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Ionic Regulation Ability in *Rutilus frisii kutum* Fingerlings During Sea Water Adaptation

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ABSTRACT

Experiments were conducted to determine the effects of weight on the ionic regulation ability of reared *Rutilus frisii kutum* fingerlings during adaptation to the seawater and downstream migration. Accordingly, the ionic regulation ability of Cl^-, K^+, Na^+ and Mg^{2+} in kutum fingerlings with weights of 1, 3, 5 and 7 g in three different salinities, that is 13‰ (the Caspian Sea salinity), 7‰ (estuarine area) and fresh water (as control, 0.3-0.5‰), were assessed. The blood samples were provided before being transferred as control (fresh water) and during adaptation to the sea and estuary water in a period of up to 336 h by a pooling method. The measurements of ions were carried out for blood serum Na^+ and K^+ and alsoplasma Cl^{-1} and Mg^{2+} by photometric methods. This investigation showed that ionic regulatory ability of kutum fingerlings depends on their weights. Results of ionic changes during the duration of 336 h (14 days) proved that unlike kutum fingerling with weights of 3, 5 and 7 g, the ionic regulation system in 1 g fingerlings were not able to expel excess ions. Further 1 g kutum were not physiologically ready (smolt) for downstream migration.

Key words: *Rutilus frisii kutum*, ionic regulation, Caspian Sea

INTRODUCTION

The *Rutilus frisii kutum* (Kamenskii 1901) commonly known as Caspian with fish is one of the most important species in the southern Caspian Sea (Keyvan *et al*., 2008). They are mostly found in the Iran and Azerbaijan's maritime borders (Kavan *et al*., 2009), especially in the area between Astara and the Gorgan River (Valipour *et al*., 2008; Valipour and Khanipour, 2009). Kutum is an anadromous fish that spends most of its adult life in seawater (Caspian Sea) and migrates to fresh water rivers and lakes to reproduce. Juvenile kutum spends smoltification process in the rivers before migrating to the Caspian Sea. Adult kutum finally returns to the rivers (fresh water) for spawning. However, the sustained overexploitation, changes in natural environment and destruction of spawning grounds of the endangered species in the past decades inevitably led to a decline in fishing (Razavi, 1995, 1998; Abdolhay *et al*., 2011).

As a result, the Iranian Fisheries Research Organization (IFRO) tried to reproduce them for rehabilitation and restocking. From 1979 to 2008, over 3,000 million kutum fingerlings were released into the Caspian Sea but due to the lack of scientific evidence the survival rate remains unclear (Abdolhay *et al*., 2011). Currently, 0.3-1 g kutum are released into rivers and estuary area without any reliable scientific background (Bartley and Rana, 1998; Abdoli, 1999; Abdolhay *et al*., 2011). While much is known regarding the physiological changes that occur during the upstream migration of adult kutums (Nikoo *et al*., 2010), information about the migration of juvenile kutums from freshwater to seawater is lacking. Parry (1961) stated that early and inappropriate time of release, results in mortality or reduces the rate of upstream migration in salmonid fishes. Seawater-exposed fish (*Oncorhynchus kisutch*) which are not prepared for the increased salinity showed large perturbations in plasma ions, decreased survival rate (Shrimpton *et al*., 1994) and reduced swimming performance (Brauner *et al*., 1992; Shrimpton *et al*., 2005). Selecting a suitable weight or size (a fully smoltified fish) for release into seawater is crucial in increasing the survival rate (Sigholt *et al*., 1995; Ugedal *et al*., 1998; Bourani *et al*., 2006). McCormick and Naiman (1984), Parry (1958) and Bourani *et al*. (2006) showed that the contributory factors to the survival of fingerlings in seawater adaptation are species and size.

Once the juvenile fish is released into seawater (hypertonic water), the level of blood ions increases. Therefore their size should have the corresponding ability for ionic regulation. Subsequently, the juvenile fish must be able to expel the extra blood ions (active transport) and keep the blood homeostasis stable via gill chloride cells and kidney (Krayushkina *et al*., 1996). If during the release process the fingerlings are not able to expel the ions due to lack of osmoregulation ability, their blood ions will increased (Nonnotte and Truchot, 1990). Rounsefell (1958) revealed that ionic regulation of Na^+, Cl^- and Mg_2^+during adaptation and survival period in seawater are strongly related to the fish size.

Very limited information is available regarding ionic regulation and osmoregulation of kutum (Nikoo *et al*., 2010). This study aims to analyze the impact of weight on regulating Cl^-, Mg^+, K^+ and Na^+ ions during seawater adaptation. The 1, 3, 5 and 7 g kutum fingerlings as recommended by IFRO (Iranian Fisheries Research Organization) were selected for analyzing the ionic changes during adaptation in Estuarine Water (EW) and Caspian Sea water (CSW). As it is not economically feasible to keep fingerling kutum for a long time, the least weight with ionic regulation ability should be selected for release.

MATERIALS AND METHODS

Rearing system: Kutum fry were prepared from IFRO hatchery, Rasht, Iran and transferred to earthen ponds in Sefidroud Fisheries Research Center laboratory, Astaneh, Iran and kept for 3 months. They were fed two times daily with Special Food for Kutum (SFK) (40 protein, 12 lipid, 13 ash and 30 carbohydrate; Mahdaneh, SFK, Karaj, Iran) to attain 1 to 7 g of weight (Valipour and Khanipour, 2009). Subsequently, the selected weight group, was sorted by a manual sorter (SADAF, Iran) and kept in 500 L fiberglass tanks, equipped with water circulation (inlet and outlet) and aeration. After two weeks of acclimatization, each target group was precisely weighed and divided in three groups. Group 1 was transferred directly from 500 to 100 L FW tanks as control. Groups 2 and 3 were transferred from 500 L tank to CSW and EW 100 L tanks as 13 and 7‰ treatments. Three replicates were performed for each treatment. All the tanks were equipped with an aeration system adapted from McCormick and Naiman (1984) and all treatments were exposed to a 12 h light-dark photoperiod using overhead fluorescent lights. During the

experiment, feeding was done once per day with SKF (40% protein, 12% lipid, 13% ash and 30% carbohydrate; Mahdaneh, SFK, Karaj, Iran) based on 7% body weight (Abdoli, 1999). Water changes were carried out daily on a level of 20% and the tanks were cleaned once a day in order to remove uneaten feed and feces.

