 L tanks with three different salinity treatments of: (i) fresh water (< 0.5 ppt) (ii), estuarine water (7 ppt) and (iii) Caspian Sea water (13 ppt) all equipped with aeration system (adapted from McCormik and Naiman, 1984, “preservation system”). For the four weight groups of the fingerlings, three different salinities and 9 experimental times, a total of 108 tanks (volume 100 L) each with 3 replicates were prepared. The biomass was determined as 10 g/L (Razavi, 1995).

Implementation phase of study

To prepare different salinities, the sea water was collected from the Caspian Sea at depths of 2 to 3 m and 50 m from shore, while estuarine water (7 ppt) was collected from the Sefidrud River mouth and inshore of the Caspian Sea (Figure). The waters were filtered before transferring them into 100-L tanks. Also, 20% of the water from the tanks was changed daily and the fingerlings were fed twice a day with Special Food for Kutum (SFK), with an amount approximating 7% of body weight. The salinity of the waters was controlled daily with a portable salinometer and the tanks were kept under ambient light conditions. On the other hand, some physical and chemical parameters were measured, including pH, DO, CO2, nitrite and N-NH4 (ammonium) (ASTM, 1989).

Blood sampling

The blood samples were collected by cutting the peduncle with capillary tubes -without anticoagulant- in chronic durations of 0, 12, 24, 48, 72, 96, 144, 240 and 336 h and kept in Eppendorf tubes labeled with the necessary specifications. After blood sampling, the samples were centrifuged with refrigerated centrifuge (3500 rpm/15 min), then the serum of blood was removed using microsampler. The serum osmotic pressure immediately measured by osmometer (ROBELING, NR-13, GERMANI) and the value expressed as milliosmoles per liter (mOsm L^{-1}) (Keys and Hill, 1934; Sayyad Bourani, 2007).

Statistical analysis

The treatments were laid out in factorial on the basis of completely randomized block design (RCBD). All the treatments were analyzed in triplicate. Treatments means were compared by using Duncan’s multiple range test (DMRT) (P<0.05). Data are presented as mean ± SE (standard error).

RESULTS

Water physical and chemical parameters

The results of water physical and chemical parameters are tabulated and shown in Table 1. During the experiment, the qualities of waters were kept constant by daily changes of water (20%) and the presence of the aeration system.

Rate of mortality

The rates of mortality (in percentage) of the fingerlings are shown in Figure 2. The highest mortality belongs to 1 g kutum preserved in the CSW treatment (13.44%), followed by the same weight group kept in EW (10.62%). The lowest mortality was for the 5 g kutum fingerlings in FW treatment.

Blood osmotic pressure changes in 1 g kutum

The results for osmotic pressure changes in 1 g kutum in the CSW and EW treatments indicated a significant increase in time duration of 0, 12, 48, 24, 144, 240 and 360 h compared to the control treatment (Figure). In the CSW treatment, the blood osmotic pressure level at 0 h (immediately after release) was measured as 303.7

