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Study on salinity tolerance and some physiological indicators of ion-osmoregulatory system in juvenile beluga, *Huso huso* (Linnaeus, 1758) in the south Caspian Sea: Effect of age and size

S.M.V. Farabi[1*] ; A. Hajimoradloo[2] and M. Bahmani[3]

Smv_Farabi@hotmail.com

1- Caspian Sea Ecological Research Centre, P.O.Box: 961 Sari, Iran

2- Department of Fisheries, Gorgan University of Agriculture Sciences and Natural Resources, P.O.Box: 49138-15749, Gorgan, Iran

3- Dr. Dadman International Sturgeon Research Institute, P.O.Box: 41635–3464 Rasht, Iran

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Abstract: The salinity tolerance and hydromineral regulation capabilities of juvenile great sturgeon, *Huso huso*, of different age and size groups (I: 35days,BW: 0.60± 0.02g &TL: 4.80±0.13cm ; II: 35 days, BW: 2.23±0.20g & TL: 7.19±0.17cm; III: 35days, BW: 9.91±0.23g & TL: 11.86±0.24cm; IV: 50 days, BW: 3.97±0.25g & TL: 9.34±0.18cm ; V:50 days, BW:22.27±1.9g & TL: 17.25±0.35cm ; VI: 65 days, BW: 6.3±0.67g & TL: 11.1±0.37cm ; n = 90 for each group) in freshwater (FW:0.5‰), estuary water (EW:9.5 ‰) and the Caspian Sea water (C_SW:12.5‰) were investigated. The fishes are directly transferred from FW to EW and C_SW. The possible repercussions of osmoregulatory processes on some indicators of classical were examined at the end of 168 hours fish acclimation. Mortality was observed (higher than 50% after 72 hours) only in Ist group in EW and C_SW. Some haematological parameters, namely haematocrit(Hct), red and white blood cells count (RBC, WBC), mean cell volume (MCV), haemoglobin concentration (Hb), mean cell haemoglobin concentration (MCHC), the amount of haemoglobin per erythrocyte (MCH), levels of cortisol, osmolarity and ion concentration (Na^+, K^+,

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[*] **Corresponding author**

Ca^{+2} and Mg^{+2}) in the plasma were determined. The functional levels of the mechanism of osmotic and ionic homeostasis were similar in different groups (II to VI) but differed in experimental media ($P<0.05$). Significant differences were observed between the levels of serum Na^{+} concentration in different groups in EW and C_SW media ($p<0.05$). Serum Na^{+} and Ca^{+2} concentrations were higher than those of FW and EW media, but lower than in C_SW media. The Hct, MCV and MCH decreased with increase in fish age, and decreased from FW media to C_SW media with increasing salinity; RBC, WBC and MCHC did not change. The trend of increasing levels of cortisol were observed at higher salinity in each group ($P<0.05$). However, Hct, MCV, MCHC, cortisol, osmolarity and ion concentration values did not return to initial values ($P<0.05$), showing that osmoregulatory processes caused major physiological changes in this species.

Keywords: *Huso huso*, Haematological parameters, Ion, Cortisol, Osmolarity, Caspian Sea, Iran

Introduction

Most acipenserids are anadromous or semi-anadromous, that they live in oceanic or brackish waters and then migrate to freshwater from several months to several years, depending on the species and then migrate to the sea. The great sturgeon, *Huso huso*, inhabits the entire Caspian Sea and enters the rivers (the Volga, Ural, Kura, Terek, Sefidrud) to spawn. Since stocks are maintained mostly by artificial breeding, this sturgeon has been proposed for inclusion in the "Red Book of the U.S.S.R." which forms the basis for measures to protect species (Pavlov *et al.*, 1985; Mina, 1992). However Lelek (1987) and Birstein (1993) list this species as vulnerable to endangered. Kiabi *et al.* (1999) consider this species to be endangered in the south Caspian Sea basin according to IUCN criteria.