Water source, treatment and quality: To prepare different salinity treatment waters, CSW 13‰ was collected from the Caspian Sea at depths of 2-3 m and 50 m from the shore while estuarine water (7‰) was collected from the Sefidrud River mouth and inshore of the Caspian Sea. Fresh water was obtained by bore hole on the Sefidrud River (<0.5‰) at 70 m depth. Treatment waters (FW, EW, CSW) were filtered and then transferred into 100 L tanks (60 L water per tank). The important physical and chemical parameters such as temp, DO, pH, nitrite and NH_4^+were measured. Salinity of tanks was monitored once a day (ASTM, 1989).

Blood sampling and analysis: Kutum fingerlings were anaesthetized by immersion in Tricaine Methanesulphonate (MS-222) at 200 mg L^{-1}. Their body weights and lengths were measured by electronic balance (OHAUS, China) and biometric board, respectively (Table 2). Approximately 1.5-2.0 mL blood were taken from the caudal vein of kutum fingerlings in durations of 0, 24, 48, 96, 144 and 336 h, using capillary tubes (McCormick and Naiman, 1984; Madsen and Naamansen, 1989; Avella *et al*., 1990; Seidelin *et al*., 2000). The blood samples were preserved in special eppendorf tubes labeled with treatment specifications. Due to small amounts of blood sample from each fish, blood samples from 5-12 specimens, belonging to the same treatment and with similar conditions were used as a single pooled sample (Bower and Evelyn, 1988; Jones *et al*., 1993). The blood samples were immediately transferred to centrifuge (5,500 rpm/5 min) and then the plasma was extracted by microsampler and kept in Eppendorf tubes. All the tubes were kept frozen at -18°C for further analysis (McCormick and Naiman, 1984).

The kutum fingerling plasma magnesium was measured by Xylidyl blue colorimetric method. The concentration of magnesium in plasma was analyzed by autoanalyzer (TECNICON, RA100, USA) and expressed as $^{mEq}/_{L}$ (Thomas, 1998; CLSI, 2008). Plasma chloride was measured using a colorimetric, mercury thiocyanate method. The amount of chloride was determined and recorded by autoanalyzer (TECNICON, RA100, USA) and expressed as $^{mEq}/_{L}$ (Henry *et al*., 1974). The concentration of the blood sodium and potassium (for the different treatments at different hours) were measured by flame photometer (BWB, UK) and expressed as $^{mEq}/_{L}$ (Williams and Twine, 1960).

Statistical analysis: The treatments were laid out in factorial on the basis of completely Randomized Block Design (RCBD) and analyzed in triplicate. Treatment means were compared by using Duncan's Multiple Range Test (DMRT) ($p<0.05$). Data into the tables are presented as Mean±SE (St andard Error).

RESULTS

Physical and chemical parameters: With daily changes of water (about 20%) and continuous aeration, water quality parameters were kept constant; pH values of 7.9-8.1; DO in a level of 8.4-9.5 mg L^{-1}; NO2 in a range of 0.0067-0.0110 mg L^{-1}; NH_4^+ about 0.01 mg L^{-1} (Table 1).

Biometry: The mean weight and length of kutum fingerlings in weight groups of 1, 3, 5 and 7 g in different salinity water treatments, consisting of CSW (Caspian Sea Water), EW (Estuary Water)

Table 1: Physical and chemical parameters of the water; Values are expressed as Mean±SE

Treatments	pH	Salinity (‰)	O_2 (mg L^{-1})	NO_2 (mg L^{-1})	NH_4 (mg L^{-1})
Caspian Sea water	8.04±0.09	12.68±0.70	8.37±0.64	0.0067±0.019	0.010±0.003
Estuary water	7.90±0.11	7.10±0.86	9.31±0.02	0.0076±0.003	0.013±0.05
Fresh water	8.10±0.16	0.35±0.03	9.46±1.15	0.0110±0.022	0.014±0.007

Table 2: Mean weight and length of 1, 3, 5 and 7 g fingerling kutum in the treatments of caspian sea water (CSW), estuary water (EW) and fresh water (FW); Values are expressed as Mean±SE

Treatment	Weight group (g)	Number	Mean weight (g)	Mean length (cm)
Seawater	7	91	7.12 ± 0.10^{d}	8.04 ± 0.14^{a}
	5	126	5.0 ± 0.07^{c}	7.73 ± 0.10^{bc}
	3	139	3.44 ± 0.076^{b}	6.98 ± 0.89^{cd}
	1	192	1.46 ± 0.058^{a}	5.41 ± 0.06^{d}
Estuary water	7	87	7.16 ± 0.13^{d}	8.49 ± 0.17^{a}
	5	99	5.16 ± 0.89^{c}	7.71 ± 0.11^{bc}
	3	147	3.32 ± 0.067^{b}	7.04 ± 0.07^{cd}
	1	187	1.51 ± 0.062^{a}	5.35 ± 0.06^{d}
Fresh water	7	94	7.21 ± 0.52^{d}	7.90 ± 0.85^{a}
	5	117	5.28 ± 0.33^{c}	7.83 ± 0.35^{b}
	3	141	3.41 ± 0.32^{b}	6.70 ± 0.22^{cd}
	1	181	1.49 ± 0.13^{a}	5.62 ± 0.20^{cd}

*Different small letters at the same columns present significant difference between mean weights ($p<0.05$)

and FW (Fresh Water) are presented in Table 2. Statistical analysis revealed that there were no significant differences between the mean weights of each group in the different salinity treatments ($p>0.05$).