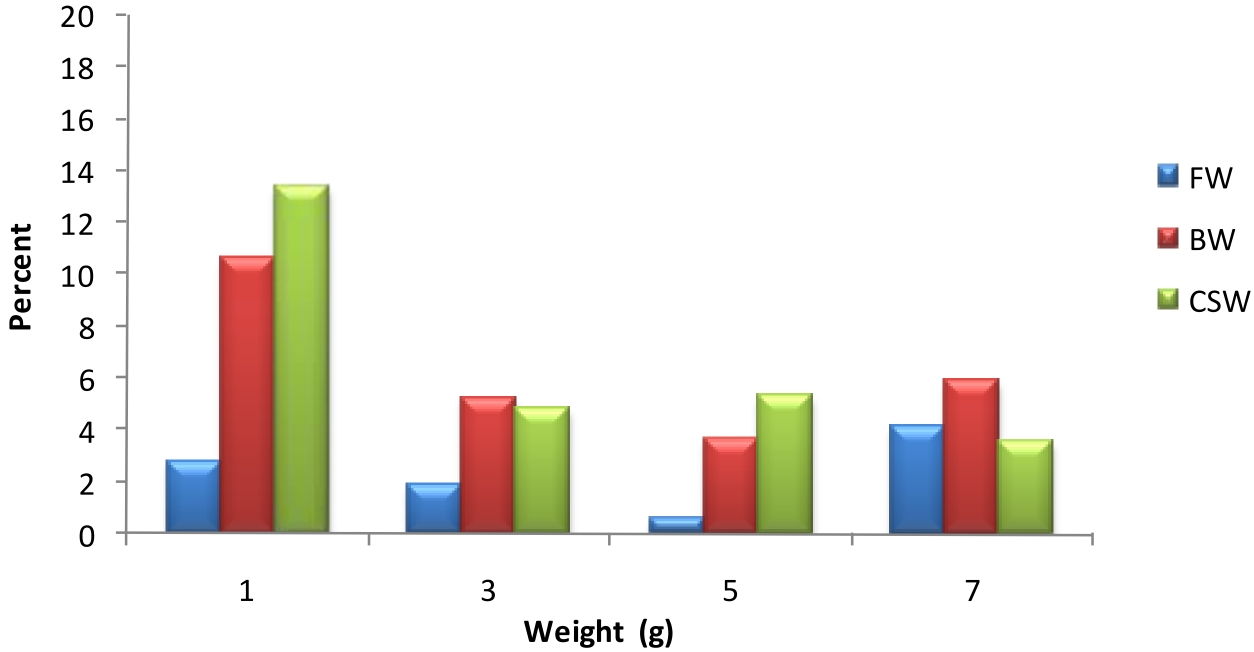

Figure 2. Percentage of mortality of the fingerling kutum in fresh water (FW), estuary water (EW) and Caspian Sea water (CSW) treatments during 336 h.

mOsm L^{-1} (15.02% difference with control level). After 24 h, it reached a maximum level of 325.7 mOsm L^{-1} (23.51%) before showing a descending trend until 48 h and reaching 281.3 mOsm L^{-1} (7.79%). Then an ascending trend from 48 to 72 h was recorded. A fluctuating trend up to the end of the experiment (336 h) was observed. After 336 h, 22.26% difference with osmotic pressure level of FW treatment (control) was found.

The changes for EW (7 ppt) treatment were higher than the control in all experimental times, in which the maximum change was 21.36% (320 mOsm L^{-1}) over 48 h. After 14 days, osmotic pressure level was 302.0 mOsm L^{-1} namely with 15.27% difference compared to the control level. Statistical analysis showed that the osmotic pressure level of CSW treatment was significantly higher than EW treatment in the time series of 24, 96 and 336 h ($p<0.05$). Comparing CSW and EW treatments, it can be mentioned that 1 g kutum have higher adaptation to estuarine water (Figure 3).

Blood osmotic pressure changes in 3 g kutum

In this weight groups, level of serum osmotic pressure in the CSW treatment (13 ppt) at time series of 0, 24, 48, 72, 96 and 144 h increased compared to the control level with values of 18.8, 15.18.9, 13.89, 20.99, 17.75 and 19.35%, respectively. The maximum changes were found after 48 h of transferring from freshwater to Caspian Sea water (20.99% difference with control). For the period of 120 up to 336 h, this weight group decreased in the osmotic pressure to a level without any significant difference from the values with FW treatment.

According to Figure 4, it can be seen that after transferring the fingerlings from FW to EW in time durations of 0, 12, 24, 48, 72 and 96 h, the osmotic pressure increased significantly. On the contrary, throughout the 12 to 24 h period, the trend was descending as seen in a change from 309.7 mOsm L^{-1} (16.56%) in 12 h to 282 mOsm L^{-1} (6.68%) at 24 h. However, it was a temporary decrease after which it increased to 301 mOsm L^{-1} (15.62%) in 48 h. At the end of the experiment, 240 and 336 h, the osmotic pressure level reached the control level ($P>0.05$).

Blood osmotic pressure changes in 5 g kutum

The osmotic pressure changes in 5 g kutum in CSW treatment showed significant increase in time durations of 0, 12, 24, 48 and 72 h compared to the control treatment (Figure 5). The maximum differences were found at 12, 48 and 72 h ($P<0.05$). As shown in Figure 5, there is a sharp decrease during 12 to 24 h, which can be due to sudden decrease of blood ions. In EW (7 ppt) treatment, significant increases were observed in 0, 12, 24, 48 and 72 h compared to the control treatment ($P<0.05$). The maximum change in proportion to the primary level was observed for 48 h (10.79%). There were no significant changes observed among different salinity treatments of FW, EW and CSW in 96, 144, 240 and 336 h duration. This shows the ability of 5 g kutum to decrease the osmotic pressure within the 4 days (96 h) period.