Khodorevskaya and Novikova (1995) point out that cooperation among all the Caspian Sea states is needed to maintain this species along with an annual release of at least 20 million young from hatcheries. Fisheries in the Caspian Sea are almost entirely dependent upon hatchery-released fish (Abdolhay, 1996). Nezami *et al.* (2000) maintain that despite artificial spawning and fingerling production, restoration of this species in Iran was not very successful. Studies from around the world have shown that hatchery reared fish have lower survival rates and provide lower returns to anglers than wild fish (Wales, 1954; Heggberget *et al.*, 1992). The difference in mortality levels between hatchery reared and wild fish is especially large if one considers the size or age classes at which hatchery fish are typically released (Maynard *et al*., 1995).

One of the major problems with the viability of restocking is the dramatic level of mortality of newly released individuals (Suboski & Templeton, 1989; Olla *et al*., 1998). Berejikian *et al*. (2000) suggest that perhaps one of the problems of previous attempts to assess the effects of training fingerlings before release is that both trained and untrained fish have been released together.

Controlling the induced acclimation to the seawater is a first step to solve the problem. Therefore, a study on the physiological condition of juveniles during primary stages of life is necessary to understand restocking management. Age and body size have been postulated as determining factors of the salinity tolerance of the fish (Krayushkina & Dyubin, 1974; Kraushkina, 1983a; McEnroe & Cech, 1985; García-Gallego *et al*., 1998).

Some aspects of these osmoregulatory processes (plasma osmolarity, Na^+, K^+, Mg^{2+},Ca^{2+} concentrations, cortisol level, morpho-functional changes of gills, kidneys and thyroid gland) have been previously studied in several sturgeon species: in *Acipenser gueldenstaedtii, A. stellatus, Huso huso* from North and Middle parts of Caspian Sea (Krayushkina, 1974; Krayushkina *et al*., 1976,1996 ; Krayushkina & Semenova, 2006), in *A. transmontanus* (McEnroe & Cech, 1985), in *A. naccari* (Cataldi *et al*., 1995,1997,1998; Sanchez de Lamadrid *et al*.,1998), in *A. bravirostrum* and *A. oxyrhynchus* (Krayushkina, 1998; Krayushkina, *et al*., 2001), in *A. persicus* (Jabbarzadeh *et al*., 2000; Kazemi *et al*., 2003). The purpose of present investigation was the study of osmotic and ionic regulation in *Huso huso* fingerlings from the south part of the Caspian Sea where the salinity is higher than in North and Middle Caspian Sea. In this investigation red and white blood cells count (RBC&WBC), hemoglobin concentration (Hb), hematocrit values (Hct) were studied and erythrocyte indices were calculated. The changes of these parameters during acclimation of acipenserids to different salinities are not yet expressed in the works of other authors.

Materials and Methods

Source and maintenance of fish

Juveniles of beluga were obtained from artificially spawned wild broodstock at fish hatchery centers of Shaeed Marjani and Shaheed Rajaei, located in the margins of the southern Caspian Sea. The experiments were carried out at the Ecological Institute of the Caspian Sea.

Experimental design

Juveniles used in this experiment comprised three groups in 35 days of age, two groups in 50 days of age and one group in 65 days of age after the beginning of exogenous nutrition. All treatments had three replications each having 30 fingerlings. The primary objectives of this study were to determine the tolerance of *Huso huso* juveniles to different salinities: freshwater (FW: $0.5_{‰}$), estuary water (EW: $9.5_{‰}$) and the Caspian Sea water (C_SW: $12.5_{‰}$) with the effects of age and size. Juveniles transferred directly from FW to saline water (EW and C_SW), using FW as control. Juveniles were not fed throughout the experimental period. Survival of juveniles (JS) was estimated by recording the number of dead fish at 12h, 48h, 72h and 168h intervals during experimental periods (Table 1). At the initiation of each experimental period, the total length (L) and weight (W) of fish were measured by using a calibrated board (±1mm) and digital balance (±0.1g), respectively; the condition factor (cf) was computed by Fulton's index (Riker, 1975): $cf = W*100/L^3$.