Rate of mortality: Figure 1 shows the percentage of mortality among kutum fingerlings in Fresh Water (FW), estuary water with salinity of 7 ‰ (EW) and seawater with salinity of 13 ‰ (CSW) in weight groups of 1, 3, 5 and 7 g throughout the 336 h. As evident from Fig. 1, the highest mortality rate was 12.41%, belonging to the weight group of 1 g treated with Caspian Sea water. The second highest rate of mortality recorded was 9.67%, also belonging to this weight group (1 g) in 7‰ salinity of water. The weight groups of 3 and 7 g were found to have the lowest rate of mortality in the water treatment of the CSW (Caspian Sea water). Further, the lowest rate of mortality during the experimental period of EW (estuary water) treatment was recorded in 5 g kutum.

Changes in ionic concentration of plasma and serum: The results of the changes in Na^+ for different treatments are summarised in Table 3. The concentration of Na^+ for 1 g fingerling kutum in Caspian Sea water salinity treatment (13‰), at time series of 0, 24, 48, 96, 144 and 336 h increased compared to the control level (fresh water) with values of 2.7, 8.9, 8.4, 13.89, 10.63 and 10.47%, respectively. The concentration of blood Na^+ recorded were 134.7, 141.4, 140.8, 148.1, 143.9 and 144.9 $^{mEq}/_{L}$, respectively. Meanwhile, in the other weight groups (3, 5, 7 g), the level of Na^+ showed an ascending trend up to 48 h but subsequently decreased until it reached the control level (FW). The maximum level in CSW treatment occurred after 96 h in the 1 g weight group (148.1 $^{mEq}/_{L}$). With respect to EW treatment (7‰) for the weight group of 1 g after 48 h, the concentration showed an increase upon reaching 138.8 $^{mEq}/_{L}$ (5.97% different from control level) at 336 h. Likewise, the maximum level in EW treatment observed was 141.7 $^{mEq}/_{L}$ (8.91%) at 96 h.

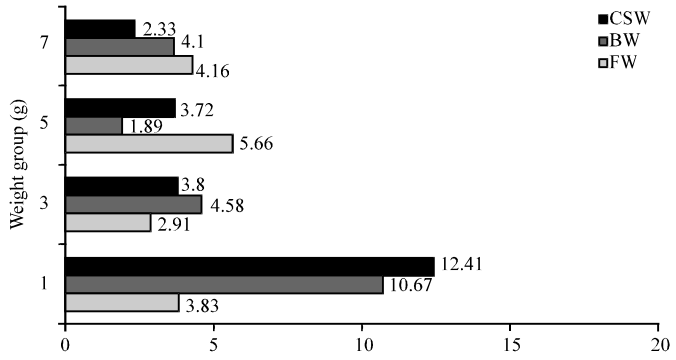

Fig. 1: Percentage of mortality of the fingerling kutum in fresh water (FW), estuary water (EW) and Caspian Sea water (CSW) treatments during 336 h

Table 3: Concentration of blood Na^+ for 1, 3, 5 and 7 g kutum in different Salinity treatment: Fresh water (FW), the estuary water (EW) and Caspian Sea water (CSW); Values are expressed as Mean±SE

		Time (h)					
Weight (g)	Water	0	24	48	96	144	336
1	FW	131.1±1.8^{a}	129.7±3.2^{a}	129.4±0.7^{a}	130.0±2.0^{a}	130.1±0.1^{a}	131.1±0.9^{a}
	EW	131.6±0.8^{a}	137.0±1.7^{a}	138.1±1.2^{de}	141.7±1.7^{e}	138.6±1.3^{g}	138.8±2.5^{d}
	CSW	134.7±1.3^{a}	141.4±2.6^{a}	140.8±0.2^{e}	148.1±0.7^{f}	143.9±0.7^{h}	144.9±1.6^{e}
3	FW	134.0±1.3^{a}	132.7±1.7^{a}	130.1±0.3^{a}	134.3±0.5^{bc}	133.8±0.6^{bcd}	134.0±0.5^{abc}
	EW	134.9±0.7^{a}	136.1±0.5^{a}	135.6±0.2^{cd}	135.7±0.50^{cd}	134.9±0.2^{cde}	135.9±0.4^{bcd}
	CSW	134.5±0.3^{a}	141.2±1.7^{a}	141.0±1.1^{e}	138.5±0.7^{de}	135.1±0.5^{def}	135.6±0.7^{bcd}
5	FW	132.2±0.4^{a}	132.3±0.3^{a}	129.2±0.4^{a}	132.1±0.6^{ab}	132.2±0.3^{ab}	132.7±0.3^{ab}
	EW	133.1±0.4^{a}	136.0±1,0^{a}	135.4±0.5^{cd}	135.8±0.3^{cd}	137.3±0.2^{fg}	136.0±1.5^{bcd}
	CSW	133.0±0.2^{a}	137.9±0.8^{a}	135.9±0.7^{cd}	135.2±1.3^{bc}	134.2±1.2^{bcd}	135.7±0.3^{bcd}
7	FW	133.0±1.0^{a}	133.1±0.4^{a}	132.3±0.3^{ab}	132.2±1.4^{ab}	132.5±0.4^{bc}	132.8±0.2^{ab}
	EW	133.8±0.4^{a}	138.5±0.3^{a}	133.9±0.2^{bc}	135.1±0.2^{bc}	133.8±0.5^{bcd}	134.4±0.7^{abc}
	CSW	134.3±0.2^{a}	138.7±0.7^{a}	135.1±0.6^{e}	134.2±0.2^{bc}	134.7±1.3^{cd}	135.3±0.2^{bcd}

Means with similar letters at the same columns are not significantly different ($p>0.05$). Based on two-way ANOVA, no significant interaction was found in 0 and 24 h

Statistical analysis on the level of Na^+showed that there were significant interactions between weight groups and salinity treatments during 48, 96, 144 and 336 h. After 14 days, the highest level of sodium was found in 1 g fingerling kutum compared with 3, 5 and 7 g weight groups. The results of the study on the amount of plasma sodium in EW and CSW treatment showed that the concentration of Na^+ in 1 g fingerlings, unlike other groups, did not decrease as much as the control group (fresh water) level.