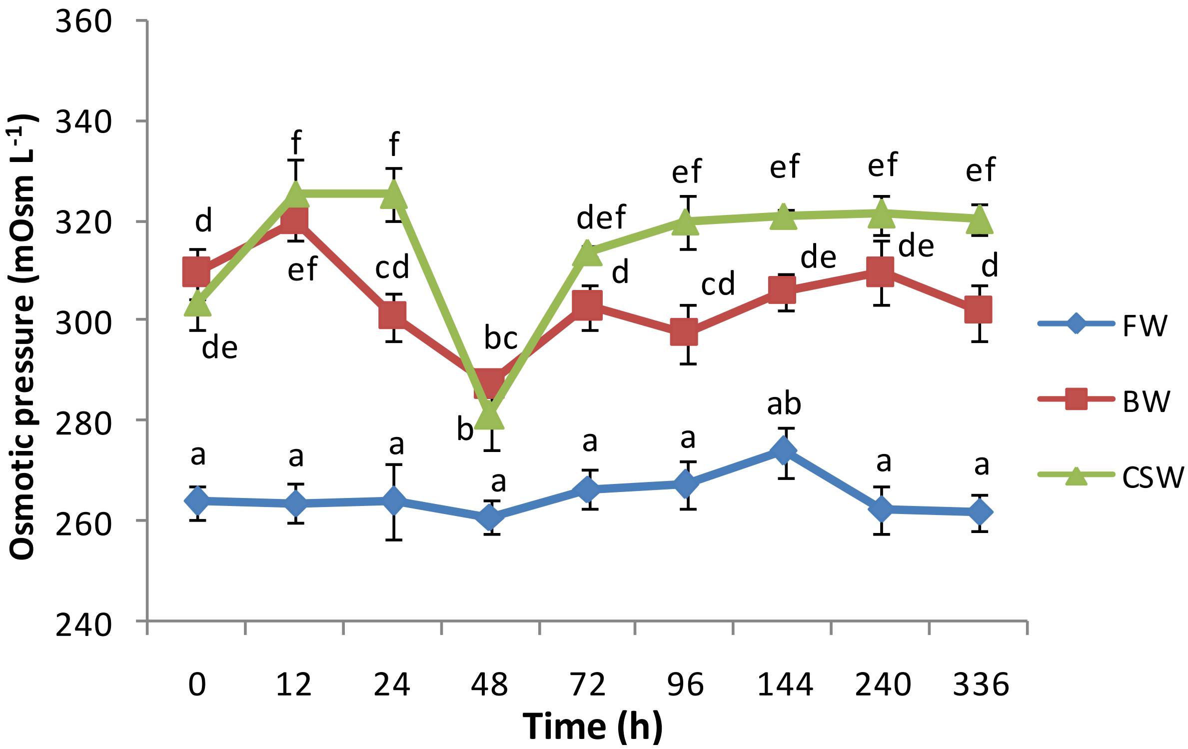

Figure 3. The trend of osmotic pressure changes in 1 g kutum and in different salinity treatments: FW (fresh water, 0.5 ppt), EW (estuarine area water, 7 ppt), CSW (Caspian Sea water, 13 ppt). Data are presented as means ± SE; means with similar letters are not significantly different (P>0.05).