If juvenile survival was above 50% in each experimental group, blood samples from the surviving individuals would be drawn by cutting peduncles with heparinized micro-capillary tube at the end of each experimental period (168 hours).

Analytical methods

Blood parameters

Red blood cells (RBC), white blood cells (WBC), hematocrit (Hct) and haemoglobin concentration (Hb) were recorded in every group. The blood samples was extracted using centrifuged (Hettich-D7200) Tuttlingen: Germany) at 453.6g for 5min and preserved in Eppendorf tubes for analyses of plasma osmolarity and then frozen at –20°C for analyses of plasma ions (Na^+, K^+, Ca^{2+} and Mg^{2+}) and cortisol. Heparinized microhematocrit capillary tubes were centrifuged at 16329.6g for 5min in a clinical centrifuge (Hettich-D7200) Tuttlingen: Germany) for Hct. Globular counting was performed by microscope and haemocytometers (standard Neubauer cell counting chamber) over cells suspended in Rees-Escher's solution. Total haemoglobin concentration (Hb) was measured using the cyanmethaemoglobin method with spectrophotometry (CECIL-CE1020: Germany) at 540 nm; mean cell volume (MCV) was computed as $MCV=HCT*10*RBC^{-1}$, mean cell haemoglobin concentration (MCHC) as $MCHC=Hb*100*HCT^{-1}$, and the amount of haemoglobin per erythrocyte (MCH) as $MCH=Hb*10*RBC^{-1}$, (Ameri Mahabadi, 1999 ; Blaxhall & Daisley, 1973). Plasma osmolarity was measured by cryoscopy method by an osmometer (Roebling Nr.9610003.Type 13: Germany). Sodium and potassium concentrations were measured with flame photometer (Corning 405C: IRI); Magnesium and calcium concentrations were measured with an absorption spectrophotometer (UNICO 3115233: USA). Serum cortisol was assayed on a competitive enzyme immunoassay (Stat fax–Avernest, 330plus: USA). This test used with automatic instrument for ELISA kits on microplate.

Water quality:

Water was obtained from the Caspian Sea offshore as well as the Tajan estuary. Water salinity was measured by salinimeter (Electrosolemer, GM-65M: Russia). Every experimental tank was well aerated by using aerator. The experimental tanks were maintained at room temperature (20±1°C) and their waters replaced every 12h to prevent accumulation of ammonia and other toxic metabolites.

Statistical analysis:

The differences in parameters were tested for significance by one-way analysis of variance (ANOVA) using SPSS.V10. Subsequent significance between groups was delineated by Duncan's test. A value of $P<0.01$ or $P<0.05$ were taken for significance in all statistical tests.

Results

The survival rate (JS>50%) is considered as an index of the success/failure of the adaptation process to saltwater. JS was observed less than 50% in group (I) (Age: 35 days, TW = 0.60±0.02g, TL = 4.80±0.13) in EW and C_SW media. Thereby, the blood parameter was not measured in group (I). In the other groups, the observed JS was more than 50% for 168 hours. After 72 hours mortality was not observed (Table 1). A strong correlation (99%) between W and L in all groups was found. CF in the juvenile *Huso huso* after 35 days of age had decreased significantly ($P<0.01$), except group (I), (Fig. 1).

FW juveniles of different sizes and ages had a blood Hct of 20.75±2.24%, an average plasma osmolality of 244.16±14.4 mOsml/l, and plasma ion concentrations of 128.34±1 mEq/l for Na^+, 2.59±0.01 mEq/l for K^+, 5.93±0.05 mEq/l for Ca^{+2} and 0.49 ±0.02 mEq/l for Mg^{+2} (Table 2&3). At 168 hours after transfer of FW fish to different salinities (EW and C_SW), serum osmolarity, ion concentration, serum cortisol levels had increased and Hct, MCV, MCHC had decreased significantly ($P<0.05$) (Table 2&3).