The changes in Mg^{2+} concentration for different salinity and weight treatments are shown in Table 4. In CSW treatment, the level of Mg^{2+} at all the experimental times-except zero hour showed significantly higher values compared to the control (fresh water) level, where the maximum increase of $4.92^{mEq}/_{L}$ (123.1%) was observed after 24 h. The sole increment in EW treatment was observed after 24 h with 1.89-fold (89.42%) of changes, compared with the control group level. The maximum difference with fresh water (control) treatment (214%) was found in 96 h. Significant interaction between the weight groups and salinity treatments were found in 24, 48, 96, 144 and

336 h. After 336 h, the maximum level of blood Mg^{2+} in CSW treatment was observed in 1 g kutum compared with other weight groups. Likewise, the statistical analysis showed that the concentration of Mg^{2+} in 3, 5 and 7 g kutum reached the primary level. There was no significant difference between FW, EW and CW treatments after 336 h ($p>0.05$).

The changes in Cl^{-1}-level in kutum fingerlings are presented in Table 5. Statistical analysis (two-way ANOVA) revealed that there were significant interactions between weight groups and salinity treatments during 0, 48, 96, 144 and 336 h. Concentration of chloride for 1 g kutum in CSW and EW treatments showed significant increase over the FW treatment, during the duration of 48, 96, 144 and 336h ($p<0.05$). The concentration of Cl^{-1} in 1 g kutum was recorded at 118.1 $^{mEq}/_{L}$ (16.43%) for CSW and 113.3 $^{mEq}/_{L}$ (11.66%) for EW treatments after 14 days (336 h). Also, the statistical analysis showed a significant difference between the levels of chloride in CSW and EW.

Table 4: Concentration of blood Mg^{+2} for 1, 3, 5 and 7 g kutum in different Salinity treatment: fresh water (FW) or control, the estuary water (EW) and the Caspian Sea water (CSW); Values are expressed as Mean±SE

		Time (h)					
Weight (g)	Water	0	24	48	96	144	336
1	FW	2.43±0.49^{a}	2.21±0.10^{a}	1.64±0.13^{a}	1.28±0.16^{a}	1.31±0.09^{a}	2.01±0.29^{a}
	EW	1.45±0.36^{a}	4.18±0.25^{def}	4.14±0.14^{cd}	4.01±0.18^{cde}	3.40±0.42^{bcd}	3.61±0.19^{de}
	CSW	1.19±0.16^{a}	4.92±0.04^{g}	4.51±0.05^{de}	4.88±0.13^{fg}	4.71±0.24^{f}	4.80±0.23^{h}
3	FW	2.96±0.03^{a}	2.85±0.04^{b}	2.66±0.37^{b}	2.98±0.13^{b}	2.91±0.23^{b}	2.94±0.07^{b}
	EW	3.05±0.03^{a}	4.14±0.15^{def}	4.68±0.10^{de}	3.07±0.13^{b}	3.06±0.08^{bc}	3.09±0.16^{bcd}
	CSW	3.15±0.23^{a}	3.74±0.37^{cd}	5.03±0.25^{e}	3.52±0.29^{bcd}	3.15±0.09^{bc}	3.11±0.24^{bc}
5	FW	3.06±0.13^{a}	3.05±0.05^{b}	3.04±0.08^{b}	3.11±0.09^{b}	3.11±0.04^{bc}	3.06±0.06^{bc}
	EW	3.33±0.21^{a}	3.31±0.23^{bc}	4.14±0.23^{cd}	4.13±0.18^{de}	3.54±0.16^{cd}	3.58±0.09 ^{cde}
	CSW	3.42±0.03^{a}	4.64±0.22^{fg}	6.01±0.16^{f}	3.41±0.35^{bc}	4.25±0.06^{ef}	3.25±0.26^{c}
7	FW	3.88±0.03^{a}	3.80±0.03^{cde}	3.92±0.05^{c}	4.01±0.24^{cde}	3.74±0.05^{de}	3.90±0.05^{ef}
	EW	4.11±0.05^{a}	4.32±0.13^{ef}	4.63±0.04^{de}	4.49±0.26^{ef}	4.15±0.05^{ef}	4.22±0.07^{fg}
	CSW	4.24±0.13^{a}	4.64±0.03^{fg}	5.84±0.22^{f}	4.45±0.08^{ef}	4.63±0.08^{f}	4.13±0.06^{f}

Means with similar letters at the same columns are not significantly different ($p>0.05$). Based on two-way ANOVA, no significant interaction was found in 0 h

Table 5: Concentration of blood Cl-for 1, 3, 5 and 7 g kutum in different Salinity treatment: fresh water (FW), the estuary water (BW) and the Caspian Sea water (CSW); Values are expressed as Mean±SE

		Time (h)					
Weight (g)	Water	0	24	48	96	144	336
1	FW	100.9±0.3^{a}	101.0±0.2^{a}	101.3±1.0^{a}	101.0±0.3^{a}	100.8±0.3^{a}	101.4±1.0^{a}
	EW	102.0±0.2^{ab}	109.7±1.0^{a}	110.1±1.0^{cd}	114.4±1.1^{e}	113.2±1.1^{e}	113.3±0.8^{e}
	CSW	102.8±1.2^{bcd}	110.7±0.1^{a}	111.5±0.4^{d}	117.9±1.0^{f}	117.7±1.5^{f}	118.1±0.2^{f}
3	FW	102.9±0.3^{bcd}	102.3±1.0^{a}	102.2±0.2^{a}	102.5±0.7^{ab}	102.4±0.3^{a}	102.6±0.5^{a}
	EW	105.1±0.4^{ef}	110.9±1.3^{a}	110.7±1.1^{d}	108.7±0.9^{d}	108.0±1.2^{bc}	108.2±0.7^{cd}
	CSW	107.5±0.2^{g}	116.6±0.5^{a}	114.1±0.6^{e}	111.6±0.2^{e}	110.1±0.7^{cd}	105.2±0.4^{ab}
5	FW	103.1±0.2^{bcd}	102.5±1.0^{a}	103.1±0.6^{a}	103.8±0.5^{b}	103.2±0.5^{a}	103.1±0.2^{a}
	EW	103.7±0.2^{cd}	112.0±0.8^{a}	112.2±0.9^{de}	106.1±0.3^{c}	105.7±0.3^{b}	104.3±0.4^{ab}
	CSW	104.1±0.2^{de}	113.9±0.9^{a}	107.9±1.3^{bc}	109.2±0.9^{d}	107.8±0.4^{bc}	105.1±0.2^{ab}
7	FW	102.3±1.0^{abc}	102.9±0.8^{a}	103.2±0.7^{a}	103.0±0.5^{ab}	102.7±0.9^{a}	103.8±0.2^{ab}
	EW	105.0±0.3^{ef}	110.1±1.0^{a}	107.4±1.0^{b}	108.9±0.8^{d}	107.1±0.9^{b}	107.2±0.7^{bc}
	CSW	106.2±0.2^{fg}	115.2±1.1^{a}	110.9±0.8^{d}	108.4±0.7^{d}	105.9±0.7^{b}	105.4±0.7^{ab}