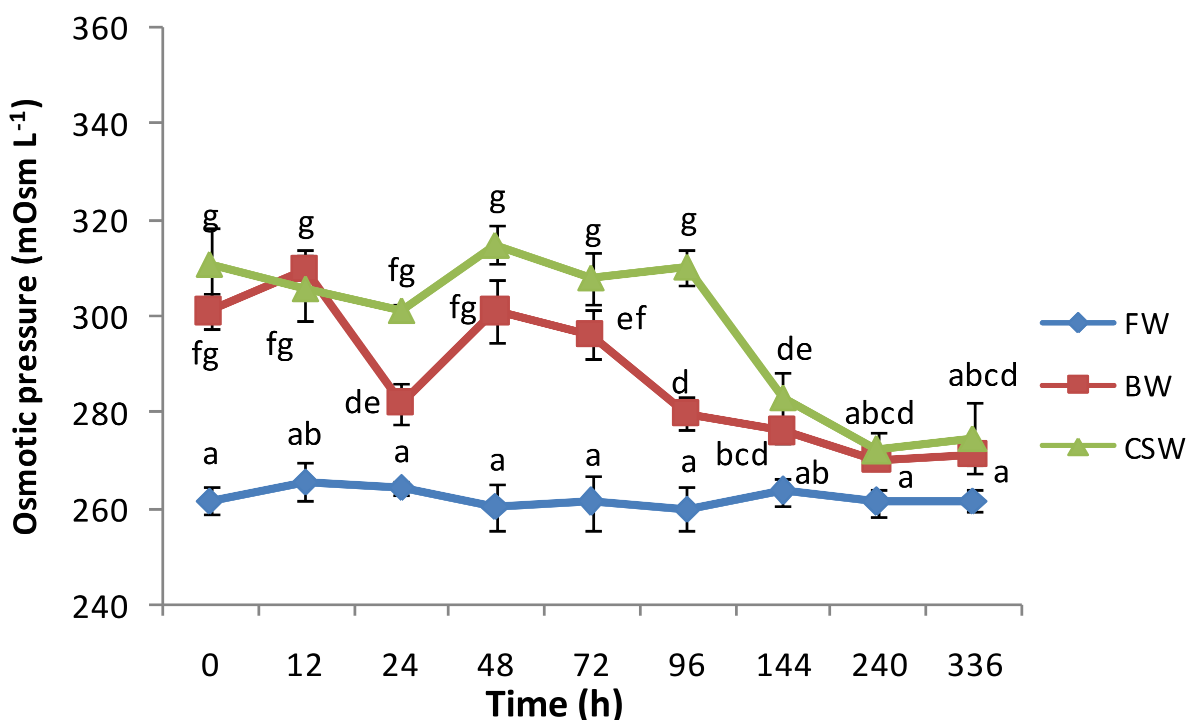

Figure 4. The trend of osmotic pressure changes in 3 g kutum and in different salinity treatments: FW (fresh water, 0.5 ppt), EW (estuarine area water, 13 ppt), CSW (Caspian Sea water, 11 ppt). Data are presented as means ± SE; means with similar letters are not significantly different (P>0.05).

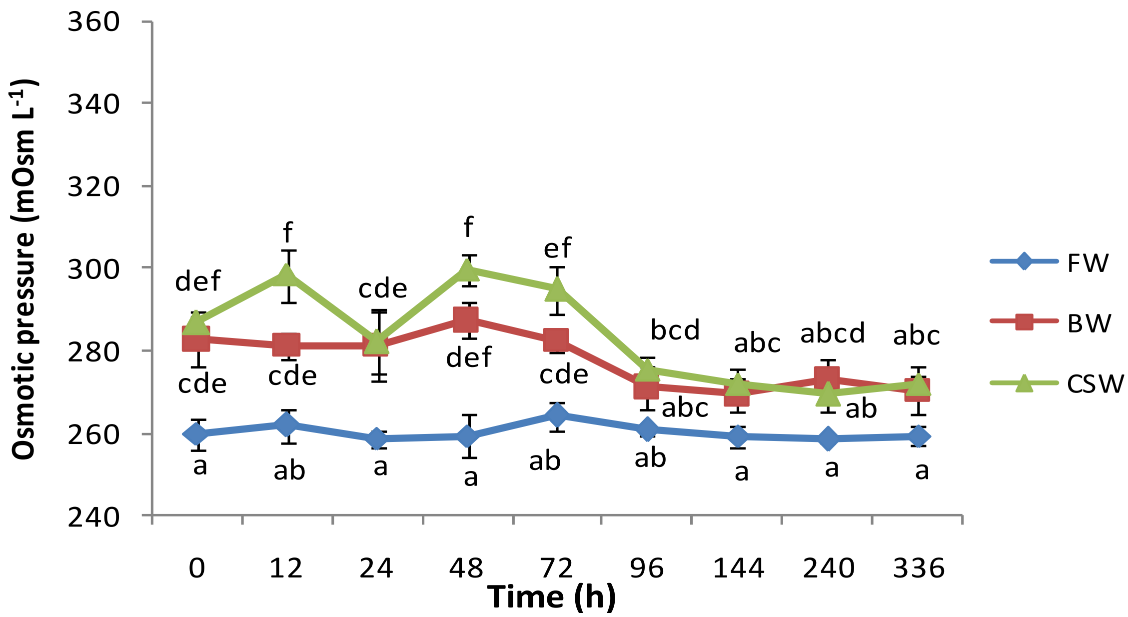

Figure 5. The trend of osmotic pressure changes in 5 g kutum and in different salinity treatments: FW (fresh water, 0.5 ppt), EW (estuarine area water, 13 ppt), CSW (Caspian Sea water, 11 ppt). Data are presented as means ± SE; means with similar letters are not significantly different (P>0.05).