Ion concentrations in blood serum as well as the three media showed significant differences found ($P<0.01$), (Table 3). Significant differences between serum Na^+ concentrations were observed in different age and size groups ($p<0.01$) only in two media (EW and C_SW), but, for other cations not significant differences ($P>0.05$), (Table 3). Sodium concentration in serum was higher than in FW and EW, but, it was lower than in C_SW (Table 3). Potassium concentrations in serum were higher than in FW, EW and C_SW (Table 3).Calcium concentrations in serum were lower than in FW and EW, but, it was higher than in C_SW (Table 3). Magnesium concentrations in serum were lower than in FW and C_SW, but, it was a little higher than in EW (Table 3). Osmolarity have shown significant differences between three experimental media in the water and blood serum ($P<0.01$), (Table 2). Blood serum osmolarity of fish acclimated to FW and EW was higher than the osmolarity of corresponding media, but, it was lower than media of C_SW (Table 2).Therefore, the juvenile's *Huso huso* were hypertonic in FW and EW, while in C_SW they were hypotonic.

Table 1: Comparative survival of juveniles *Huso huso* in different size/age and different media (FW :< 0.5 ‰ - EW: 9.5 ‰ - C_SW: 12.5 ‰) groups.

Juveniles Survival %: n= 90	-	12 hours			48 hours			72 hours		
Age (days): n=30,df=28, $r_{0.01}$ = 0.46 Wg±SD , Lcm±SD	cf	FW	EW	C_SW	FW	EW	C_SW	FW	EW	C_SW
I. 35 days: $0.60\pm0.02g^{f}$, 4.80 ± 0.13 cm^{f}, r= 0.67	0.54 ± 0.03^{b}	90	45	30	75	10	0	75	0	0
II. 35 days: $2.23\pm0.20g^{f}$, $7.19\pm0.17cm^{e}$, r= 0.53	0.60 ± 0.05^{a}	100	95	70	100	80	60	100	80	55
III. 35 days: $9.91\pm0.23g^{b}$, $11.86\pm0.24cm^{b}$, r= 0.48	0.60 ± 0.03^{a}	100	100	100	100	100	100	100	100	95
IV. 50 days: $3.97\pm0.25g^{d}$, $9.34\pm0.18cm^{d}$, r= 0.65	0.49 ± 0.03^{c}	100	100	90	100	100	90	100	100	90
V. 50 days: $22.27\pm1.9g^{a}$, $17.25\pm0.35cm^{a}$, r= 0.63	0.50 ± 0.03^{c}	100	100	100	100	100	100	100	100	95
VI. 65 days: $6.3\pm0.67g^{c}$, $11.1\pm0.37cm^{c}$, r= 0.89	0.46 ± 0.02^{c}	100	100	100	100	100	95	100	100	95

Note: n= The number of fish and values are means ± SD. Values with different superscript letters within each column are significantly different (P<0.01)

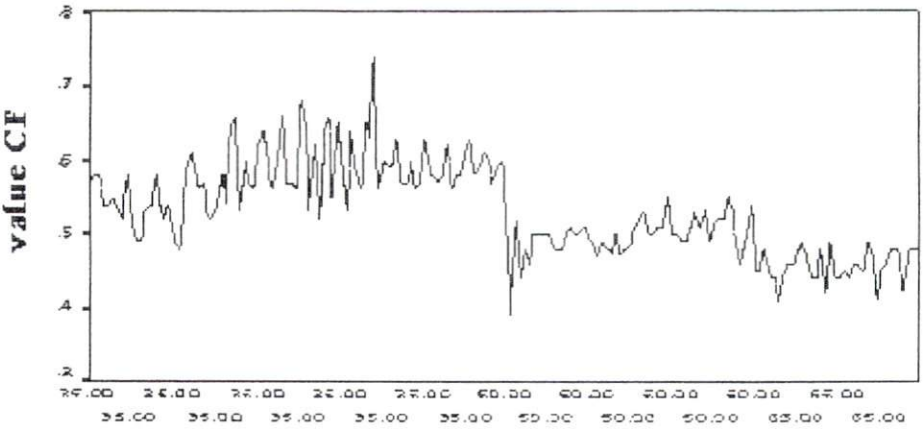

Figure 1: Condition Factors of juvenile *Huso huso* at different times after the beginning of exogenous nutrition (n=30 at each group)