Means with similar letters at the same columns are not significantly different ($p>0.05$). Based on two-way ANOVA, no significant interaction was found in 24 h

Table 6: Concentration of blood K^+ of 1, 3, 5 and 7 g kutum in different Salinity treatment: fresh water (FW), the estuary water (BW) and the caspian sea water (CSW); Values are expressed as Mean±SE

		Time (h)					
Weight (g)	Water	0	24	48	96	144	336
1	FW	1.43±0.03^{a}	1.47±0.07^{b}	1.43±0.09^{b}	1.50±0.06^{a}	1.37±0.03^{abc}	1.37±0.03^{ab}
	EW	1.43±0.04^{a}	1.60±0.06^{cd}	1.73±0.03^{cd}	1.93±0.19^{a}	1.70±0.06^{e}	1.70±0.03^{e}
	CSW	1.47±0.03^{a}	1.83±0.09^{e}	1.90±0.06^{d}	2.00±0.06^{a}	1.86±0.03^{f}	1.87±0.06^{f}
3	FW	1.43±0.03^{a}	1.50±0.00^{bc}	1.43±0.07^{b}	1.50±0.00^{a}	1.47±0.03^{bcde}	1.43±0.03^{bc}
	EW	1.47±0.03^{a}	1.80±0.00^{e}	1.63±0.03^{c}	1.63±0.03^{a}	1.53±0.03^{cde}	1.50±0.05^{c}
	CSW	1.50±0.06^{a}	1.83±0.03^{e}	1.73±0.03^{cd}	1.70±0.00^{a}	1.57±0.03^{de}	1.57±0.09^{c}
5	FW	1.20±0.06^{a}	1.23±0.03^{a}	1.20±0.06^{a}	1.20±0.06^{a}	1.23±0.03^{a}	1.27±0.05^{a}
	EW	1.30±0.10^{a}	1.70±0.00^{de}	1.70±0.00^{c}	1.57±0.03^{a}	1.33±0.03^{ab}	1.41±0.07^{cd}
	CSW	1.37±0.07^{a}	1.97±0.03^{f}	1.77±0.03^{cd}	1.67±0.03^{a}	1.47±0.09^{bcde}	1.50±0.06^{bc}
7	FW	1.30±0.06^{a}	1.37±0.03^{b}	1.27±0.07^{ab}	1.27±0.03^{a}	1.40±0.06^{bcd}	1.28±0.09^{a}
	EW	1.47±0.03^{a}	1.37±0.03^{b}	1.43±0.07^{b}	1.43±0.12^{a}	1.43±0.09^{bcde}	1.43±0.03^{cd}
	CSW	1.50±0.06^{a}	1.63±0.03^{d}	1.70±0.06^{c}	1.53±0.03^{a}	1.47±0.03^{bcde}	1.43±0.03^{cd}

Means with similar letters at the same columns are not significantly different ($p>0.05$). Based on two-way ANOVA, no significant interaction was found in 0 and 96 h

Perhaps, this might be due to greater adaptation of 1 g kutum to salinity of 7‰. In addition, the level of chloride for 3, 5 and 7 g fingerlings in CSW treatment (13‰) after 336 h was found to be 105.2 $^{mEq}/_{L}$ (2.5%), 105.1 $^{mEq}/_{L}$ (1.9%) and 105.4 $^{mEq}/_{L}$ (1.6%), respectively. The value for EW treatment (7‰) was measured as 108.2 $^{mEq}/_{L}$ (5.4%), 104.3 $^{mEq}/_{L}$ (1.1%) and 107.2 $^{mEq}/_{L}$ (3.3%), respectively.

Changes of K^+ concentration for different salinity and weight treatments are shown in Table 6. Significant interactions between weight groups and salinity treatments were found at 24, 48, 144 and 336 h. In the both salinity treatments, the maximum levels of K^+ belonged to 5 and 7 g kutum at 12 and 24 h. However, during 48, 144 and 336 h, the highest level of blood K^+ was recorded in 1 g weight group. After 14 days exposure to CSW treatment, K^+concentrations in 1, 3, 5 and 7 g fingerling kutum were recorded as 1.87 (36.5%), 1.57(9.3%), 1.50 (18.4%) and 1.43 $^{mEq}/_{L}$ (11.9%) and the values for EW treatment (7‰) was measured 1.73 $^{mEq}/_{L}$ (24.4%), 1.50 $^{mEq}/_{L}$ (6.9%), 1.41 $^{mEq}/_{L}$ (7.8%) and 1.43 $^{mEq}/_{L}$ (11.7%), respectively.

DISCUSSION

Ionic regulation and osmoregulation studies are important to determine the suitable weight (smoltified) for downstream migration in anadromous species such as kutum. As *Rutilus frisii kutum* is one of IFRO's main targets for rehabilitation and restocking (Abdolhay *et al*., 2011), it is crucial to determine the suitable weight for release to increase the survival rate. Studies showed that the changes in osmolarity and ionic compounds brought about by changes in salinity occur during two stages (He *et al*., 2009; Zhao *et al*., 2011):

- **First stage (first reaction):** The level of ions increase in the first few days (0 to 48 h) after transfer to reach the environmental concentration level (Nikoo *et al*., 2010)
- **Second stage (second reaction):** After adaptation, the concentration of blood ions decreases to a low level (after 48 h) (Krayushkina *et al*., 1996; Barton, 2002).