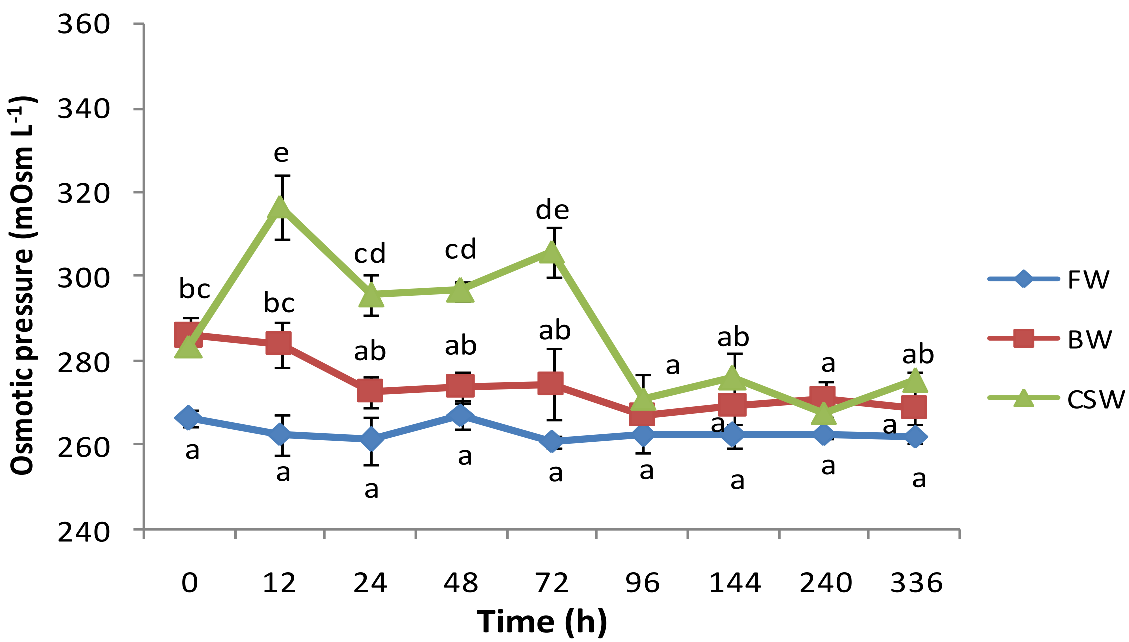

Figure 6. The trend of osmotic pressure changes in 7 g kutum and in different salinity treatments: FW (fresh water, 0.5 ppt), EW (estuarine area water, 13 ppt), CSW (Caspian Sea water, 11 ppt). Data are presented as means ± SE; means with similar letters are not significantly different (P>0.05).

Blood osmotic pressure changes in 7 g kutum

The amount of osmotic pressure in 7 g kutum in CSW and EW treatment is presented in Figure. Results for EW treatment showed that except 0 and 12 h, there is no significant difference between the level of blood osmotic pressure in all the experiment times and control level. In CSW treatment, the level of serum osmotic pressure was higher than EW and FW treatments at 12, 24, 48 and 72 h, significantly (P<0.05). The maximum increase was observed in the time period of 12 h with value of 20.55%. A comparison between three treatments of CSW, EW and FW during the experimental period showed that 7 g kutum fingerlings were successful in adapting themselves to new conditions with 7 and 13 ppt salinities.

DISCUSSION

One of the most important methods to determine the suitable downstream migration time in juvenile fish is to study the osmoregulation ability. Determination of this physiological index will help us to find the downstream migration time and also select the suitable weight for release and sea culture. This study attempted to determine these facts for *R. frisii kutum* fingerlings via investigation of the level of their blood osmotic pressure.