Table 2: Hematological parameters and concentration of cortisol and osmolarity in blood serum of juvenile *Huso huso* in the southern Caspian Sea after acclimation for 168 hours to different media

Juvenile's group	Hct (%)	RBC (10^3cellsμl^{-1})	WBC (10^3cellsμl^{-1})	Hb (g/dl)	MCV (μm^3 or fl)	MCH (μμg or pg)	MCHC (%)	Cortisol (ng/ml)	Osmolarity (mOsml/l)
In FW: <0.5 ‰ and Osmolarity: 5±2 mOsml/l									
II.35days: *n*=10	$21.7\pm0.67^{b}_{a}$	$838\pm24.85^{ab}_{a}$	$19.15\pm1.61^{a}_{a}$	$5.42\pm0.28^{b}_{a}$	$258.98\pm4.81^{a}_{a}$	$64.65\pm1.94^{a}_{a}$	$24.97\pm0.77^{c}_{c}$	$16.5\pm0.55^{b}_{c}$	$236.5\pm3.6^{b}_{c}$
III. 35 days: n=10	$24.49\pm0.74^{a}_{a}$	$872\pm39.66^{a}_{a}$	$19.2\pm0.92^{a}_{a}$	$5.85\pm0.26^{a}_{a}$	$286.11\pm15.88^{b}_{a}$	$67.45\pm4.43^{a}_{a}$	$23.59\pm1.11^{c}_{c}$	$17.06\pm0.35^{a}_{c}$	$240.5\pm2.84^{b}_{c}$
IV. 50 days: n=10	$19.7\pm0.67^{c}_{a}$	$826\pm50.81^{b}_{a}$	$19.05\pm0.68^{a}_{a}$	$5.5\pm0.33^{b}_{a}$	$239.68\pm22.17^{c}_{a}$	$66.89\pm6.67^{a}_{a}$	$27.94\pm1.79^{ab}_{c}$	$16.52\pm0.51^{b}_{c}$	$283.3\pm7.02^{b}_{c}$
V.50days: *n*=10	$19.4\pm0.52^{c}_{a}$	$836\pm41.15^{ab}_{a}$	$19.3\pm1.06^{a}_{a}$	$5.65\pm0.39^{ab}_{a}$	$232.55\pm12.71^{c}_{a}$	$67.75\pm5.97^{a}_{a}$	$29.16\pm2.39^{a}_{b}$	$16.76\pm0.54^{ab}_{c}$	$269.7\pm51.1^{a}_{b}$
VI. 65 days: n=10	$19.8\pm0.79^{c}_{a}$	$844\pm32.72^{ab}_{a}$	$19.5\pm0.97^{a}_{a}$	$5.41\pm0.28^{b}_{a}$	$234.93\pm13.3^{c}_{a}$	$64.22\pm4.73^{a}_{a}$	$27.37\pm1.91^{ab}_{c}$	$16.47\pm0.76^{b}_{c}$	$237.5\pm3.92^{b}_{c}$
Average:	20.75±2.24	843.2±17.35	19.24±0.17	5.57±0.19	250.45±22.48	66.19±1.64	26.6±2.27	16.66±0.25	244.16±14.4
In EW: 9.5 ‰ and Osmolarity: 297.8±5.89 mOsml/l									
II. 35 days: n=10	$19.6\pm1.43^{a}_{b}$	$825\pm19.57^{a}_{a}$	$19.05\pm0.76^{a}_{a}$	$5.22\pm0.19^{a}_{ab}$	$234.53\pm13^{a}_{b}$	$62.5\pm0.99^{a}_{b}$	$26.71\pm1.21^{b}_{b}$	$24.3\pm1.61^{a}_{b}$	$282.8\pm7.1^{a}_{b}$
III. 35 days: n=10	$20.3\pm1.16^{a}_{b}$	$846\pm29.88^{a}_{a}$	$19.15\pm1.31^{a}_{a}$	$5.32\pm0.21^{a}_{b}$	$240.23\pm16.24^{a}_{b}$	$62.98\pm3.79^{a}_{b}$	$26.26\pm1.49^{b}_{b}$	$24.86\pm0.87^{a}_{b}$	$278.1\pm6.26^{a}_{b}$