In this study, taking into account the weight groups and three salinity treatments (fresh water, 7 and 13‰), two stages of the effects on fingerlings could be observed. In the first stage, an

increase in the amounts of ions was noted after release into Caspian Sea water (hypertonic water). As Tables 3 to 6 show, significant increases in Na^+, Mg^{2+}, Cl^- and K^+ concentration in all the weight groups were observed in the first stage (0 to 48 h) ($p<0.05$). This situation was only temporary in 3, 5 and 7 g (except 1 g) kutum. This is because in the second stage (after 48 h), the ionic regulation activity start and the level of ions reach the control level. This reaction could be accompanied by development of osmoregulatory organs, increase in hormones and some osmoregulator factors such as, Na^+, K^+ ATPase and IGF-I (McCormick and Naiman, 1984); Mccormick and Saunders, 1987). Studies indicated that in 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).

However, compared to the other weight groups the important factor was the different reaction of 1 g fingerlings to sea and estuary water treatments. For instance, statistical analysis showed that the concentration of Na^+in the 1 g kutum in the Caspian Sea water treatment (Table 3) was significantly higher than 3, 5 and 7 g kutum in durations of 96, 144 and 336 h ($p<0.05$). This finding indicates that the second reaction (after 48 h) of 1 g kutum was not well adjusted to expel the excess ions. On the other hand, this incomplete development of ionic regulation was observed for 1 g kutum in the estuary water treatment. It could probably be due to the disability of this system to decrease the plasma ions concentration as much as fresh water (control) treatment level (Table 3). Bolton *et al*. (1987) showed that plasma Na levels of freshwater-adapted rainbow trout peaked 24 h after transfer to seawater and remained high for at least 48 h. They also indicated that during 24 h after transfer, plasma Na^+ levels were inversely correlated to their body weight.

Moreover, statistical analysis proved that maximum level of Mg_2^+ (214%) was recorded in 1 g Caspian Sea treatment. The 1 g kutum was not able to significantly decrease the concentration of Mg_2^+ after 14 days, during sea and estuarine water treatment as much as the control treatment. The 3, 5 and 7 g weight groups were able to reduce the concentration of blood Mg_2^+ until it reached the control level. It might be due to ionic regulation and development of organs dealing with osmoregulation (Nikoo *et al*., 2010). Based on the earlier-mentioned results and comparing the levels of Mg^{2+} among different groups during the relevant experiment times, it could be concluded that 1 g fingerlings could not adapt themselves to Caspian Sea and estuary water salinity (Table 4). A 350% increase in the amount of magnesium was reported by McCormick and Naiman (1984) in the Brook trout after 7 days exposure to seawater. They also showed that Mg^{2+} constituted less than 1% of the total plasma osmolarity. Furthermore, during first 30 h, SW exposed coho salmon showed a transient rise in plasma Mg^{2+} level (Miles and Smith, 1968).

The results of the changes in blood chloride showed that 3, 5 and 7 g kutum were able to expel the extra plasma Cl^{-1} font when exposed to sea and estuarine waters ($p<0.05$). Comparatively, maximum levels of the chloride appeared in 1 g kutum among all the weight groups in both the CSW and EW treatments (118.1, 113.3 $^{mEq}/_{L}$) after 336 h. This demonstrates that secondary reaction (secondary mechanism) had not begun until that time (Table 5). Further, the monitoring of K^+ concentration in 1 g kutum showed an incapable ionic regulation function after 48 h (Table 6). However a suitable osmoregulation reaction (or secondary mechanism) was found in the 3 g kutum fingerling. Congruent with our findings, He *et al*. (2009) revealed that 7-months-old juvenile Chinese sturgeon was successful in decreasing the level of blood Na^+, Cl^-, Ca^{2+} and K^+ during the stabilization period (step two). Franklin *et al*. (1992) also showed that smolt sockeye salmon (*Oncorhynchus nerka*) was able to decrease plasma chloride and sodium concentration after

24 to 48 h. However in the salmon that failed to adapt to sea water, the plasma ionic concentrations were not regulated and the salmon became severely dehydrated and eventually died.

With respect to the results of the ionic changes in the blood of kutum fingerling, it can be concluded that 1 g weight group kutum under the effect of ionic regulatory ability was not able to excess the extra blood ions (during step two) significantly. Accordingly, the high mortality rate observed in 1 g kutum than in the other weight groups could be justified (Fig. 1). After 7 days exposure to sea water, a significant increase in mortality rate in the least weight group of *Salvelinus alpinus* was found by Halvorsen *et al.* (1994). It appears reasonable to assume that the migratory behaviors of kutum fingerlings are directly related to body weight. In this regard, other articles also showed that the migration time for juvenile fishes depends on their weight or size (McCormick and Naiman 1984; Mccormick and Saunders, 1987; Ugedal *et al.*, 1998). 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. Thorpe *et al.* (1994), Rikardsen *et al.* (2004) and Davidsen *et al.* (2009) indicated that the period of sea entry and the first few days of marine life is a critical period in the life of the juvenile Atlantic salmon (Salmo salar) and occasionally characterized by very high mortality.

In addition to physiological factors like suitable weight for release, the economical aspects should also be taken into consideration. Since the ionic regulation systems in 1 g kutum fingerlings are undeveloped, they cannot be considered as the suitable weights for release into the Caspian Sea water. On the other hand, preserving kutum of this size for five to seven months to reach 3 or 5 g weight is not economically feasible (Abdolhay *et al.*, 2011). Due to economic reasons, releasing 1 g kutum, after finding a proper place in the natural habitat should be considered. Since the estuary area is always in close contact with the rough conditions of the sea, it causes changes in salinity level (Abdoli, 1999). Therefore selecting this region for releasing 1 g kutum, with no seawater adaptation ability (smoltification), is not recommended. The upstream of rivers is deemed more appropriate for this purpose. The advantages of releasing 1 g (or less) kutum into upstream of rivers are as follows:

- The up streams have a constant salinity level (0.3-0.5‰) being fresh water (control water in this study). Therefore, in these regions there would be a lack of salinity stress (Table 3-6)
- The release of fingerlings into upstream of rivers enables them to select their migration time towards the estuary

CONCLUSION

It is suggested that due to the low volume flow rate (flowrate) of upstream of rivers compared with estuaries (Shchevyev and Bogoyavlensky, 1990), the release of *Rutilus frisii kutum* fingerlings should be controlled by scientific programs. To reduce the mortality rate, it should be performed step by step with a small number of fishes in each release program. However there is a need for proper studies to identify a safe region and the feasibility of releasing the fingerlings.