Considering the results of this study and other articles, it should be noted that the ionic and osmotic changes after transferring the fingerlings to sea water occur in two stages and are affected by two mechanisms (Krayushkina et al., 1999; Sayyad Bourani et al., 2009). In the first stage, blood osmotic pressure of the fingerlings in the primary days increased after transferring to sea water (Nikoo et al., 2010). When fish ingest sea water into their gut, the mono and divalent ions are absorbed into the blood through gut system membranes and also a high level of ions is transferred via gills. This causes the sudden increase of blood osmotic pressure (Sayyad Bourani 2007; He et al., 2009; Hwang et al., 2009). Studies showed that in this stage, the first mechanism to be activated are related to the activity of stress hormones (example cortisol) which increase the renal-gill filtration phenomenon and lead to decrease in the amount of blood ions (McCormik and Naiman, 1984; McCormik and Sanders, 1987). This process caused a temporary decrease in the blood osmolarity and subsequently, the blood osmotic pressure increased after 24 h (Figures 3 to 6). Since this mechanism is temporary and insufficient, it is replaced by a major sustainable and well-developed mechanism that can establish a permanent homeostasis. In this way the second mechanism or osmoregulation system is activated (Krayushkina et al., 1999). Studies indicated that in the second mechanism stage, mostly the monovalent ions are transferred in cooperation with ATPase enzyme through gills (Uchida et al., 2000; Hwang, 2009) compared to lower concentration of divalent ions that are excreted through the kidney (Beyenbach, 2000). In the interval between the end of the first stage and the beginning of the second stage, there is a sensible decrease and then an increase of osmotic pressure (Figures 3 to 6). This sensible decrease is due to the activity of the primary (first) mechanism and the following increase is due to termination of this short-term mechanism (Sayyad Bourani, 2007).

This trend (decrease and fast increase) in the 1 g weight group was recorded in time durations of 24 to 72 and 12 to 72 h for CSW and EW treatment, respectively. However, this fact for both salinity treatments (CSW and EW) of 3 and 5 weight groups occurred in durations of 12 to 48, while for 7 g kutum was found out during 12 to 72 h, respectively. Based on all measurements, the first mechanism progressed up to 48 h in all weight groups and then the activity of the second mechanism is started (Figures 3 to 6). The statistical results show that 3, 5 and 7 g kutum were able to decrease the blood osmotic pressure after 48 h in both CSW and EW treatments. It can be concluded that they were successful in expelling the access ions in opposition to the gradient slop (Figures 4 to 6). However, the results show that 1 g kutum did not have enough ability to decrease the blood osmotic pressure in the time duration of 48 to 336 h (Figure 3). This means that hypo-osmoregulation did not occur because of lack of second mechanism activity, and it can be concluded that this weight group was not physiologically ready for downstream migration.

With respect to the results of the blood osmoregulation of the fingerling, the high mortality rate observed in 1 g kutum could be justified (Figure 2). After 7 days exposure to sea water, a significant increase in mortality rate in the least weight group of *Salvelinus alpinus* was also found by Halverson et al. (1993). Also, Enayat Gholampoor et al. (2011) showed that 1 g kutum during 60 days exposure with 7 and 13 ppt salinity were unable to increase growth indices (daily growth, specific growth and weight gain) as well as 2 and 4 ppt salinity treatments significantly. In addition, Ugedal et al. (1998), McCormik and Sanders (1987) and McCormik and Naiman (1984) indicated that migration time for juvenile fishes depends on their weight and size. Furthermore, Sayyad Bourani et al. (2006, 2009) reported that the fingerlings of Caspian Sea trout with weights less than 5 g, could not adapt to the Caspian Sea salinity. Sayyad Bourani et al. (2007) showed the levels of the osmotic pressure for 20, 15, 10 and 5 g kutum after 240 h exposure in the Caspian Sea water were measured as 329, 321, 325.3 and 346.5 mOsm L^{-1}, respectively. Osmoregulatory ability of 7-month-old juvenile Chinese sturgeon (*Acipenser sinensis* Gray) was studied by He et al. (2009). The results mentioned that the fish was successful in adapting themselves to brackish water (10 ppt), significantly. They also revealed the fact that the level of serum osmotic pressure significantly decreased during the 10 days after transferring to brackish water compared to first days.

Boeuf et al. (1982) showed that smoltified salmonids could reduce the osmotic stress, while brook trout yearlings were unable to control the osmotic stress. Similar to results of our study, Arnesen et al. (1993) showed that osmoregulation in *Salvelinus alpinus* depends on their weight. They indicated that higher osmoregulation ability occurs in large weight groups. Parry (1985) and McCormik and Naiman (1984) proved that the contributory factors to the survival of the fingerlings in seawater adaptation are “species and size”. It means each species has different osmoregulation ability.

Conclusion

In general, it can be concluded that the osmoregulatory ability of juvenile kutum depends on their weights. Despite, high ability of brackish water adaptation in 3, 5 and 7 g kutum, 1 g fingerlings were not able to adapt to the Caspian Sea and estuarine waters. Therefore, they cannot be considered as suitable sizes to be released into the brackish and marine environments.

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