IV. 50 days: n=10	$17.6\pm0.7^{b}_{b}$	$836\pm46.95^{a}_{a}$	$19.1\pm1.04^{a}_{a}$	$5.41\pm0.35^{a}_{a}$	$211.11\pm14.17^{b}_{b}$	$64/81\pm4.33^{a}_{a}$	$30.76\pm1.99^{a}_{b}$	$23.99\pm1.35^{a}_{b}$	$313.6\pm7.46^{a}_{b}$
V. 50 days: n=10	$17.4\pm0.52^{b}_{b}$	$818\pm48.02^{a}_{a}$	$19.25\pm1.37^{a}_{a}$	$5.31\pm0.25^{a}_{b}$	$213.48\pm15.79^{b}_{b}$	$65.1\pm4.61^{a}_{ab}$	$30.56\pm2.01^{a}_{ab}$	$23.83\pm0.68^{a}_{b}$	$282.1\pm6.64^{a}_{b}$
VI. 65 days: n=10	$17.8\pm0.63^{b}_{b}$	$840\pm38^{a}_{a}$	$19.05\pm1.23^{a}_{a}$	$5.35\pm0.27^{a}_{a}$	$212.43\pm14.8^{b}_{b}$	$63.77\pm3.69^{a}_{a}$	$30.09\pm1.81^{a}_{b}$	$24.07\pm1.55^{a}_{b}$	$283.3\pm7.27^{a}_{b}$
Average:	18.54±1.32	835±10.4 4	19.12±0.08	5.32±0.07	222.36±13.89	63.83±1.12	28.88±2.2	24.21±0.4	281.92±2.2
C_SW: 12.5 ‰ and Osmolarity: 407±2.92 mOsml/l									
II. 35 days: n=10	$18.6\pm0.7^{a}_{c}$	$824\pm23.19^{a}_{a}$	$19.3\pm0.78^{a}_{a}$	$5.18\pm0.21^{a}_{c}$	$225.74\pm5.79^{a}_{c}$	$62.85\pm1.06^{a}_{b}$	$27.85\pm0.67^{b}_{a}$	$28.18\pm1.07^{ab}_{a}$	$312.3\pm7.39^{a}_{a}$
III. 35 days: n=10	$18.6\pm0.52^{a}_{c}$	$851\pm43.06^{a}_{a}$	$19.1\pm0.99^{a}_{a}$	$5.28\pm0.21^{a}_{b}$	$218.95\pm10.18^{a}_{c}$	$62.21\pm4.37^{a}_{b}$	$28.4\pm1.2^{b}_{a}$	$29.14\pm0.68^{a}_{a}$	$312.6\pm5.58^{a}_{a}$
IV.50 days: n=10	$15.9\pm0.74^{b}_{c}$	$817\pm30.93^{a}_{a}$	$19.15\pm0.62^{a}_{a}$	$5.27\pm0.24^{a}_{a}$	$194.97\pm13.42^{b}_{c}$	$64.63\pm4.43^{a}_{a}$	$33.22\pm2.27^{a}_{a}$	$27.45\pm1.3^{b}_{a}$	$312.2\pm3.39^{a}_{a}$
V.50 days: n=10	$16.2\pm0.63^{b}_{c}$	$854\pm40.6^{a}_{a}$	$18.95\pm0.44^{a}_{a}$	$5.24\pm0.3^{a}_{b}$	$190.13\pm12.76^{b}_{c}$	$61.35\pm1.73^{a}_{b}$	$32.4\pm2.36^{a}_{a}$	$28.24\pm1.04^{ab}_{a}$	$312.4\pm5.82^{a}_{a}$
VI.65 days: n=10	$15.9\pm0.74^{b}_{c}$	$837\pm58.69^{a}_{a}$	$19\pm1.15^{a}_{a}$	$5.24\pm0.26^{a}_{a}$	$190.86\pm16.73^{b}_{c}$	$62.76\pm3.61^{a}_{a}$	$33.01\pm2.32^{a}_{a}$	$27.35\pm1.31^{b}_{a}$	$311.5\pm8.05^{a}_{a}$
Average:	17.04±1.43	836.6±16.23	19.1±0.13	5.24±0.04	204.13±16.9	62.76±1.2	30.98±2.63	28.07±0.72	312.48±0.75