REFERENCES

ASTM, 1989. American St andard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC.

Abdolhay, H.A., S.K. Daud, S.R. Ghilkolahi, M. Pourkazemi, S.S. Siraj and M.K.A. Satar, 2011. Fingerling production and stock enhancement of Mahisefid (*Rutilus frisii kutum*) lessons for others in the south of Caspian Sea. Rev. Fish Biol. Fish., 21: 247-257.

Abdoli, A., 1999. The Inl and Water Fishes of Iran. Natural Museum and World Wild of Iran, Iran, ISBN: 964-6902-01-4, (In Persian).

Avella, M., G. Young, P. Prunet and C.B. Schreck, 1990. Plasma prolactin and cortisol concentrations during salinity challenges of coho salmon (*Oncorhynchus kisuich*) at smolt and post-smolt stage. Aquaculture, 91: 359-372.

Bartley, D. and K.J. Rana, 1998. Evaluation of artificial rehabilitation of the Caspian sea fisheries and genetic resources management. FAO Report Prepared for the SHILAT (Fisheries Department).

Barton, B.A., 2002. Stress in fishes a diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Boil., 42: 517-525.

Beyenbach, K.W., 2000. Renal h andling of magnesium in fish: From whole animal to brush border membrane vesicles. Front Biosci., 5: 712-719.

Bolton, J.P., N.L. Collie, H. Kawauchi and T. Hirano, 1987. Osmoregulatory actions of growth hormone in rainbow trout (*Salmo gairdneri*). J. Endocrinol., 112: 63-68.

Bourani, M.S., B. Abtahi, M. Bahmani, L. Krayushekina, A. Yousofi and M. Ahmadnezhad, 2006. Determination of suitable size of *Salmo trutta* caspius for releasing or marine culture by evaluation of osmotic ability. Proceedings of the International Conference on Coastal Oceanography and Sustainable Marine Aquaculture, May 2-4, 2006, Sabah, Malaysia.

Bourani, M.S., B. Abtahi, M. Bahmani, L. Krayushekina, A. Yousofi and M. Ahmadnezhad, 2009. Thyroid gl and function in *Salmo trutta* caspius fingerlings under changing salinity conditions. Asian-Pacific Aquaculture 2009 - Meeting Abstract. 504. World Aquaculture Society, https://www.was.org/WASMeetings/Meetings/ShowAbstract.aspx?Id=18755.

Bower, S.M. and T.P. Evelyn, 1988. Acquired and innate resistance to the haemoflagellate cryptobia salmositica in sockeye salmon (*Oncorhynchus nerka*). Dev. Comp. Immunol., 12: 749-760.

Brauner, C.J., J.M. Shrimpton and D.J. R andall, 1992. Effect of short-duration seawater exposure on plasma ion concentrations and swimming performance in coho salmon (*Oncorhynchus kisutch*) Parr. Can. J. Fish. Aqua. Sci., 49: 2399-2405.

CLSI, 2008. Evaluation of precision performance of quantitative measurement methods: Approved guideline: Second Edition. CLSI Document EP5-A2, ISBN: 1-56238-542-9. Clinical and Laboratory St andards Institute, Wayne, PA, USA. http://www.clsi.org/source/orders/Product_Display .cfm?section=Shop&SKU=EP05A2.

Davidsen, J.G., A.H. Rikardsen, E. Halttunen, E.B. Thorstad and F. Okl and *et al.*, 2009. Migratory behaviour and survival rates of wild northern Atlantic salmon *Salmo salar* post-smolts: Effects of environmental factors. J. Fish Biol., 75: 1700-1718.

Franklin, C.E., M.E. Forster and W. Davison, 1992. Plasma cortisol and osmoregulatory changes in sockeye salmon transferred to sea water: Comparison between successful and unsuccessful adaptation. J. Fish Biol., 41: 113-122.

Halvorsen, M., A.M. Arnesen, K.J. Nilssen and M. Jobling, 1994. Osmoregulatory ability of anadromous Arctic char, *Salvelinus alpinus* (L.), migrating towards the sea. Aquacult. Res., 25: 199-211.

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

Henry, R.J., D.C. Cannon and J.W. Winkelman, 1974. Clinical Chemistry Principles and Techniques. 2nd Edn., Harper and Row, Hagerstrown, pp: 712.

Hwang, P.P., 2009. Ion uptake and acid secretion in zebra fish (*Danio rerio*). J. Exp. Biol., 212: 1745-1752.

Jones, S.R., M. Palmen and W.B. van Muiswinkel, 1993. Effects of inoculum route and dose on the immune response of common carp, *Cyprinus carpio* to the blood parasite, *Trypanoplasma borreli*. Vet. Immunol. Immunopathol., 36: 369-378.

Kavan, L.S. S.R. Gilkolaei, G. Vossoughi, S.M.R. Fatemi, R. Safari and S. Jamili, 2009. Population genetic study of *Rutilus frisii kutum* (Kamansky 1901) from the Caspian Sea; Iran and Azerbaijan regions, using microsatellite markers. J. Fish. Aquat. Sci., 4: 316-322.

Keyvan, A., S. Moini, N. Ghaemi, A.A. Haghdoost, S. Jalili and M. Pourkabir, 2008. Effect of frozen storage time on the lipid deterioration and protein denaturation during Caspian Sea white fish (*Rutilus frisi kutum*). J. Fish. Aquat. Sci., 3: 404-409.

Krayushkina, L.S., A.A. Panov, A.A. Gerasomov and W.T.W. Potts, 1996. Changes in sodium, calcium and magnesium ion concentrations in sturgeon (*Huso Huso*) urine and in kidney morphology. J. Comp. Biol. B, 165: 527-533.