Note 1: Values with different superscript and subscript letters within each column are significantly different in age and size classes of different media (FW, EW, C_SW) (Means ± SD and $P<0.05$).

Table 3: Ion concentration of blood serum in juvenile *Huso huso* in the southern part of the Caspian Sea after fish acclimation to different salinity for 168 hours. (means ± SD)

Media	Juvenile's group		II. 5 days: n=10	III. 35 days: n=10	IV.50 days: n=10	V.50 days: n=10	VI.65 days: n=10	Average	Water N=5
In Fresh Water: <0.5 ‰$^{(C)}$	Ion concentration mEq/1	Na^+	129.7±2.95	128.8±2.82	128.3±2.83	127.1±4.12	127.8±3.05	128.34±1	28.8±2.86
		K^+	2.6±0.03	2.59±0.04	2.59±0.03	2.58±0.06	2.57±0.03	2.59±0.01	0.39±0.03
		Ca_{2+}	5.89±0.15	5.97±0.15	5.95±0.17	5.98±0.13	5.86±0.12	5.93±0.05	2.04±0.15
		Mg_{2+}	0.47±0.07	0.5±0.04	0.48±0.07	0.51±0.03	0.5±0.05	0.49±0.02	1.11±0.16
In Estuary Water: <9.5 ‰ $^{(b)}$	Ion concentration mEq/1	Na^+	$143.8±3.74^a$	$139.9±2.77^b$	$139.9±3.54^b$	$142.3±1.34^{ab}$	$140.3±2.67^b$	141.24±1.74	135.42±5.18
		K^+	2.7±0.04	2.71±0.03	2.68±0.04	2.69±0.02	2.98±0.03	2.69±0.01	2.51±0.45
		Ca_{2+}	6.48±0.08	6.51±0.06	6.49±0.09	6.49±0.03	6.49±0.06	6.49±0.01	4.46±1.39
		Mg_{2+}	1.45±0.13	1.51±0.06	1.48±0.09	1.51±0.04	1.48±0.06	1.49±0.03	1.16±0.11
In Caspian Water: <12.5 ‰$^{(a)}$	Ion concentration mEq/1	Na^+	$155.1±3.41^{ab}$	$152.3±3.23^b$	$153.7±4.19^{ab}$	$155.8±2.39^a$	$154.4±2.59^{ab}$	154.6±0.85	175.88±4.56
		K^+	2.8±0.05	2.8±0.03	2.79±0.04	2.81±0.02	2.8±0.02	2.8±0.01	2.41±0.35
		Ca_{2+}	7.09±0.17	7.17±0.12	7.1±0.13	7.1±0.11	7.06±0.13	7.1±0.04	20.46±0.82
		Mg_{2+}	2.1±0.15	2.16±0.1	2.1±0.12	2.18±0.8	2.16±0.1	2.14±0.04	63.3±3.06

Note: Values with different superscript letters within each column and first row are significantly different ($P<0.01$)

Discussion

If the internal perturbation of the fish, either directly or as a result of alterations of the environment, overwhelms the physiological mechanisms of the animal for response and adaptation to new conditions, survival can be threatened and death can result (Martinez-Álvarez *et al.*, 2002). Thus, anadromous fish must develop complex osmoregulatory mechanisms to survive successfully both in hypoosmotic environments (e.g. rivers) and hyperosmotic environments (e.g. estuaries and open sea). In previous investigations, it was noted that adult and juvenile acipenserids of euryhaline species stabilize the serum osmolarity and ionic concentration after the transfer to sea water from freshwater more slowly, during 7-10 days (Potts & Rudy, 1972; Krayushkina, 1974, 1983a) than teleost fish, in particular salmonids, during 1-3 or 5 days (Folmar & Dickhoff, 1980; Krayushkina, 1983b). In this study, we observed that the mortality decreased with the increasing fish age and size after their transfer to EW and C_SW for 168 hours (Table 1). The same phenomenon was also found in other *Acipenser* species, such as *A. gueldenstaedtii, A. stellatus* (Krayushkina, 1983a), *A. transmontanus* (MacEnroe & Cech, 1985), *A. naccarii* (Garcia-Gallego *et al.*, 1998).

Any environmental disturbance can be considered as a potential source of stress, as it prompts a number of responses in the animal to deal with the physiological changes triggered by exterior changes. In theory, these responses can be detected in fish and in other vertebrates in the form of changes in hormonal or substrate concentrations in the plasma or alterations in erythrocyte parameters, such as cell volume or enzyme activities (Donaldson, 1981). Species specific normal ranges of such parameters can be established as useful guidelines for interpreting stress-induced physiological changes (Clark *et al.*, 1979; Roche & Boge, 1996). Thereby control treatments (FW) were for evaluation parameters.

The functional levels of the mechanism of osmotic and ionic homeostasis were similar in different groups (II to VI) but differed in experimental media (FW, EW, C_SW) ($P<0.05$) (Table 2&3). As a result, fluctuation range of osmolarity in blood serum of fish were increased from FW (244±14.4mOsml/l) to C_SW (312.48± 0.752mOsml/l) media ($P<0.05$) (Table 2). In sea water the diadromous acipenserids after their transfer from fresh water, have ability to support blood serum osmolarity lower than environmental osmolarity (Krayushkina & Semenova, 2006). In this study, the fishes have ability to support blood serum osmolarity higher than FW and approximate equal with EW osmolarity, but lower than C_SW osmolarity, (Table 2) (Krayushkina, 1983a).

In FW, EW and C_SW, we found that the great sturgeon regulate its blood Na^+ concentration at a similar level to teleosts. In FW adapted teleost the plasma Na^+ content generally lies between 110 and 130mEq/l, in SW it range between 150 and 180mEq/l. In FW adapted *Huso huso* the plasma Na^+ content was 128.8± 2.86mEq/l, and in EW 141.24±1.74mEq/l, and in C_SW 154.6±0.85mEq/l. Based on our data and that of others (Krayushlina, 1974; McEnroe & Cech, 1985; Kazemi *et al*, 2003; Krayushkina & Semenova, 2006) there are good similarity in K^+, Ca^{+2} and Mg^{+2} ions concentrations between FW, EW and C_SW adapted sturgeon, indicating a high degree of intracellular body fluid regulation .The significant differences were only between the levels of the Na^+ concentration in blood serum in different groups in the EW and C_SW media ($P<0.05$) (Table 3). Serum Na^+ and Ca^{2+} concentrations were found higher than in FW and EW media, but lower than in C_SW media (Table 3). Serum Mg^{+2} concentration (2.14± 0.04mEq/l) was found much lower than C_SW media (63.3±3.06mEq/l) (Krayushkina, 1983a; Krayushkina & Semenova, 2006) (Table 3).

Some authors suggest that no correlation between body size and haematological parameters such as HCT, erythrocyte size and number, and haemoglobin concentration are found (Shmidt-Nielsen, 1984; Calder, 1984; Garland & Carter, 1994). This is supported by the results of the present study. But in this study, the Hct, MCV and MCH decreased with the increasing of fish age, and decreased from FW media to C_SW media with increasing salinity, and RBC, WBC and MCHC did not change (Nespolo & Rosenmann, 2002)($P<0.05$) (Table 2).

In the present study, the trend of increasing levels of cortisol at higher salinity in juvenile *H. huso* (Table 2) indicates that for this chondrostean, the role of cortisol must be similar to that in teleosts. Besides, the increase of serum cortisol levels is considered to be a primary indicator of stress response (Cataldi *et al.*, 1998; Martinez-Alvarez *et al.*, 2002).

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