Madsen, S.S. and E.T. Naamansen, 1989. Plasma ionic regulation and gill Na^+/K^+-ATPase changes during rapid transfer to sea water of yearling rainbow trout, *Salmo gairdneri*: Time course and seasonal variation. J. Fish Biol., 34: 829-840.

McCormick, S.D. and R.J. Naiman, 1984. Osmoregulation in the brook trout, *Salvelinus fontinalis*: II. Effects of size, age and photoperiod on seawater survival and ionic regulation. Comp. Biochem. Phys. A: Physiol., 79: 17-28.

Mccormick, S.D. and R.L. Saunders, 1987. Preparatory physiological adaptations for marine life of salmonids: Osmoregulation, growth and metabolism. Am. Fish. Soc. Symp., 1: 211-229.

Miles, H.M. and L.S. Smith, 1968. Ionic regulation in migrating juvenile coho salmon, *Oncorhynchus kisutch*. Comp. Biochem. Physiol., 26: 381-398.

Nikoo, M., B. Falahatkar, M. Alekhorshid, B.N. Haghi, A. Asadollahpour, M. Zarei dangsareki and H.F. Langroudi, 2010. Physiological stress responses in kutum *Rutilus frisii kutum* subjected to captivity. Int. Aquat. Res., 2: 55-60.

Nonnotte, G. and J.P. Truchot, 1990. Time course of extracellular acid-base adjustments under hypo-or hyperosmotic conditions in the euryhaline fish *Platichthys flesus*. J. Fish Biol., 36: 181-190.

Parry, G., 1958. Size and osmoregulation in salmonid fishes. Nature, 181: 1218-1219.

Parry, G., 1961. Osmotic and ionic changes in blood and muscle of migrating salmonids. J. Exp. Biol., 38: 411-427.

Razavi, S.B., 1995. Mahisefied. Iranian Fisheries Research Organization, Tehran, Iran, pp: 164.

Razavi, S.B., 1998. Ecology of the Caspian Sea. Iranian Fishery Research Organisation, Tehran, Iran, pp: 90.

Rikardsen, A.H., M. Haugl and, P.A. Bjorn, B. Finstad and R. Knudsen *et al*., 2004. Geographical differences in marine feeding of Atlantic salmon post-smolts in Norwegian fjords. J. Fish Biol., 64: 1655-1679.

Rounsefell, G.A., 1958. Anadromy in North American Salmonidae. United States Government Printing Office, USA., pp: 171-185.

Seidelin, M., S.S. Madsen, H. Blenstrup and C.K. Tipsmark, 2000. Time-course changes in the expression of Na^+, K^+-ATPase in gills and pyloric caeca of brown trout (*Salmo trutta*) during acclimation to seawater. Physiol. Biochem. Zool., 73: 446-453.

Shchevyev, Y.L. and N.Y. Bogoyavlensky, 1990. Calculation of the average flow velocity in rivers. Proceedings of the Beijing Symposium on The Hydrvlogical Basis for Water Resources Management, October 1990, IAHS Publ., pp: 89-91.

Shrimpton, J.M., N.J. Bernier, G.K. Iwama and D.J. R andall, 1994. Differences in measurements of smolt development between wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) before and after saltwater exposure. Can. J. Fish. Aqua. Sci., 51: 2170-2178.

Shrimpton, J.M., D.A. Patterson, J.G. Richards, S.J. Cooke, P.M. Schulte, S.G. Hinch and A.P. Farrell, 2005. Ionoregulatory changes in different populations of maturing sockeye salmon *Oncorhynchus nerka* during ocean and river migration. J. Exp. Biol., 208: 4069-4078.

Sigholt, T., M. Staurnes, H.J. Jakobsen and T. Asgard, 1995. Effects of continuous light and short-day photoperiod on smolting, seawater survival and growth in Atlantic salmon (*Salmo salar*). Aquaculture, 130: 373-388.

Thomas, L., 1998. Clinical Laboratory Diagnostics. 1st Edn., TH-Books Verlagsgesellschaft, Frankfurt, Germany, pp: 208-214.

Thorpe, J.E., N.B. Metcalfe and N.H.C. Fraser, 1994. Temperature Dependence of Switch between Nocturnal and Diurnal Smolt Migration in Atlantic Salmon. In: High Performance Fish, MacKinlay, D. (Ed.). University of British Columbia, Vancouver, pp: 83-86.

Uchida., K., T. Kaneko, H. Miyazaki, S. Hasegawa and T. Hirano, 2000. Excellent salinity tolerance of mozambique tilapia (*Oreochromis mossambicus*): Elevated Chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated seawater. Zool. Sci., 17: 149-160.

Ugedal, O., B. Finstad, B. Damsgard and A. Mortensen, 1998. Seawater tolerance and downstream migration in hatchery-reared and wild brown trout. Aquaculture, 168: 395-405.

Valipour, A. and A.A. Khanipour, 2009. Kutum, *Rutilus frisii kutum*, The Jewel of the Caspian Sea. Iranian Fisheries Research Organisation, Tehran, Iran, pp: 95.

Valipour, A.R., A.A. Khanipour and M.S. Bourani, 2008. The first restocking of autumn form *Rutilus frisii kutum*, in Iranian Costal of caspian sea. World Aquaculture 2008 - Meeting Abstract, 833, World Aquaculture Society, https://www.was.org/Meetings/Abstract Data.asp ? AbstractId=16328.

Williams, V. and S. Twine, 1960. Flame Photometric Method for Sodium, Potassium and Calcium. In: Modern Methods of Plant Analysis, Peach, K. and M.V. Tracey (Eds.). Vol. 5, Springer Verlag, Berlin, Heidelberg, pp: 3-5.

Zhao, F., L. Qu, P. Zhuang, L. Zhang, J. Liu and T. Zhang, 2011. Salinity tolerance as well as osmotic and ionic regulation in juvenile Chinese sturgeon (*Acipenser sinensis* Gray, 1835) exposed to different salinities. J. Applied Ichthyol., 27: 231-234.